

## 1. INORGANIC BIO-MOLECULES

*C h a p t e r*

# 19

## The Inorganic Chemistry of Biological Systems

The chemistry of life can ultimately be referred to two chemical processes: (1) the use of radiant solar energy to drive chemical reactions that produce oxygen and reduced organic compounds from carbon dioxide and water, and (2) the oxidation of the products of (1) with the production of carbon dioxide, water, and energy. Alternatively, living organisms have been defined as systems capable of reducing their own entropy at the expense of their surroundings (which must gain in entropy).<sup>1</sup> An important feature of living systems is thus their unique dependence upon kinetic stability for their existence. All are thermodynamically unstable—they would burn up immediately to carbon dioxide and water if the system came to thermodynamic equilibrium. Life processes depend upon the ability to restrict these thermodynamic tendencies by controlled kinetics to produce energy as needed. Two important aspects of life will be of interest to us: (1) the ability to capture solar energy; (2) the ability to employ catalysts for the controlled release of that energy. Examples of such catalysts are the enzymes which control the synthesis and degradation of biologically important molecules. Many enzymes depend upon a metal ion for their activity. Metal-containing compounds are also important in the process of chemical and energy transfer, reactions which involve the transport of oxygen to the site of oxidation and various redox reactions resulting from its use.

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### Energy Sources for Life

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It may be somewhat surprising that most of the reactions for obtaining energy for living systems are basically inorganic. Of course, the reactions are mediated and made possible by complex biochemical systems.

### Nonphotosynthetic Processes

Even though almost all living organisms depend either directly (green plants) or indirectly (saprophytes and animals) upon photosynthesis to capture the energy of the sun, there are a few reactions, relatively unimportant in terms of scale but extremely

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<sup>1</sup> These reductionist definitions of life are not meant to imply that life processes or living organisms are simplistic or any the less interesting. A similar definition of physics and chemistry might be "the study of the interactions of matter and energy." None of these definitions hints at the fascination of some of the problems presented by these branches of science.

interesting in terms of chemistry, utilizing *inorganic* sources of energy. Even these may be indirectly dependent upon photosynthesis, since it is believed that all free oxygen on earth has been formed by photosynthesis.

Chemolithotrophic<sup>2</sup> bacteria obtain energy from various sources. For example, *iron bacteria* produce energy by the oxidation of iron(II) compounds:

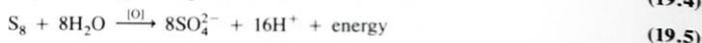


*Nitrifying bacteria* are of two types, utilizing ammonia and nitrite ion as nutriments:



Though they are photolithotrophs (Gr. *photos*, "light") and thus more closely related to the chemistry of normal photosynthesis (see page 916), the *green sulfur bacteria* and the *purple sulfur bacteria* are included here to demonstrate the diverse bacterial chemistry based on sulfur paralleling the more common biochemistry involving water and oxygen. Light energy is used to split hydrogen sulfide into sulfur, which is stored in the cells, and hydrogen which forms carbohydrates, etc., from carbon dioxide.

To return to the chemolithotrophs, there are species of sulfur bacteria that obtain energy from the oxidation of various states of sulfur:



These latter reactions are the source of energy for a unique fauna, one completely isolated from the sun on the floor of the oceans. These ecosystems have been discovered at certain rifts in the earth's crust on the ocean's floor, where large amounts of sulfide minerals are spewed forth from hydrothermal vents.<sup>3</sup> The sulfide concentration, principally in the form of hydrogen sulfide, ranges routinely up to 100  $\mu\text{M}$  depending upon the dilution of vent water by surrounding sea water. The  $\text{H}_2\text{S}$  has been shown to be depleted, along with  $\text{O}_2$ , in the midst of the aggregated organisms, and it is the energetic basis of these communities.<sup>4</sup> The sulfide is oxidized by bacteria as shown above. It is of considerable interest that the enzymes, mechanisms, and products of this chemically driven synthesis are essentially identical to those of photosynthesis (page 916), except that the source of electrons for the reduction of water to carbohydrates is sulfur(-II) rather than photoactivated chlorophyll. In addition to free-living bacteria, many of the vent animals contain endosymbiotic bacteria that serve them as primary energy sources as well as the source of reduced carbon compounds. The parallel between these endosymbionts in rift animals, such as tube worms, clams, and mussels, and the chloroplasts of plants is striking.<sup>5</sup> Whether

<sup>2</sup> That is, feeding (Gr. *trophos*) on inorganic (Gr. *lithos*, "stone") chemicals.

<sup>3</sup> Spiess, F. N.; Macdonald, K. C.; Atwater, T.; Ballard, R.; Carranza, A.; Cordoba, D.; Cox, C.; Diaz Garcia, V. M.; Francheteau, J.; Guerrero, J.; Hawkins, J.; Haymon, R.; Hessler, R.; Juteau, T.; Kastner, M.; Larson, R.; Luyendyk, B.; Macdougall, J. D.; Miller, S.; Normark, W.; Orcutt, J.; Rangin, C. *Science* **1980**, *207*, 1421-1433. Hekinian, R.; Fevrier, J. L.; Picot, P.; Shanks, W. C. *Ibid.* **1980**, *207*, 1433-1444. Edmond, J. M.; Von Damm, K. L.; McDuff, R. E.; Measures, C. I. *Nature (London)* **1982**, *297*, 187.

<sup>4</sup> Johnson, K. S.; Beehler, C. L.; Sakamoto-Arnold, C. M.; Childress, J. J. *Science* **1986**, *231*, 1139-1141.

<sup>5</sup> Childress, J. J.; Felbeck, H.; Somero, G. N. *Sci. Am.* **1987**, *256*(5), 114-120.

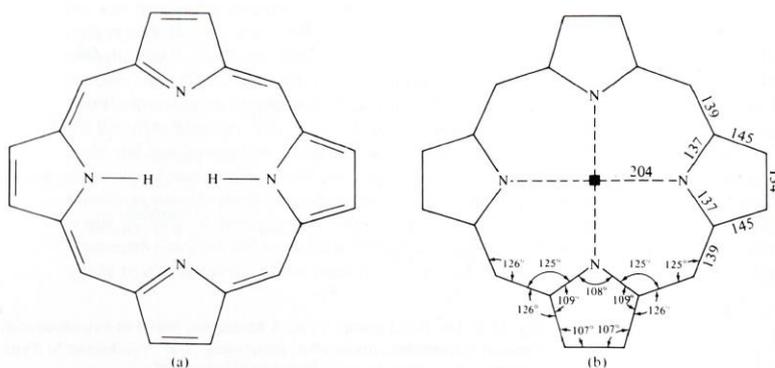
this parallelism results from an adaptation of the cycle from photosynthetic bacteria, or whether these chemolithotrophic bacteria are possibly ancestral to photosynthetic organisms presents the age-old phylogenetic problem—which came first, the chicken or the egg? The entire community, including predator species such as crabs, is entirely independent of photosynthesis except for the use of by-product dioxygen. There is even evidence that some of the animals such as gutless clams can metabolize hydrogen sulfide independently, simultaneously detoxifying it and using it as an energy source.<sup>6</sup>

### Metalloporphyrins and Respiration

#### Cytochromes

Some of the simplest bioinorganic compounds are the various cytochromes. In terms of overall structure and molecular weight they are anything but simple. However, the inorganic chemistry of several of them is very simple coordination and redox chemistry. The active center of the cytochromes is the *heme* group. It consists of a porphyrin ring chelated to an iron atom. The porphyrin ring consists of a macrocyclic pyrrole system with conjugated double bonds (Fig. 19.1) and various groups attached to the perimeter. We shall not be concerned with the nature and variety of these substituents except to note that by their electron-donating or electron-withdrawing ability they can "tune" the delocalized molecular orbitals of the complex and thus vary its redox properties. The porphyrin can accept two hydrogen ions to form the +2 diacid or donate two protons and become the -2 dianion. It is in the latter form that the porphyrins complex with metal ions, usually divopositive, to form metalloporphyrin complexes.

From the covalent bond radii (Table 8.1) we can estimate that a bond between a nitrogen atom and an atom of the first transition series should be about 200 pm long. The size of the "hole" in the center of the porphyrin ring is ideal for accommodating metals of the first transition series (Fig. 19.1b). The porphyrin system is fairly rigid,



**Fig. 19.1** (a) The porphyrin molecule. Porphyrins have substituents at the eight pyrrole positions. (b) A "best" set of parameters for an "average" porphyrin skeleton. Distance in pm. [From Fleischer, E. B. *Acc. Chem. Res.* **1970**, *3*, 105. Reproduced with permission.]

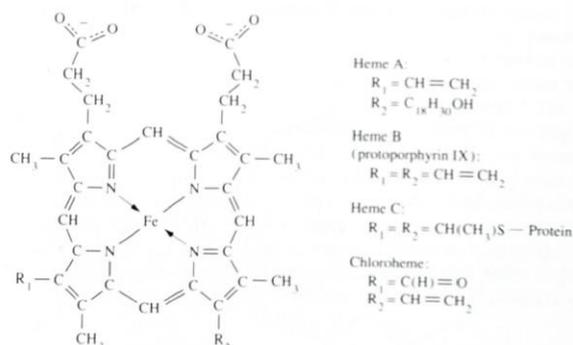
<sup>6</sup> Powell, M. A.; Somero, G. N. *Science* **1986**, *233*, 563.

and the metal–nitrogen bond distance does not vary greatly from 193–196 pm in nickel porphyrins to 210 pm in high spin iron(II) porphyrins. The rigidity of the ring derives from the delocalization of the  $\pi$  electrons in the pyrrole rings. Nevertheless, if the metal atom is too small, as in nickel porphyrinates, the ring becomes ruffled to allow closer approach of the nitrogen atoms to the metal. At the other extreme, if the metal atom is too large, it cannot fit into the hole and sits above the ring which also becomes domed (see page 903).

The order of stability of complexes of porphyrins with +2 metal ions is that expected on the basis of the Irving–Williams series (see Chapter 9), except that the square planar ligand favors the  $d^8$  configuration of  $\text{Ni}^{2+}$ . The order is  $\text{Ni}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Zn}^{2+}$ . The kinetics of formation of these metalloporphyrins has also been measured and found to be in the order  $\text{Cu}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Ni}^{2+}$ .<sup>7</sup> If this order holds in biological systems, it poses interesting questions related to the much greater abundance of iron porphyrins (see below). What might have been the implications for the origin and evolution of biological systems if the natural abundance of iron were not over a thousandfold greater than those of cobalt and copper?

The porphyrin ring or modifications of it are important in several quite different biological processes. The reason for the importance of porphyrin complexes in a variety of biological systems is probably twofold: (1) They are biologically accessible compounds whose functions can be varied by changing the metal, its oxidation state, or the nature of the organic substituents on the porphyrin structure; (2) it is a general principle that evolution tends to proceed by modifying structures and functions that are already present in an organism rather than producing new ones *de novo*.

The heme group is a porphyrin ring with an iron atom at the center (Fig. 19.2). The oxidation state<sup>8</sup> of the iron may be either +2 or +3, and the importance of the



**Fig. 19.2** The heme group: Type A hemes are found in cytochrome *a*; Type B hemes are found in hemoglobin, myoglobin, peroxidase, and cytochrome *b*; Type C hemes are found in cytochrome *c*; chloroheme is found in chlorocruorin.

<sup>7</sup> Bishop, D. G.; Reed, M. L. *Photochem. Photobiol. Rev.* **1976**, *1*, 1.

<sup>8</sup> The term *heme* refers to the neutral group containing Fe(II), either isolated or in a protein. When the isolated heme is oxidized to Fe(III), there will be a net positive charge and an associated, coordinated anion, often the chloride ion. When oxidized, the term *hemin* is applied, as in hemin chloride. *Hematin*, long thought to be "hemin hydroxide," is actually a  $\mu$ -oxo dimer (see page 896).

cytochromes lies in their ability to act as redox intermediates in electron transfer. They are present not only in the chloroplasts for photosynthesis but also in mitochondria to take part in the reverse process of respiration.

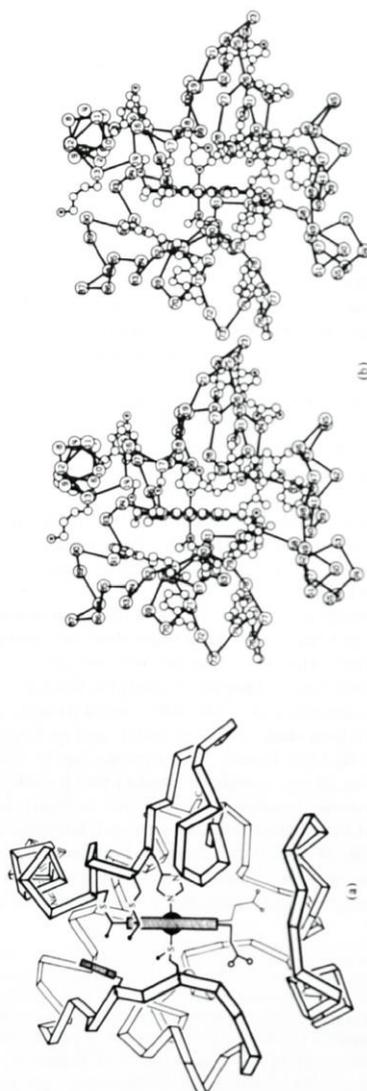
The heme group in cytochrome *c* has a polypeptide chain attached and wrapped around it (Fig. 19.3). This chain contains a variable number of amino acids, ranging from 103 in some fish and 104 in other fish and terrestrial vertebrates to 112 in some green plants. A nitrogen atom from a histidine segment and a sulfur atom from a methionine segment of this chain are coordinated to the fifth and sixth coordination sites of the iron atom.<sup>9</sup> Thus, unlike the iron in hemoglobin and myoglobin (see below), there is no position for further coordination. Cytochrome *c* therefore cannot react by simple coordination but must react indirectly by an electron transfer mechanism. It can reduce the dioxygen and transmit its oxidizing power towards the burning of food and release of energy in respiration (the reverse process to complement photosynthesis). The importance of cytochrome *c* in photosynthesis and respiration indicates that it is probably one of the oldest (in terms of evolutionary history) of the chemicals involved in biological processes. An interesting "family tree" of the evolution of living organisms can be constructed from the differences in amino acid sequences in the peptide chains between the various types of cytochrome *c* found, for example, in yeasts, higher plants, insects, and humans. Despite these differences, however, it should be noted that cytochrome *c* is evolutionarily conservative. Cytochrome *c* from any eucaryotic species will react with the cytochrome oxidase of any other eucaryotic species, plant or animal, though at reduced rates.<sup>10</sup>

There is quite a variety of cytochromes, most of which have not been as well characterized as cytochrome *c*. Depending upon the ligands present, the redox potential of a given cytochrome can be tailored to meet the specific need in the electron transfer scheme, whether in photosynthesis or in respiration. The potentials are such that the electron flow is  $b \rightarrow c \rightarrow a \rightarrow O_2$ . At least some of the *a* type (cytochrome *c* oxidase) are capable of binding dioxygen molecules and reducing them. They are thus the last link in the respiratory chain of electrons flowing from reduced foodstuffs to oxygen. Therefore, they must be five coordinate (in the absence of  $O_2$ ) in contrast to cytochrome *c*. They are responsible for the unusually severe and rapid toxicity of the cyanide ion,  $CN^-$ . The latter binds strongly to the sixth position and stabilizes the Fe(III) to such an extent that it can no longer be readily reduced and take part in the electron shuttle. The cyanide ion is isoelectronic with the carbon monoxide molecule and it might be thought that it could bind tightly to hemoglobin as does CO. However, cyanide binds well only to Fe(III) hemoglobin (methemoglobin<sup>11</sup>), an aberrant form usually present only in small quantities. Cyanide poisoning is thus not the result of lack of hemoglobin function (as is CO poisoning). In fact, the standard treatment for cyanide poisoning is inhalation of amyl nitrite or injection of sodium nitrite to oxidize some of the hemoglobin to methemoglobin (see page 907). The latter,

<sup>9</sup> For the complete structures of ferrocycytochrome *c* ( $Fe^{2+}$ ), see Takano, T.; Trus, B. L.; Mandel, N.; Mandel, G.; Kallai, O. B.; Swanson, R.; Dickerson, R. E. *J. Biol. Chem.* 1977, 252, 776-785.

<sup>10</sup> See Stryer, L. *Biochemistry*, 3rd ed.; Freeman: New York, 1981; pp 328-329. Dickerson, R. E. *Sci. Amer.* 1972, 226(4) pp 58-72. *Eucaryotic* cells have their DNA in true nuclei, as opposed to *procaryotic* cells (bacteria and blue-green algae) which do not.

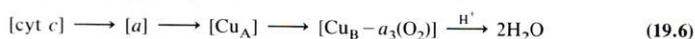
<sup>11</sup> The prefix *met-* is used to signify that the iron atom, normally in the +2 oxidation state, has been oxidized to +3.



**Fig. 19.3** (a) Schematic view of cytochrome *c*. The heme group is viewed edge on, with the iron atom (large black atom) coordinated to a sulfur atom from a methionine residue and a nitrogen atom from a histidine residue. (b) Stereoview of the cytochrome *c* molecule. Each number represents an amino acid in the protein chain. Note the complete coordination sphere of the iron atom as well as the protection afforded by the encircling protein chains. [Courtesy of R. E. Dickerson and from Takano, T.; Trus, B. L.; Mandel, N.; Mandel, G.; Kallai, O. B.; Swanson, R.; Dickerson, R. E. *J. Biol. Chem.* 1977, 252, 776-785. Reproduced with permission.]

although useless for dioxygen transport, binds cyanide even more tightly than hemoglobin or cytochrome oxidase and removes it from the system.<sup>12</sup>

The structure of cytochrome *c* oxidase is not known completely. It contains two heme groups of the cytochrome type (*a* and *a*<sub>3</sub>) and two copper atoms (Cu<sub>A</sub> and Cu<sub>B</sub>). When the reduced Fe(II) oxidase is treated with carbon monoxide, the *a*<sub>3</sub> moiety binds it and gives a myoglobin-carbon monoxide-like spectrum. The *a* site does not bind carbon monoxide, indicating a six-coordinate, cytochrome-*c*-like structure. The oxidized, Fe(III) form binds cyanide at *a*<sub>3</sub>, but not at *a*, supporting this interpretation. (Metmyoglobin and methemoglobin will also bind cyanide, but cytochrome *c* will not.) The EPR spectra of iron and copper show that *a*<sub>3</sub> and Cu<sub>B</sub> are antiferromagnetically coupled, and EXAFS (see page 913) measurements indicate that these Fe and Cu atoms are about 370 pm apart, compatible with a sulfide bridge. The electron flow is probably:<sup>13</sup>



### Dioxygen Binding, Transport, and Utilization

#### The Interaction between Heme and Dioxygen

While all of the biochemical uses of the heme group are obviously important, the one that has perhaps attracted the most attention because of its central biological role and its intricate chemistry is the binding of the dioxygen molecule, O<sub>2</sub>. This has been mentioned briefly above with regard to the binding and reduction of dioxygen by cytochrome oxidase. Before this step occurs, vertebrates<sup>14</sup> have already utilized two other heme-containing proteins: Hemoglobin picks up the dioxygen from the lungs or gills and transports it to the tissues where it is stored by myoglobin. The function of hemoglobin in the red blood cells is obvious, that of myoglobin is more subtle. Besides being a simple repository for dioxygen, it also serves as a dioxygen reserve against which the organism can draw during increased metabolism or oxygen deprivation.<sup>15</sup> Other suggested functions include facilitation of dioxygen flow within the cell and a "buffering" of the partial pressure within the cell in response to increasing or decreasing oxygen supply.<sup>16</sup>

Dioxygen is far from a typical ligand. It probably resembles the carbon monoxide, nitrosyl, and dinitrogen ligands more than any others. None of these has a significant dipole moment contributing to the σ bond, but the electronegativity difference between the atoms in CO and NO enhances π\* interactions (see Chapter 11). Dinitrogen and dioxygen lack this advantage, but may be considered soft ligands with some π-bonding capacity. Iron(II), d<sup>6</sup>, is not a particularly soft metal cation, but the "soften-

<sup>12</sup> See Hanzlik, R. P. *Inorganic Aspects of Biological and Organic Chemistry*; Academic: New York, 1976, p 152. Ochiai, E-I. *Bioinorganic Chemistry*; Allyn & Bacon: Boston, 1977; p 483.

<sup>13</sup> See various articles on cytochrome oxidase in *Electron Transport and Oxygen Utilization*; Ho, C., Ed.; Elsevier North Holland: New York, 1982; Karlin, K.; Gultneh, Y. *Progr. Inorg. Chem.* **1987**, *35*, 310-311.

<sup>14</sup> There is an exception in certain "bloodless" Antarctic fishes (Chaenichthyidae) in which the metabolism is so low and the oxygen solubility so high, both resulting from the extremely low water temperatures, that oxygen carriers are not necessary.

<sup>15</sup> Diving mammals such as whales have a large amount of myoglobin in their tissues which presumably enables them to remain submerged for extended periods of time.

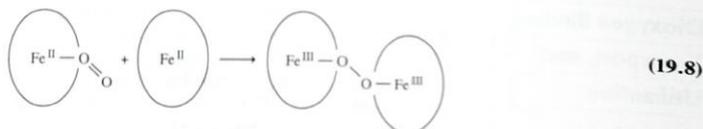
<sup>16</sup> See Cole, R. P. *Science* **1982**, *216*, 523.

ing" (sybiotic) action of the tetrapyrrole ring system probably facilitates dioxygen binding. Note that the heme group binds the truly soft ligand carbon monoxide even more tightly, resulting in potentially lethal carbon monoxide poisoning.<sup>17</sup>

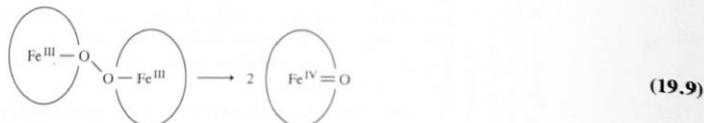
However, there is another potentially fatal flaw in the binding of dioxygen by heme: irreversible oxidation. If free heme in aqueous solution is exposed to dioxygen, it is converted almost immediately into a  $\mu$ -oxo dimer known as hematin. The mechanism of this reaction has been worked out in detail.<sup>18</sup> The reactions are as follows, where the heme group is symbolized by the circle about an iron atom. The first step is the binding of the dioxygen molecule, as in hemoglobin:<sup>19</sup>



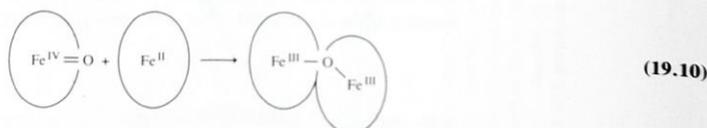
The bound dioxygen can now coordinate to a second heme, forming a  $\mu$ -peroxo complex:



Cleavage of the peroxo complex results in two molecules of a ferryl complex with the iron in the +4 formal oxidation state:



Finally, attack of the ferryl complex on another heme results in the formation of hematin:



<sup>17</sup> Carbon monoxide poisoning may be treated by flooding the system with oxygen. Nevertheless, the binding of CO is about 500 times stronger than the binding of O<sub>2</sub>. It could be worse. Carbon monoxide binds even more strongly (by about two orders of magnitude) to free heme. The steric hindrance about the heme in hemoglobin and myoglobin may favor the bent O<sub>2</sub> over the (optimally) linear carbon monoxide. (Stryer, L. *Biochemistry*, 2nd ed.; Freeman: New York, 1981; p 54.)

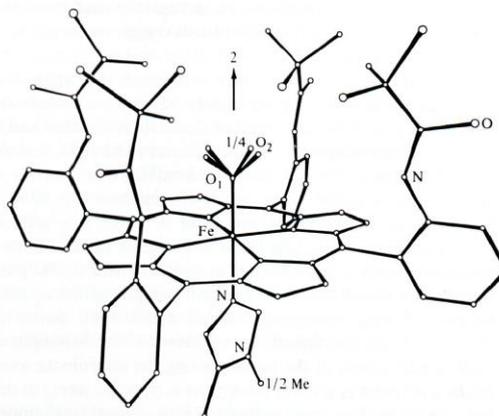
<sup>18</sup> Balch, A. L.; Chan, Y.-W.; Cheng, R. J.; La Mar, G. N.; Latos-Grazynski, L.; Renner, M. W. *J. Am. Chem. Soc.* **1984**, *106*, 7779-7785; Penner-Hahn, J. E.; Eble, K. S.; McMurry, T. J.; Renner, M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *Ibid.* **1986**, *108*, 7819.

<sup>19</sup> The oxidation states may occasionally be ambiguous—the adduct in Eq. 19.7 may be formulated as heme(II)-dioxygen or as heme(III)-superoxide. See Problem 19.21.

Obviously, living systems have found a way to frustrate reactions 19.7-19.10; otherwise all of the heme would be precipitated as hematin rather than shuttling electrons in the cytochromes or carrying dioxygen molecules in oxyhemoglobin (and storing them in oxymyoglobin). There may be more than one mechanism in effect here, but certainly the primary one is *steric hindrance*: The globin part of the molecule prevents one oxoheme from attacking another heme. This was first illustrated over thirty years ago by embedding the heme group in a polymer matrix that allowed only restricted access to the iron atom: The embedded heme will reversibly bind dioxygen.<sup>20</sup> More recently this same result has been achieved by "picket-fence" hemes and related compounds (Fig. 19.4) that reversibly bind dioxygen<sup>21</sup> and not only confirm the steric hypothesis with regard to the stability of hemoglobin, but allow detailed structural measurements to be made of a heme model compound. Thus the angular or bent coordination of dioxygen to heme (in hemoglobin and myoglobin) was first indicated by the structure shown in Fig. 19.4. It has since been confirmed in myoglobin and hemoglobin (see below).

### The Binding of Dioxygen to Myoglobin

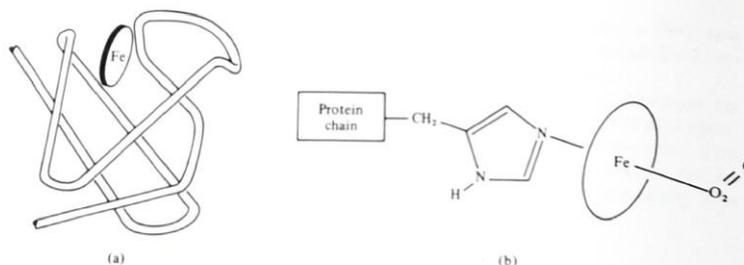
Myoglobin is a protein of molecular weight of about 17,000 with the protein chain containing 153 amino acid residues folded about the single heme group (Fig. 19.5). This restricts access to the iron atom (by a second heme) and reduces the likelihood of formation of a hematin-like Fe(III) dimer. The microenvironment is similar to that in cytochrome *c*, but there is no sixth ligand (methionine) to complete the coordination



**Fig. 19.4** Perspective view of picket-fence dioxygen adduct. The apparent presence of four different O<sub>2</sub> atoms results from a four-way statistical disorder of the oxygen atoms on different molecules responding to the X-ray diffraction. [From Collman, J. P.; Gagne, R. R.; Reed, C. A.; Robinson, W. T.; Rodley, G. A. *Proc. Natl. Acad. Sci. U. S. A.* **1974**, *71*, 1326-1329. Reproduced with permission.]

<sup>20</sup> Wang, J. H. *J. Am. Chem. Soc.* **1958**, *80*, 3168; *Acc. Chem. Res.* **1970**, *3*, 90.

<sup>21</sup> Jameson, G. B.; Molinaro, F. S.; Ibers, J. A.; Collman, J. P.; Brauman, J. I.; Rose, E.; Suslick, K. S. *J. Am. Chem. Soc.* **1980**, *102*, 3224-3237. For a review of sterically hindered biomimetic porphyrins, see Morgan, B.; Dolphin, D. *Struct. Bonding (Berlin)* **1987**, *64*, 115.



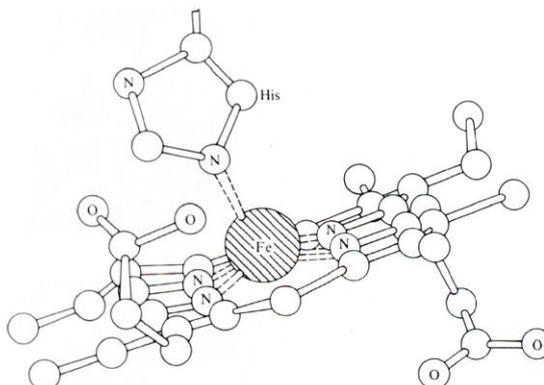
**Fig. 19.5** The myoglobin molecule: (a) the folding of the polypeptide chain about the heme group (represented by the disk); (b) close-up view of the heme environment. [Modified from Kendrew, J. C.; Dickerson, R. E.; Strandberg, B. E.; Hart, R. G.; Davies, D. R.; Phillips, D. C.; Shore, V. C. *Nature* **1960**, *185*, 422-427. Reproduced with permission.]

sphere of the iron atom. Thus there is a site to which a dioxygen molecule may reversibly bind.

Note how the differences in structure between the dioxygen-binding molecules (myoglobin, hemoglobin, and cytochrome oxidase) and the electron carriers (various cytochromes, including cytochrome oxidase which performs both functions) correlate with their specific functions. In myoglobin and hemoglobin the redox behavior is retarded, and there is room for the dioxygen molecule to coordinate without electron transfer taking place.<sup>22</sup>

Myoglobin contains iron(II) in the high spin state. Iron(II) is  $d^6$  and, when high spin, has a radius of approximately 92 pm in a pseudo-octahedral environment (the square pyramidal arrangement of heme in myoglobin and hemoglobin may be considered an octahedron with the sixth ligand removed), and the iron atom will not fit into the hole of the porphyrin ring. The iron(II) atom thus lies some 42 pm above the plane of the nitrogen atoms in the porphyrin ring (see Fig. 19.6). When a dioxygen molecule binds to the iron(II) atom, the latter becomes low spin  $d^6$  (cf. the extremely stable  $\text{Co}^{3+}$  complexes with  $2.4\Delta$  LFSE). The ionic radius of low spin iron(II) with coordination number six is only 75 pm, in contrast with the 92 pm of high spin iron. Why the difference? Recall that in octahedral complexes the  $e_g$  orbitals are those aimed at the ligands. If they contain electrons, which they do in the high spin case ( $t_{2g}^4 e_g^2$ ), they will repel the ligands as opposed to the low spin case ( $t_{2g}^6 e_g^0$ ), which allows unhindered access of the ligands along the coordinate axes. Thus the effective radius of the iron atom is greater (along the  $x$ ,  $y$ , and  $z$  axes) in the high spin state than in the low spin state. The result is that the iron atom shrinks upon spin pairing and drops into the hole in the porphyrin ring. All of the ligands (including the proximal histidine) are able to approach the iron atom more closely. The net effect in myoglobin is minimal, but the process is an important one for the transmission of dioxygen from the lungs to the tissues by hemoglobin. The spin pairing of the normally paramagnetic dioxygen molecule is also of interest, though often overlooked (see Problem 19.9).

<sup>22</sup> Indeed, hemoglobin has been dubbed a "frustrated oxidase" [Winterbourn, C. C.; French, J. K. *Biochem. Soc. Trans.* **1977**, *5*, 1480; French, J. K.; Winterbourn, C. C.; Carrell, R. W. *Biochem. J.* **1978**, *173*, 19].



**Fig. 19.6** Close-up of the heme group in myoglobin and hemoglobin. Note that the iron atom does *not* lie in the plane of the heme group.

A knowledge of the exact molecular arrangement of dioxygen in oxymyoglobin and oxyhemoglobin has been desirable in order to understand the chemistry of dioxygen transport and storage. Unfortunately, this has been difficult to achieve because of the high molecular weight of the molecules and the low resolution of the X-ray-determined structures. The structure that has been determined to the greatest resolution is that of erythrocyruorin which has been refined to a resolution of 140 pm.<sup>23</sup> The dioxygen is bonded to the iron with an angle of  $\sim 150^\circ$  and an Fe—O bond length of  $\sim 180$  pm. Oxymyoglobin (sperm whale)<sup>24</sup> and oxyhemoglobin (human)<sup>25</sup> have not been resolved as highly (210 pm), but the Fe—O bond lengths are similar. All of these are compatible with the more accurate value of 190 pm in the picket-fence adduct.<sup>26</sup> However, the Fe—O—O bond angles vary considerably, from  $\sim 115^\circ$  in myoglobin to  $153^\circ$  in human hemoglobin (for more details, see Table 19.1, page 905) with the most accurate value being  $131^\circ$  in the model picket-fence compound. The source of the differences is not clear, but calculations<sup>27</sup> indicate that the bond energy changes but little with bond angle, and so other factors such as steric effects or hydrogen bonding with a neighboring group could be important (Fig. 19.7).

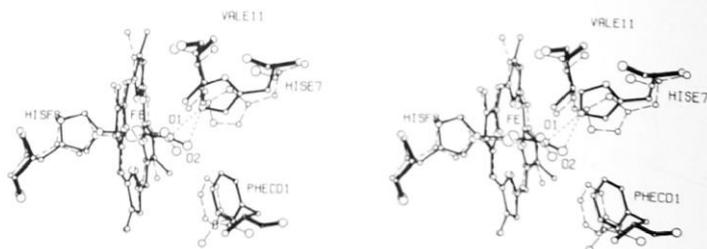
<sup>23</sup> Erythrocyruorin is a form of myoglobin found in chironomid midges (flies). In general, the greater the resolution (the smaller this value), the more accurate the structural determination, but it should be realized that the refinement of structures of proteins containing tens of thousands of atoms is far more complicated than the almost routine determination of structures of molecules containing a few dozen atoms at most. Often assumptions must be made with a resulting shift of values: The M—O—O bond angle in erythrocyruorin was corrected from  $170^\circ$  (extraordinary!) to  $150^\circ$  when such assumptions were changed (Steigemann, W.; Weber, E. *J. Mol. Biol.* **1979**, *127*, 309).

<sup>24</sup> Phillips, S. E. V. *Nature (London)* **1978**, *273*, 247; *J. Mol. Biol.* **1980**, *142*, 531.

<sup>25</sup> Shaanan, B. *Nature (London)* **1982**, *296*, 683; *J. Mol. Biol.* **1983**, *171*, 31.

<sup>26</sup> Jameson, G. B.; Rodley, G. A.; Robinson, W. T.; Gagne, R. R.; Reed, C. A.; Collman, J. P. *Inorg. Chem.* **1978**, *17*, 850-857.

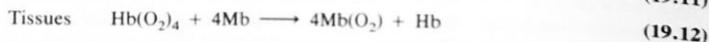
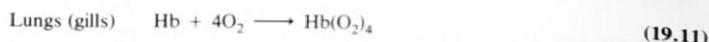
<sup>27</sup> Hoffmann, R.; Chen, M. M.-L.; Thorn, D. L. *Inorg. Chem.* **1977**, *16*, 503-511. Kirchner, R. F.; Loew, G. H. *J. Am. Chem. Soc.* **1977**, *99*, 4639.



**Fig. 19.7** Stereoview of superimposed heme environments in oxyhemoglobin and oxymyoglobin. Solid lines denote  $\text{HbO}_2$  and dashed lines  $\text{MbO}_2$ . Note the difference in the  $\text{Fe}-\text{O}_1-\text{O}_2$  bond angles and the presumed hydrogen bond (dotted line) to the histidine (His E7). [From Shaanan, B. *Nature (London)* **1982**, 296, 683. Reproduced with permission.]

### The Physiology of Myoglobin and Hemoglobin

In vertebrates dioxygen enters the blood in the lungs or gills<sup>28</sup> where the partial pressure of dioxygen is relatively high [21% oxygen =  $0.21 \times 1.01 \times 10^5 \text{ Pa}$  (760 mm Hg) =  $2.1 \times 10^4 \text{ Pa}$  (160 mm Hg)] under ideal conditions; in the lungs with mixing of inhaled and nonexhaled gases, the value is closer to  $1.3 \times 10^4 \text{ Pa}$  (100 mm Hg). It is then carried by red blood cells (Fig. 19.8a) to the tissues where the partial pressure is considerably lower [of the order of  $2.5 \times 10^3$  to  $6.5 \times 10^3 \text{ Pa}$  (20–50 mm Hg)]. The reactions are as follows:

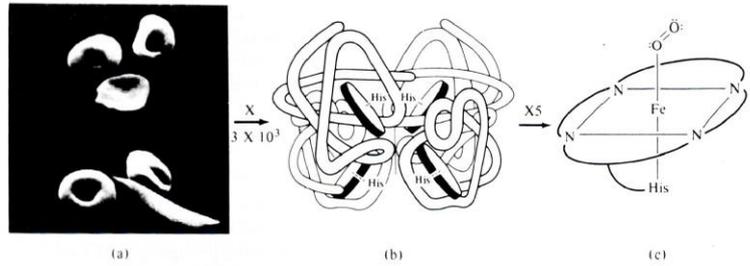


Note that hemoglobin has an ambivalent function: It should bind dioxygen tightly and carry as much as possible to the tissues, but once there it should, chameleon-like, relinquish it readily to myoglobin which can store it for oxidation of foodstuffs. Hemoglobin serves this function admirably as shown by Fig. 19.9: (1) Myoglobin must have a greater affinity for dioxygen than hemoglobin in order to effect the transfer of dioxygen at the cell. (2) The equilibrium constant for the myoglobin-dioxygen complexation is given by the simple equilibrium expression:

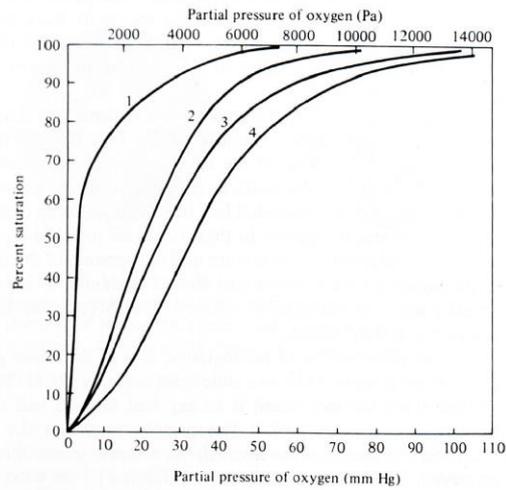
$$K_{\text{Mb}} = \frac{[\text{Mb}(\text{O}_2)]}{[\text{Mb}][\text{O}_2]} \quad (19.13)$$

If the total amount of myoglobin ( $[\text{Mb}] + [\text{Mb}(\text{O}_2)]$ ) is held constant (as it must be in the cell) while the concentration of oxygen is varied (in terms of partial pressure), the

<sup>28</sup> Small organisms require no oxygen transport system beyond simple diffusion. There is a family of lungless salamanders, the Plethodontidae, which have neither gills nor lungs (as adults) and rely upon oxygen exchange through the skin and through buccopharyngeal ("mouth and throat") exchange. Some worms and mollusks have proteins related to hemoglobin for oxygen transport and storage. Some polychaete worms employ chlorocruorin which turns green upon oxygenation. Sipunculid worms and some other species utilize nonheme iron proteins, the hemerythrins, for these functions (see page 908). Lobsters, crabs, spiders, cephalopods, and some snails use a copper-containing protein (hemocyanin, see page 909) for oxygen transport.



**Fig. 19.8** Relative scale of (a) red blood cells, the *biological* unit of dioxygen transport; (b) the hemoglobin molecule, the *biochemical* unit of dioxygen transport; and (c) the dioxygen-heme group, the *inorganic* unit of dioxygen transport. The relative sizes are given by the factors over the arrows. [Scanning electron micrograph courtesy of M. Barnhart, Wayne State University of Medicine. Hemoglobin molecule modified from Perutz, M.; Rossman, M. G.; Cullis, A. F.; Muirhead, H.; Will, G.; North, A. C. T. *Nature (London)* 1960, 185, 416. Reproduced with permission.]



**Fig. 19.9** Dioxygen binding curves for (1) myoglobin and for hemoglobin at various partial pressures of carbon dioxide: (2) 20 mm Hg; (3) 40 mm Hg; (4) 80 mm Hg. Note that myoglobin has a stronger affinity for dioxygen than hemoglobin and that this effect is more pronounced in the presence of large amounts of carbon dioxide. [Modified from Bock, A. V.; Field, H., Jr.; Adair, G. S. *J. Biol. Chem.* 1924, 59, 353-378. Reproduced with permission.]

curve shown in Fig. 19.9 is obtained. Myoglobin is largely converted to oxymyoglobin even at low oxygen concentrations such as occur in the cells. (3) The equilibrium constant for the formation of oxyhemoglobin is somewhat more complicated. The expression for the curve in the range of physiological importance in the tissues is:

$$K_{\text{Hb}} = \frac{[\text{Hb}(\text{O}_2)_4]}{[\text{Hb}][\text{O}_2]^{2.8}} \quad (19.14)$$

The 2.8 exponent for dioxygen results from the fact that a single hemoglobin molecule can accept four-dioxygen molecules and *the binding of the four is not independent*. It is not the presence of four heme groups to bind four dioxygen molecules per se that is important. If they acted independently, they would give a curve identical to that of myoglobin. It is the *cooperativity* of the four heme groups that produces the curves shown in Fig. 19.9. The presence of several bound dioxygen molecules favors the addition of more dioxygen molecules; conversely, if only one dioxygen molecule is present, it dissociates more readily than from a more highly oxygenated species. The net result is that at low dioxygen concentrations hemoglobin is less oxygenated (tends to release  $\text{O}_2$ ), and at high dioxygen concentrations hemoglobin is oxygenated almost to the same extent as if the exponent were 1. This results in a sigmoid curve for oxygenation of hemoglobin (Fig. 19.9). This effect favors oxygen transport since it helps the hemoglobin become saturated in the lungs and deoxygenated in the capillaries. (4) There is a pH dependence shown by hemoglobin. This is known as the *Bohr effect*.<sup>29</sup> Hemoglobin binds one  $\text{H}^+$  for every two dioxygen molecules released. This favors the conversion of carbon dioxide, a metabolite of the tissues, into the hydrogen carbonate ion ( $\text{HCO}_3^-$ ) promoting its transport back to the lungs. Likewise, the production of carbon dioxide from cell respiration and of lactic acid from anaerobic metabolism favors the release of dioxygen to the tissues.

### Structure and Function of Hemoglobin

Hemoglobin may be considered an approximate tetramer of myoglobin. It has a molecular weight of 64,500 and contains four heme groups bound to four protein chains (Fig. 19.8b). Two of the chains, labeled beta, have 146 amino acids and are somewhat similar to the chain in myoglobin; the other two, labeled alpha, have 141 amino acids and are somewhat less like the myoglobin chain. The differences between hemoglobin and myoglobin in their behavior towards dioxygen (particularly 3 and 4 above) are related to the structure and movements of the four chains. If the tetrameric hemoglobin is broken down into dimers or monomers, these effects are lost, and the smaller units do not exhibit cooperativity. Myoglobin does not exhibit the sigmoid curve nor a Bohr effect.

Upon oxygenation of hemoglobin, two of the heme groups move about 100 pm towards each other while two others separate by about 700 pm. Perhaps a better way of describing the movement is to say that one  $\alpha\beta$  half of the molecule rotates  $15^\circ$  relative to the other half.<sup>30</sup> These movements are the result of a change in the quaternary structure of the hemoglobin and are responsible for the cooperative effects observed. The quaternary structure exhibited by the deoxy form is called the T state,

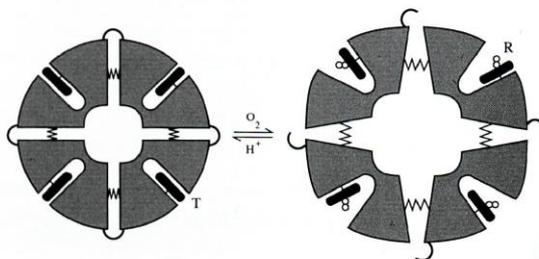
<sup>29</sup> Discovered by Christian Bohr, father of Niels Bohr, the pioneer of quantum mechanics.

<sup>30</sup> Dickerson, R. E.; Geis, I. *The Structure and Action of Proteins*; Harper & Row: New York, 1969; p 59; *Hemoglobin: Structure, Function, Evolution, and Pathology*; Benjamin/Cummings: Menlo Park, CA, 1983. Baldwin, J.; Chothia, C. *J. Mol. Biol.* **1979**, *129*, 175.

and that of the oxy form the R state.<sup>31</sup> The dioxygen affinity of the R form is about the same as that of isolated  $\alpha$  and  $\beta$  chains, but the dioxygen affinity of the T state is some 12–14 kJ mol<sup>-1</sup> lower. This fundamental difference in the energetics of dioxygen binding is responsible for the cooperativity of hemoglobin. The lower affinity of the T form is responsible for the slow start of the sigmoidal curve (lower left, Fig. 19.9), and the higher affinity of the R form causes the rapid rise in the curve (upper right, Fig. 19.9) until it almost matches that of myoglobin.

Perutz<sup>32</sup> has suggested a mechanism to account for the cooperativity of the four heme groups in hemoglobin. Basically it is founded on the idea that the interaction between a dioxygen molecule and a heme group can affect the position of the protein chain attached to it, which in turn affects the other protein chains through hydrogen bonds, etc., and eventually the tertiary and quaternary structure of the protein. It has been dubbed the Rube Goldberg effect after the marvelous mechanisms of ropes, pulleys, and levers in Goldberg's cartoons.<sup>33</sup> A simplified illustration of the Perutz mechanism is shown in Fig. 19.10.

The key or trigger in the Perutz mechanism is the high spin Fe(II) atom in a dioxygen-free heme. As we have seen, the radius of high spin Fe<sup>2+</sup> is too large to fit within the plane of the four porphyrin nitrogen atoms. The iron atom is thus forced to sit *above* the center of the heme group (Fig. 19.6; Fig. 19.11a) with an Fe—N<sub>porphyrin</sub> distance of about 206 pm. Furthermore, the heme group is domed upward towards the proximal histidine.

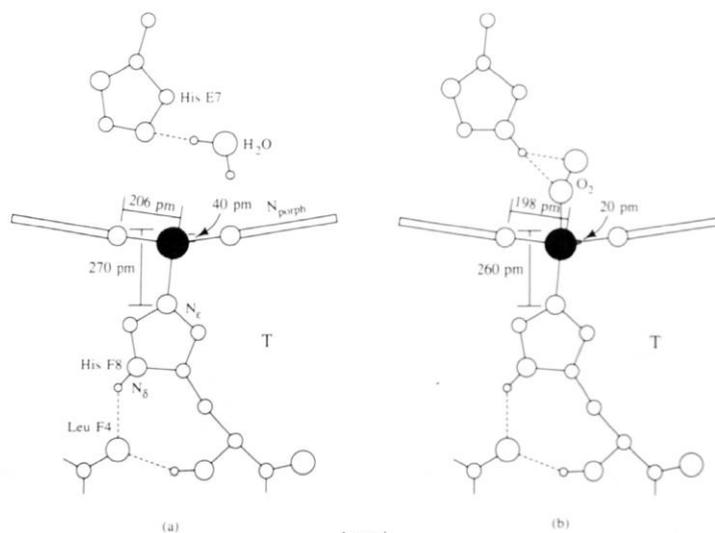


**Fig. 19.10** A schematic diagram of the Perutz mechanism in hemoglobin. The three most important factors are highlighted: (1) the environment of the heme; (2) the movement (tension) of the protein chains, and (3) the breaking of hydrogen bonds ("salt bridges"). [Courtesy of Professor M. Perutz. Reproduced with permission.]

<sup>31</sup> The terms come from the adjectives *tense* and *relaxed* that have been applied to the two structures. However, since the nature, the extent, and even the existence of "tension" in one form or the other is a matter of considerable controversy, we shall use T and R as labels for the quaternary structures without structural or mechanistic implications.

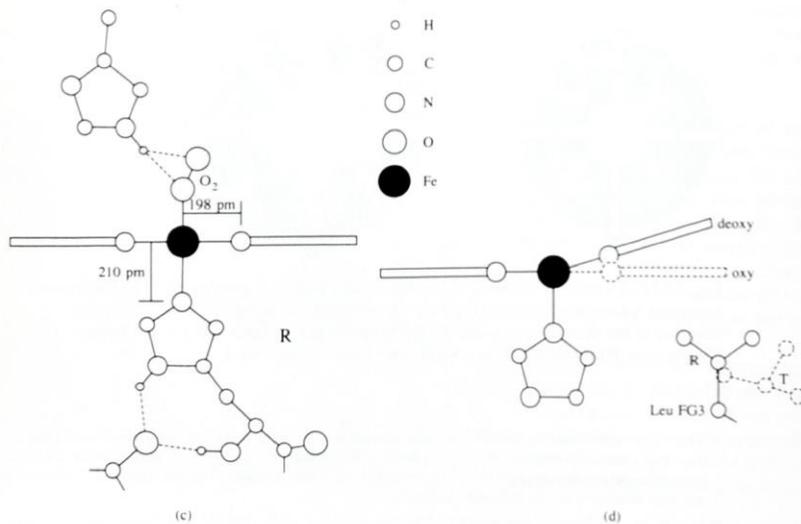
<sup>32</sup> Perutz, M. F. *Nature (London)* **1970**, 228, 726; *Br. Med. Bull.* **1976**, 32, 195. Perutz, M. F.; Fermi, G.; Luisi, B.; Shaanan, B.; Liddington, R. C. *Acc. Chem. Res.* **1987**, 20, 309. Perutz, M. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University: Cambridge, 1990.

<sup>33</sup> Huheey, J. E. In *REACTS 1973, Proceedings of the Regional Annual Chemistry Teaching Symposium*; Egolf, K.; Rodez, M. A.; Won, A. J. K.; Zidick, C., Eds.; University of Maryland: College Park, 1973; pp 52–78.



Legend:

- H
- C
- N
- O
- Fe



The coordination of the dioxygen molecule as a sixth ligand causes spin pairing to take place on the iron atom. Since the radius of low spin Fe(II) is about 17 pm smaller than high spin Fe(II), it should fit in the porphyrin hole; we expect the smaller iron atom to drop into the hole. As a matter of fact, it does move about 20 pm towards the porphyrin ring (Fig. 19.11b) and the Fe—N<sub>porphyrin</sub> distance shortens to 198 pm. However, it stops short of moving all of the way into the plane of the ring. Data for the heme in myoglobin, hemoglobin, and related species are given in Table 19.1.

Table 19.1

Distances (pm) and angles (°) in various heme adducts<sup>a</sup>

Compound <sup>b</sup>	Fe—N <sub>porph</sub> <sup>c</sup>	Fe—N <sub>prox</sub> <sup>c</sup>	Fe—P <sub>N</sub> <sup>d</sup>	P <sub>porph</sub> —N <sub>prox</sub> <sup>e</sup>	Movement (N <sub>prox</sub> ) <sup>f</sup>	Fe—O	∠ Fe—O—O
Whale Mb	203	222	42	267			
Mb(O <sub>2</sub> )	195	207	18	228	39	183	115
Human Hb	206	215	38	268			
Hb(O <sub>2</sub> ) <sub>2</sub>	204	220	18	266	2	182	153
Hb(O <sub>2</sub> ) <sub>4</sub>	198	200	0 ± 5	210	58	176 ± 10	156
Picket fence <sup>g</sup>							
(a) 1-MeIm							
O <sub>2</sub> adduct	197.9	206.8				164.5	130
(b) 2-MeIm	207.2	209.5	40	252			
O <sub>2</sub> adduct	199.6	210.7	8.6	217	31	189.8	129

<sup>a</sup> Data from Perutz, M. F.; Fermi, G.; Luisi, B.; Shaanan, B.; Liddington, R. C. *Acc. Chem. Res.* 1987, 20, 309-321.

<sup>b</sup> Average value of several methods of determination. See reference in Footnote a for details.

<sup>c</sup> Bond lengths.

<sup>d</sup> Distance between the iron atom and the plane of the nitrogen atoms in porphyrin ring.

<sup>e</sup> Distance between the nitrogen atom in the proximal histidine (or imidazoles in the picket-fence compounds) and the plane of the porphyrin ring.

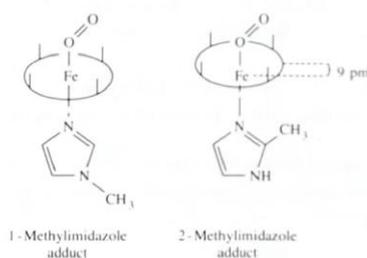
<sup>f</sup> Movement of the proximal histidine (or imidazole) towards the porphyrin ring upon oxygenation.

<sup>g</sup> "Picket fence" = tetrakis(1,1,1,1-*o*-pivalamidophenyl)porphinatoiron(II) (Fig. 19.4); 1-MeIm = 1-methylimidazole (N-methylimidazole); 2-MeIm = 2-methylimidazole.

**Fig. 19.11** The "trigger action" of the Perutz mechanism in hemoglobin. (a) Deoxy-T accepts a dioxygen molecule, O<sub>2</sub>, to form oxy-T (b) with partial movement of the iron atom into the ring, which is strained and unstable. Addition of more dioxygen molecules at other sites results in a rearrangement of oxy-T to oxy-R (c) with the iron atom moving completely into the ring. (d) The configuration about the heme group with respect to Leu FG3 in the T and R forms. Note that the flattening of the porphyrin on going from deoxy to oxy exerts a leverage on leucine FG3 and valine FG5 which lie at the switching contact between the two structures. The vertical bars indicate the distance of N<sub>his</sub> of the proximal histidine F8 from the mean plane of the porphyrin nitrogens and carbons. The horizontal bar gives the Fe—N<sub>porph</sub> distance, and the value to the right of the iron atoms gives the displacement of the iron from the plane of the porphyrin nitrogens. Note that the porphyrin is flat only in oxy-R and that the proximal histidine tilts relative to the heme normal in the T structures. Note also the water molecule attached to the distal histidine in deoxy-Hb. The differences in heme geometry between the deoxyhemes in the T and R structures shown here are closely similar to those found between sterically hindered 2-methyl- and unhindered 1-methylimidazole adducts with picket-fence porphyrin. [From Perutz, M. F.; Fermi, G.; Luisi, B.; Shaanan, B.; Liddington, R. C. *Acc. Chem. Res.* 1987, 20, 309-321. Reproduced with permission.]

The inhibition of free movement of the iron atom into the porphyrin ring has been attributed to steric interactions between the histidine ligand (which must follow the iron), the associated globin chain, and the heme group.<sup>34</sup> This apparently results in considerable strain on the oxyheme and associated tertiary structure of the globin within the T form. This discourages the addition of the first molecule of dioxygen, or more important, it "pushes" the last dioxygen molecule off in the tissues, where it is needed. Addition of a second dioxygen molecule takes place with similar results and, in effect, the hemoglobin molecule becomes spring-loaded. The structure of the bis(dioxygen)-T state has been determined and shows little movement of the iron atom and negligible movement of the histidine. The addition of a third dioxygen molecule results in interconversion to the R state. This removes the tension of the intermediate species and allows the iron atom to move freely into the center of the porphyrin ring (Fig. 19.11c). The porphyrin ring also flattens, and the histidine is free to follow the iron atom, some 50–60 pm. This change allows the fourth heme to accept a dioxygen molecule without paying the price of the protein constraint and accounts for the avidity of  $\text{Hb}(\text{O}_2)_3$  for the last dioxygen molecule. The relaxation of the globin-heme interaction in the R state versus the crowding in the oxy-T state is shown in Fig. 19.11d.

Support for the view that the globin portion of the molecule produces a constraint upon the iron atom (which would otherwise move into the heme pocket) comes from the behavior of myoglobin and model compounds (such as the picket-fence compounds with 1- or 2-methylimidazole mimicking the porphyrin and histidine), which are easier to study than the more complex hemoglobin:



In myoglobin and the sterically hindered 2-methylimidazole complex (as in hemoglobin), the iron atom does not move into the plane of the porphyrin nitrogen atoms (remaining 9 pm displaced in the complex), although it does so in sterically unhindered imidazole models, indicating that the iron atom does indeed shrink enough to fit were it not constrained. The data on the Fe—O bond length fit this picture: It is longer (and presumably weaker) in myoglobin and the 2-methylimidazole/picket-fence adduct (as it is in T hemoglobin) and shorter (and presumably stronger) in the unhindered 1-methylimidazole/picket-fence adduct (and R hemoglobin).

It was mentioned above that the deoxy-T  $\rightleftharpoons$  oxy-R equilibrium was affected by pH (Bohr effect) as well as the partial pressure of dioxygen. Other species such as a

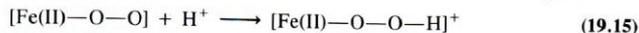
<sup>34</sup> Gelin, B. R.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* 1977, 74, 801.

single chloride ion and 2,3-diphosphoglycerate also influence the equilibrium.<sup>35</sup> Of perhaps the greatest interest is the fact that the T → R transition involves the addition of about 60 molecules of water to the hemoglobin. This hydration of newly exposed protein surfaces stabilizes the R form which might not even be capable of existence without this hydration energy.<sup>36</sup>

The above discussion has been somewhat simplified inasmuch as the number of possible interactions in a molecule as large as hemoglobin is very great. On the other hand, even as presented in abbreviated form, it is quite complicated. Various workers have placed varying degrees of weight upon different factors.<sup>37</sup> Nevertheless, one should not lose sight of the fact that the iron atom *does* undergo a change in spin state that causes it to move, and the net result is a change in the quaternary structure from T to R. And lest we get too involved in the biomechanical "trees" and forget to look at the biological "forest," recall that it is the *reduced affinity* of the T form that is nature's device that makes it possible for hemoglobin to *push the dioxygen molecule off in the tissues and transfer it to myoglobin*. We can thus look at dioxygen transport at several levels (go back to Fig. 19.8 and review).

Before leaving the subject of the binding of dioxygen to hemoglobin, two molecular (genetic) diseases should be mentioned. One is *sickle cell anemia* (SCA): Upon stressful deoxygenation of the blood, the hemoglobin (Hb<sub>S</sub>) polymerizes and precipitates, resulting in severe deformation of the red blood cells.<sup>38</sup> The genetic defect responsible is the replacement of hydrophilic glutamic acid at β-6 with the hydrophobic valine. The exposure of the latter upon R → T conversion reduces the solubility of hemoglobin S compared to normal adult hemoglobin, hemoglobin A.

It was mentioned above that heme(Fe<sup>3+</sup>) will not bind dioxygen. Heme is always susceptible to oxidation when in the presence of dioxygen. This reaction results from the nucleophilic displacement of superoxide by water, and it is acid catalyzed:<sup>39</sup>



The globin chain gives some protection by providing a hydrophobic environment, but still about 3% of the hemoglobin is oxidized to methemoglobin daily. The enzyme methemoglobin reductase returns the oxidized heme to the +2 state ordinarily. However, some individuals have an inborn metabolic defect that prevents the reduc-

<sup>35</sup> For an excellent discussion of the action of these *heterotropic ligands*, see Perutz, M. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University: Cambridge, 1990.

<sup>36</sup> Colombo, M. F.; Rau, D. C.; Parsegian, V. A. *Science* **1992**, *256*, 655-659.

<sup>37</sup> For reviews of the subject from different points of view, see Footnote 32 and Collman, J. P.; Halpert, T. R.; Suslick, K. S. In *Metal Ion Activation of Dioxygen*; Spiro, T. G., Ed.; Wiley: New York, 1980; Chapter 1; Bertini, I.; Barton, J. K.; Ellis, W. R., Jr.; Forsen, S.; George, G.; Gray, H. B.; Ibers, J. A.; Jameson, G. B.; Kordel, J.; Lippard, S. J.; Luchinat, C.; Raymond, K. N.; Stiefel, E. I.; Theil, E. C.; Valentine, J. S. *Bioinorganic Chemistry*; University Science Books: Mill Valley, CA; in press.

<sup>38</sup> Notice that the red blood cell in the lower right-hand corner of Fig. 19.8 is badly sickled. Erythrocytes that are sickled cannot flow as readily through the capillaries as normal red blood cells, and they are more susceptible to mechanical damage. These factors are responsible for the symptoms of sickle cell anemia.

<sup>39</sup> Shikama, K. *Coord. Chem. Rev.* **1988**, *83*, 73.

tion of methemoglobin.<sup>40</sup> In addition, any individual, but especially an infant,<sup>41</sup> may be stressed by nitrite/nitrate intoxication, in which case methemoglobin is produced faster than it can be reduced. In either case the iron(III) impairs dioxygen transport and causes cyanosis disproportionate to its abundance. This is because iron(III) is small enough to fit into the porphyrin ring without binding a sixth ligand, making methemoglobin very similar in structure to oxyhemoglobin. The presence of two or three iron(III) atoms can lock a hemoglobin molecule into the R state so that even the heme group(s) carrying dioxygen cannot release it readily. Recall that R hemoglobin has about the same dioxygen affinity as myoglobin, so the cooperativity mechanism has been defeated.<sup>42</sup>

### Other Biological Dioxygen Carriers

Hemerythrin is a nonheme, dioxygen-binding pigment utilized by four phyla of marine invertebrates. Its chief interest to the chemist lies in certain similarities to and differences from hemoglobin and myoglobin. Like both of the latter, hemerythrin contains iron(II) which binds oxygen reversibly, but when oxidized to methemerythrin ( $\text{Fe}^{3+}$ ) it does not bind dioxygen. There is an octameric form with a molecular weight of about 108,000 that transports dioxygen in the blood. In the tissues are lower molecular weight monomers, dimers, trimers, or tetramers.<sup>43</sup> And just as hemoglobin consists of four chains each of which is very similar to the single chain of myoglobin, octameric hemerythrin consists of eight subunits very similar in quaternary structure to myohemerythrin. A major difference between the hemoglobins and hemerythrin is in the binding of dioxygen: Each dioxygen-binding site (whether monomer or octamer) contains two iron(II) atoms, and the reaction takes place via a redox reaction to form iron(III) and peroxide ( $\text{O}_2^{2-}$ ). Oxyhemerythrin is diamagnetic, indicating spin coupling of the odd electrons on the two iron(III) atoms. Mössbauer data indicate that the two iron(III) atoms are in different environments in oxyhemerythrin. This could result from the peroxide ion coordinating one iron atom and not the other, or from each of the iron atoms having different ligands in its coordination sphere. The first evidence concerning the nature of the ligands came from an X-ray study of methemerythrin.<sup>44</sup> It indicated that the two iron atoms have approximately octahedral coordination and are bridged by an oxygen atom (from water, hydroxo, or oxo), aspartate, and glutamate. The remaining ligands are three histidine residues on one iron atom and two histidines on the other.<sup>45</sup> This is a rather small difference, but it can be reconciled with the other

<sup>40</sup> The classic case is of the "blue Fugates" of Troublesome Creek, KY, descendants of a single couple, each of whom carried the recessive gene. Mansouri, A. *Am. J. Med. Sci.* **1985**, 289, 200.

<sup>41</sup> These "blue babies" result because infants have fetal hemoglobin, hemoglobin F.  $\text{Hb}_F$  differs from  $\text{Hb}_A$  (adult hemoglobin) and has a higher oxygen affinity that facilitates dioxygen transfer *in utero* from the mother's  $\text{Hb}_A$ . Hemoglobin F, which is gradually replaced during the first year of life, is more susceptible to oxidation than adult hemoglobin, methemoglobin is produced more readily, and oxygen transport is reduced. A different, curious aspect of  $\text{Hb}_F$  is that it protects the infant (temporarily) from the SCA problems of hemoglobin S!

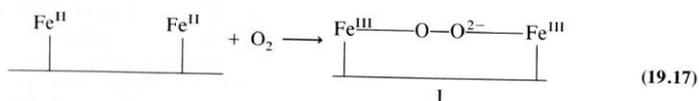
<sup>42</sup> For a review of methemoglobinemia, see Senozan, N. M. *J. Chem. Educ.* **1985**, 62, 181.

<sup>43</sup> It should be remembered that what the chemist glibly calls "hemerythrin" is not necessarily the same from one species to the next: The four phyla in which hemerythrin is found comprise thousands of species. Thus generalizations tend to be difficult and somewhat oversimplified. For reviews of hemerythrin chemistry, see Klotz, I. M.; Kurtz, D. M., Jr. *Acc. Chem. Res.* **1984**, 17, 16. Wilkins, P. C.; Wilkins, R. G. *Coord. Chem. Rev.* **1987**, 79, 195.

<sup>44</sup> Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, 73, 349-351.

<sup>45</sup> The first studies indicated that the second iron atom also had a tyrosine residue attached to it. Later refinement of the structure showed that it is actually pentacoordinate, but the argument remains the same.

data, and so until recently the consensus has tended to favor a simple peroxy bridge between the two iron atoms:

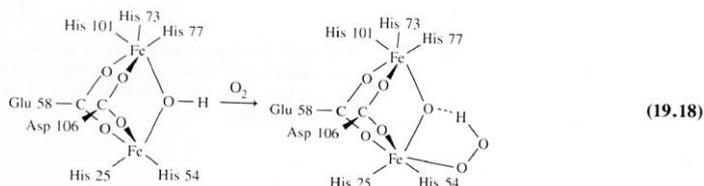


where the continuous line connecting the two iron atoms is a simplified representation of the coordination spheres and the protein chain holding the iron atoms in place. Militating against this simple structure is the fact that the Mössbauer spectrum does not distinguish the iron atoms in *deoxyhemerythrin*. If the difference in amino acid environment is sufficient to distinguish the iron atoms in the Mössbauer spectrum of oxyhemerythrin, why not in deoxyhemerythrin?

Further data on this matter came from the Raman spectrum of oxy( $^{16}\text{O}^{18}\text{O}$ ) hemerythrin, which shows the two oxygen atoms to be in *nonequivalent* positions.<sup>46</sup> Of the various alternative structures that have been proposed, the Raman data are compatible with only two:



$^{16}\text{O}^{18}\text{O}$  data, as well as other spectroscopic evidence,<sup>47</sup> are compatible with structure III, but the question was still open until the X-ray structure of oxyhemerythrin was further refined.<sup>48</sup> The proposed structures of deoxyhemerythrin and oxyhemerythrin are:



Note that the hydrogen atoms cannot be located at this level of resolution and so the hydrogen bond shown is merely one suggestion for the possible stabilization of the peroxide ion.

Another oxygen-containing pigment is confusingly named *hemocyanin*, which contains neither the heme group nor the cyanide ion; the name simply means "blue

<sup>46</sup> Kurtz, D. M., Jr.; Shriver, D. F.; Klotz, I. M. *J. Am. Chem. Soc.* **1976**, *98*, 5033-5035; *Coord. Chem. Rev.* **1977**, *24*, 145-178.

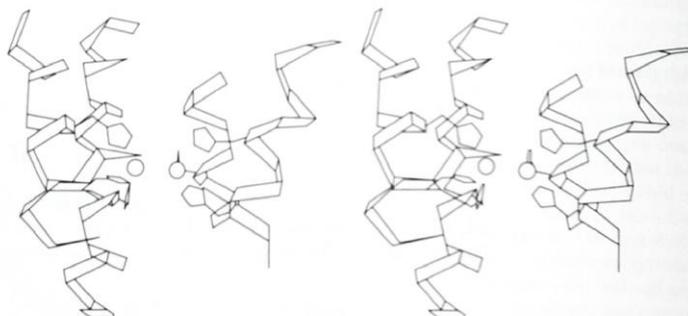
<sup>47</sup> Gay, R. R.; Solomon, E. I. *J. Am. Chem. Soc.* **1978**, *100*, 1972.

<sup>48</sup> Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H.; McCallum, J. D.; Sanders-Loehr, J. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 713-716.

blood": Whereas hemoglobin turns bright red upon oxygenation, the chromophore ( $\text{Cu}^{\text{I}}/\text{Cu}^{\text{II}}$ ) in colorless deoxyhemocyanin turns bright blue. Hemocyanin is found in many species in the Mollusca and Arthropoda.<sup>49</sup> The gross molecular structures of the hemocyanins in the two phyla are quite different, though both bind dioxygen cooperatively, and spectroscopic evidence indicates that the dioxygen-binding centers are similar. The dioxygen binding site appears to be a pair of copper atoms, each bound by three histidine ligands (Fig. 19.12). The copper is in the +1 oxidation state in the deoxy form and +2 in the oxy form.

The structure of oxyhemocyanin has recently been determined.<sup>50</sup> It presents yet a third mode of binding between oxygen-carrying metal atoms and the dioxygen molecule. The latter oxidizes each copper(I) to copper(II) and is in turn reduced to the peroxide ion ( $\text{O}_2^{2-}$ ). The two copper(II) atoms are bridged by the peroxide ion with unusual  $\mu\text{-}\eta^2\text{:}\eta^2$  bonds, i.e., each oxygen atom is bonded to both copper atoms.

The parallels and differences among hemoglobin, hemerythrin, and hemocyanin illustrate the ways in which evolution has often solved what is basically the same problem in different ways in different groups of animals.<sup>51</sup>



**Fig. 19.12** The copper–dioxygen binding site in hemocyanin from *Panulirus interruptus*. The copper atoms are indicated by open circles, the histidines by pentagons, and the protein chains by ribbons. [From Volbeda, A.; Hol, W. G. J. *J. Mol. Biol.* **1989**, *209*, 249–279. Reproduced with permission.]

<sup>49</sup> Karlin, K. D.; Gultneh, Y. *Prog. Inorg. Chem.* **1987**, *35*, 219–327. The structure of hemocyanin from an arthropod, the spiny lobster, has been determined by Volbeda, A.; Hol, W. G. J. *J. Mol. Biol.* **1989**, *209*, 249–279.

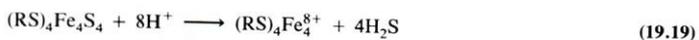
<sup>50</sup> *Note added in proof:* The complete structure of oxyhemocyanin has not yet been published, but it contains the  $\mu\text{-}\eta^2\text{:}\eta^2$  coordination mode shown above (Magnus, K. C.; Tong-That, H. *J. Inorg. Biochem.* **1992**, *27*, 20). This structure was predicted on the basis of model dicopper compounds (Blackburn, N. J.; Strange, R. W.; Farooq, A.; Haka, M. S.; Karlin, K. D. *J. Am. Chem. Soc.* **1988**, *110*, 4263–4272. Kitajima, N.; Fujisawa, K.; Fujimoto, C.; Moro-oka, Y.; Hashimoto, S.; Kitagawa, T.; Toriumi, K.; Tasumi, K.; Nakamura, A. *J. Am. Chem. Soc.* **1992**, *114*, 1277–1291). The Cu—Cu distance in oxyhemocyanin is 360 pm.

<sup>51</sup> For an interesting discussion of parallels in function, structure, and possibly evolution of hemoglobin, hemerythrin, and hemocyanins, see Volbeda, A.; Hol, W. G. J. *J. Mol. Biol.* **1989**, *206*, 531–546.

### Electron Transfer, Respiration, and Photosynthesis

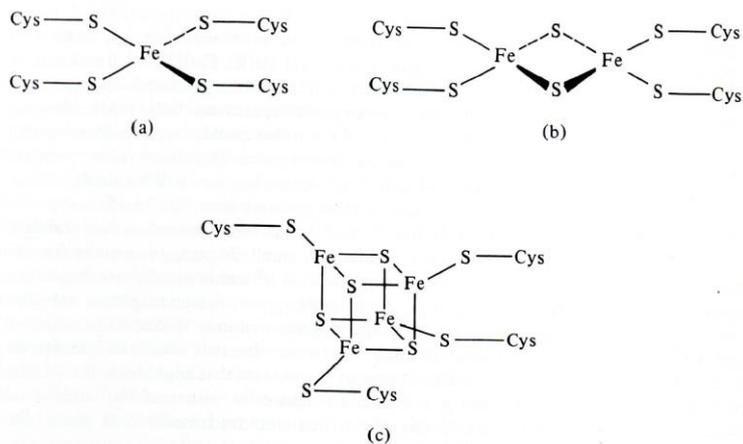
#### Ferredoxins and Rubredoxins<sup>52</sup>

There are several nonheme iron-sulfur proteins that are involved in electron transfer. They have received considerable attention in the last few years. They contain distinct iron-sulfur clusters composed of iron atoms, sulfhydryl groups from cysteine residues, and "inorganic" or "labile" sulfur atoms or sulfide ions. The latter are readily removed by washing with acid:



The cysteine moieties are incorporated within the protein chain and are thus not labile. The clusters are of several types. The simplest is bacterial rubredoxin,  $(\text{Cys-S})_4\text{Fe}$  (often abbreviated  $\text{Fe}_1\text{S}_0$ , where S stands for inorganic sulfur), and contains only nonlabile sulfur. It is a bacterial protein of uncertain function with a molecular weight of about 6000. The single iron atom is at the center of a tetrahedron of four cysteine ligands (Fig. 19.13a). The cluster in the ferredoxin molecule associated with photosynthesis in higher plants is thought to have the bridged structure  $\text{Fe}_2\text{S}_2$  shown in Fig. 19.13b. The most interesting cluster is found in certain bacterial ferredoxins involved in anaerobic metabolism. It consists of a cubane-like cluster of four iron atoms, four labile sulfur atoms, thus  $\text{Fe}_4\text{S}_4$ , and four cysteine ligands (Fig. 19.13c).

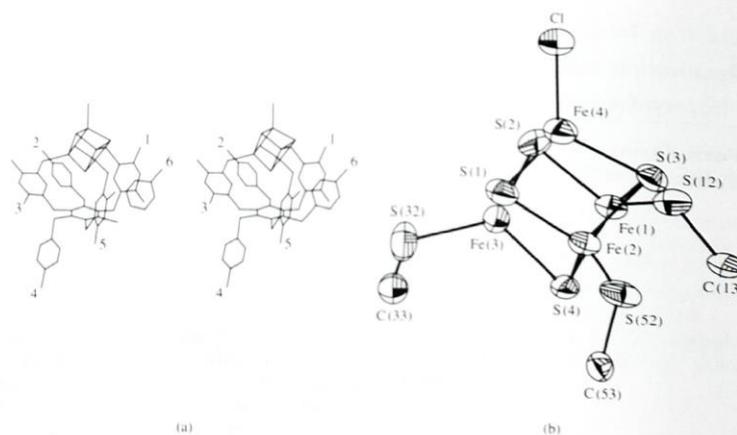
Because of the inherent chemical interest in clusters of this sort, as well as their practical significance to biochemistry, there has been considerable effort expended in making model compounds for study (Fig. 19.14). These model compounds allow direct experimentation on the cluster in the absence of the protein chain.<sup>53</sup>



**Fig. 19.13** Iron-sulfur clusters in ferredoxins: (a)  $\text{Fe}_1\text{S}_0$  in bacterial rubredoxin; (b)  $\text{Fe}_2\text{S}_2$  in photosynthetic ferredoxin; (c)  $\text{Fe}_4\text{S}_4$  in cubane-like ferredoxin.

<sup>52</sup> *Biochemistry of Nonheme Iron*; Bezkorovainy, A., Ed.; Plenum: New York, 1980; Chapter 8. *Iron-Sulfur Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1982. The entire volume of *Adv. Inorg. Chem.* **1992**, *38*, 1-487 is devoted to iron-sulfur proteins. Unfortunately, it was received too late to include much of it in this volume but it should prove to be very useful to the interested reader.

<sup>53</sup> See, for example, Liu, H. Y.; Scharbert, B.; Holm, R. H. *J. Am. Chem. Soc.* **1991**, *113*, 9529-9539; Holm, R. H.; Ciurli, S.; Weigel, J. A. *Prog. Inorg. Chem.* **1990**, *38*, 1-74, and references therein.



**Fig. 19.14** Model compound for a cubane-type  $\text{Fe}_4$  cluster with a trithiol ligand. (a) Stereoview of  $[\text{Fe}_4\text{S}_4(\text{trithiol})\text{L}]$ ; (b) close-up view of the Fe-S cluster. [From Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 2484-2494. Reproduced with permission.]

### Blue Copper Proteins

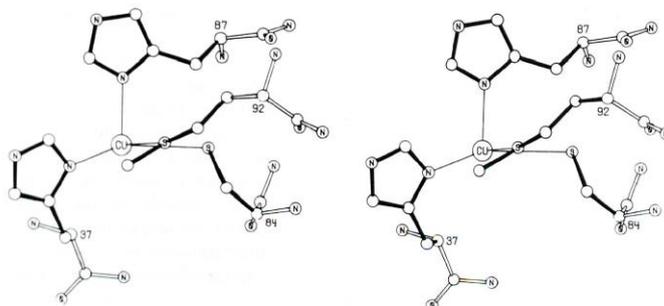
Perhaps the three most important redox systems in bioinorganic chemistry are: (1) high spin, tetrahedral Fe(II)/Fe(III) in rubredoxin, ferredoxin, etc.; (2) low spin, octahedral Fe(II)/Fe(III) in the cytochromes; and (3) pseudotetrahedral Cu(I)/Cu(II) in the *blue copper proteins*, such as stellacyanin, plastocyanin, and azurin. Gray<sup>54</sup> has pointed out that these redox centers are ideally adapted for electron exchange in that no change in spin state occurs. Thus there is little or no movement of the ligands—the Franck-Condon activation barriers will be small.

The structure of plastocyanin (Fig. 19.15) is especially instructive in this regard. Copper(I) is  $d^{10}$  and thus provides no ligand field stabilization energy in any geometry. Because it is relatively small (74 pm), it is usually found in a tetrahedral environment. In contrast, copper(II) is  $d^9$  and is usually octahedrally coordinated with Jahn-Teller distortion, often to the point of square planar coordination. In the case of plastocyanin, the copper is situated in a "flattened tetrahedron" of essentially  $C_{3v}$  symmetry, "halfway" between the two idealized geometries.<sup>55</sup> This facilitates electron transfer compared to a system that might be at the tetrahedral extreme or at the square planar extreme: Energetically, either of the latter would require reorganization towards the other when electron transfer took place. Such structural changes would inhibit the process.

The mechanism of electron transfer over the long distances (of the order of 1000 pm or more) necessitated by the large size of redox enzymes is one that is not completely clear despite much current study. These transfers are critical whether one is considering the photosynthetic center (page 917) or electron carriers such as the

<sup>54</sup> Gray, H. B. *Chem. Soc. Rev.* **1986**, *15*, 17.

<sup>55</sup> Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* **1978**, *272*, 319-324.



**Fig. 19.15.** Stereoview of the copper-binding site of plastocyanin. The four ligand residues are His 37, Cys 84, His 87, and Met 92. Note that the geometry about the copper is neither tetrahedral nor square planar, but intermediate. [From Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* 1978, 272, 319–324. Reproduced with permission.]

cytochromes and copper redox enzymes (above). The rate of electron transfer falls off exponentially with distance at long range (Chapter 13). The rate is also dependent upon the thermodynamic driving force and, as mentioned above, facilitated when structural changes are minimal. One may readily ask why the iron and copper atoms are not on the surface of the protein so that such long-range transfer would be unnecessary. Surely one reason is to prevent their irreversible “corruption” to an unusable form. And almost certainly the surrounding protein shield serves the purpose of recognition, as yet poorly understood, that allows cytochrome *c*, plastocyanin, etc., to react with the intended target species and not be “short-circuited” by reacting uselessly with the wrong redox agent.<sup>56</sup>

The determination of the structures of biologically important copper-containing redox systems illustrates the multiplicity of techniques that can be brought to bear on structural bioinorganic chemistry. Ultimately, one would like to have a highly refined, accurate structure determined by X-ray crystallography. Yet over and over in this chapter we shall see that this goal has not been met for many of the most interesting compounds. There is a wide variety of techniques that may be used instead to gain the desired information. These vary in ease of application and in the quality of the results, but by combining different techniques much can often be said about the active site.

Often the nature, number, and distances of atoms in the coordination sphere of a metal can be obtained by a relatively new method called *extended X-ray absorption fine structure* (EXAFS). It elaborates upon the long-known fact that X-ray absorption spectra show element-specific “edges” that correspond to quantum jumps of core

<sup>56</sup> Scott, R. A.; Mauk, A. G.; Gray, H. B. *J. Chem. Educ.* 1985, 62, 932. Mayo, S. L.; Ellis, W. R., Jr.; Crutchley, R. J.; Gray, H. B. *Science* 1986, 233, 948. Bowler, B. E.; Raphael, A. L.; Gray, H. B. *Prog. Inorg. Chem.* 1990, 38, 259–322. Liang, N.; Pielak, G. J.; Mauk, A. G.; Smith, M.; Hoffman, B. M. *Proc. Natl. Acad. Sci. U. S. A.* 1987, 84, 1249. Wendoloski, J. J.; Matthew, J. B.; Weber, P. C.; Salemme, F. R. *Science* 1987, 238, 794. McLendon, G. *Acc. Chem. Res.* 1988, 21, 160. See also articles by these authors in *Struct. Bonding (Berlin)* 1991, 75, 1–224. The kinetics of electron transfer of this sort is discussed in Chapter 13.

electrons to unoccupied orbitals or to the continuum. By choosing X-ray frequencies near the X-ray edge of a particular element, atoms of that element can be excited to emit photoelectrons. The wave of each electron will be backscattered by the nearest neighbors in proportion to the number and kind of the ligands and inversely proportional to the interatomic distance. If the backscattered wave is in phase with the original wave, reinforcement will occur, yielding a maximum in the X-ray absorption spectrum. Out-of-phase waves will cancel and give minima. The EXAFS spectrum consists, then, of the X-ray absorption plotted against the energy of the incident X-ray photon. The amplitudes and frequencies of the oscillations in the absorption are related to the number, type, and spacing of the ligands. Thus if one bombards heme with an X-ray frequency characteristics of an iron edge, it should, in principle, be possible to learn that there are four atoms of atomic number 7 equidistantly surrounding the iron atom.<sup>57</sup>

Some EXAFS data for copper proteins are given in Table 19.2. Confirming data from X-ray crystallography are also listed where known. Copper is particularly well suited for study by *electron paramagnetic resonance*. At the very simplest level, this

Table 19.2

The type, accuracy, and extent of information given by EXAFS and X-ray crystallography on blue copper proteins

Compound	EXAFS (pm)	X-ray (pm)
Azurin	Cu-N = 205 <sup>a</sup>	Cu-N (His-46) = 206 <sup>b</sup>
	Cu-N = 189	Cu-N (His-117) = 196
	Cu-S = 223	Cu-S (Cys-112) = 213
	Cu-S = 270	Cu-S (Met-121) = 260
Cytochrome oxidase	Cu-S = 227 <sup>c</sup>	<sup>d</sup>
Plastocyanin	Cu-N = 197 <sup>e</sup>	Cu-N (His-37) = 204 <sup>f</sup>
		Cu-N (His-87) = 210
	Cu-S = 211	Cu-S (Cys-84) = 213 Cu-S (Met-92) = 290
Stellacyanin	<sup>g</sup>	<sup>g</sup>

<sup>a</sup> Tullius, T. D.; Frank, P.; Hodgson, K. O. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75*, 4069. Groeneweld, C. M.; Feiters, M. C.; Hasnain, S. S.; Van Rijn, J.; Reedijk, J.; Canters, G. W. *Biochem. Biophys. Acta* **1986**, *873*, 214.

<sup>b</sup> Norris, G. E.; Anderson, B. F.; Baker, E. N. *J. Am. Chem. Soc.* **1986**, *108*, 2784.

<sup>c</sup> Scott, R. A.; Cramer, S. P.; Shaw, R. W.; Beinert, H.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 664.

<sup>d</sup> No crystallographic data available.

<sup>e</sup> Guss, J. M.; Freeman, H. C. *J. Mol. Biol.* **1983**, *169*, 521.

<sup>f</sup> Guss, J. M.; Harrowell, P. R.; Murata, M.; Norris, V. A.; Freeman, H. C. *J. Mol. Biol.* **1986**, *192*, 361.

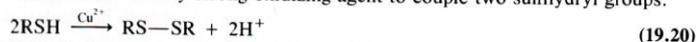
<sup>g</sup> No EXAFS or crystallographic data available. Spectroscopy indicates that three ligands are the same as in plastocyanin.

<sup>57</sup> The present discussion has been greatly simplified to give the general technique as well as the information obtained without going into the details of the analysis. For the latter as well as the experimental technique, see Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.* **1979**, *25*, 1. Hay, R. W. *Bio-Inorganic Chemistry*; Ellis Horwood: Chichester, 1984; pp 51-57; Rehr, J. J.; de Leon, J. M.; Zabinsky, S. I.; Albers, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 5135-5140.

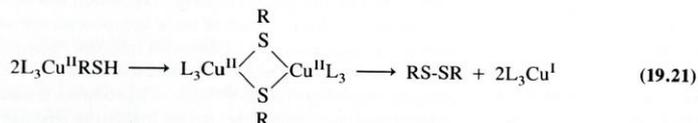
method can distinguish between the presence of an odd electron ( $\text{Cu}^{2+}$ ,  $d^9$ , EPR signal) and complete electron pairing ( $\text{Cu}^+$ ,  $d^{10}$ , no EPR signal). Ligands with nuclei having nonzero spins (such as nitrogen) will cause hyperfine splittings proportional to the number of such atoms bonded to the copper(II) atom [see page 923 with respect to Cu(II)-substituted carboxypeptidase A]. Finally, a study of the hyperfine splitting, some of it resulting from the copper atom's nonzero nuclear spin, can provide geometric information (see below).

Analysis of ligand field and charge transfer absorption bands can provide information concerning the geometry of the copper site and the nature of the ligand, though mostly of a qualitative sort; values for bond angles and bond lengths cannot be quantified. It is often useful in this regard to attempt the synthesis and structural determination of model compounds and to try to match their properties with those of the active sites in the metalloproteins. These efforts, combined frequently with theoretical calculations of the same properties, often allow predictions to be made concerning the nature of the active sites. For example, while the structures of azurin and plastocyanin have been determined by X-ray crystallography, that of the related blue copper protein stellacyanin has not because suitable crystals have not yet been grown. Spectral studies have indicated that three of the four ligands (His, His, Cys) are the same in plastocyanin and stellacyanin, but that the latter does not contain the methionine ligand found in the former.<sup>58</sup> A combination of electron paramagnetic resonance and electronic spectral data with self-consistent-field calculations has indicated that the unknown fourth ligand in stellacyanin provides a stronger field than does methionine.<sup>59</sup> It presumably results in a shorter Cu—X bond as well as a flattening of the geometry more towards a square planar arrangement.

The synthesis of model compounds has proved to be an interesting challenge. The  $\text{Cu}^{2+}$  ion is a sufficiently strong oxidizing agent to couple two sulfhydryl groups:



Thus any simple attempt to let thiols coordinate to Cu(II) will result in persulfides and Cu(I).



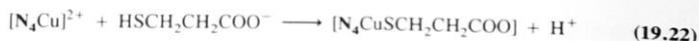
As shown in Eq. 19.21, it is thought that this reaction takes place through a dimeric, bridged intermediate of coordination number 5. Copper tends to form a maximum of five reasonably strong bonds,<sup>60</sup> so complexation with a nonlabile tetracoordinate macrocyclic ligand ( $\text{N}_4$ ) provides only one additional site for a sulfur ligand. The reaction in Eq. 19.21 is inhibited, and a thiolate complex can be isolated:<sup>61</sup>

<sup>58</sup> Gewirth, A. A.; Cohen, S. L.; Schugar, H. J.; Solomon, E. I. *Inorg. Chem.* **1987**, *26*, 1133-1146.

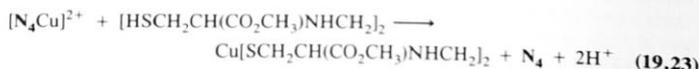
<sup>59</sup> Thomann, H.; Bernardo, M.; Baldwin, M. J.; Lowery, M. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1991**, *113*, 5911-5913. This discussion of the study of blue copper proteins has necessarily been brief. For a comprehensive discussion, see Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem. Rev.* **1992**, *92*, 521-542.

<sup>60</sup> See Chapter 12. Copper(II) undergoes Jahn-Teller distortion when six-coordinate and tends to form four strong bonds and two weak ones (Chapter 11).

<sup>61</sup> John, E.; Bharadwaj, P. K.; Potenza, J. A.; Shugar, H. J. *Inorg. Chem.* **1986**, *25*, 3065.



Finally, a pseudotetrahedral complex more closely resembling the copper site in blue copper proteins, including the presence of two cysteine groups, can be achieved by using a cysteine derivative of ethylenediamine,  $[\text{HSCH}_2\text{CH}(\text{CO}_2\text{CH}_3)\text{NHCH}_2]_2$ . It is a softer, polydentate (though not macrocyclic) ligand and will displace  $\text{N}_4$ :



The Cu—S bonds are about 10 pm longer than those in plastocyanin but about the same as those in cytochrome *c* oxidase.<sup>62</sup>

### Photosynthesis

The photosynthetic process in green plants consists of splitting the elements of water, followed by reduction of carbon dioxide:



where [4H] does not imply free atoms of hydrogen but a reducing capacity of four equivalents. The details of the reactions involved in photosynthesis are not known, although the broad outlines are fairly clear. In all dioxygen-producing organisms ranging from cyanobacteria to algae to higher plants, there are two coupled photosynthetic systems, PS I and PS II. The two differ in the type of chlorophyll present and in the accessory chemicals for processing the trapped energy of the photon. The primary product of PS I is reduced carbon, and the primary product of PS II is energy in the form of two moles of ATP<sup>63</sup> with molecular oxygen as a chemical by-product.

In addition to the chlorophyll molecules at the reaction centers of PS I and PS II, there are several other pigments associated with the light-harvesting complex. Among these are carotenoids, open-chain tetrapyrrole pigments, and others. These serve dual roles of protecting the cell from light radiation and at the same time harvesting much of it for photosynthesis. Some of these compounds are arranged in antenna-like rods that gather the light energy and funnel it into the reaction centers.<sup>64</sup>

The energy of an absorbed photon in either PS I or PS II initiates a series of redox reactions (see Fig. 19.16).<sup>65</sup> System I produces a moderately strong reducing species ( $\text{RED}_I$ ) and a moderately strong oxidizing species ( $\text{OX}_I$ ). System II provides a stronger oxidizing agent ( $\text{OX}_{II}$ ) but a weaker reducing agent ( $\text{RED}_{II}$ ).

$\text{OX}_{II}$  is responsible for the production of molecular oxygen in photosynthesis. A manganese complex, probably containing four atoms of manganese, is attached to a protein molecule. It reduces  $\text{OX}_{II}$  which is recycled for use by another excited chlorophyll molecule in PS II. In the redox reactions the manganese shuttles between two oxidation states with each manganese atom increasing (and subsequently decreasing) its oxidation state by one unit, but it is not known with absolute certainty what

<sup>62</sup> Bharadwaj, P. K.; Potenza, J. A.; Shugar, H. J. *J. Am. Chem. Soc.* **1986**, *108*, 1351.

<sup>63</sup> Adenosine triphosphate, an important energy-rich species in metabolism.

<sup>64</sup> Deisenhofer, J.; Michel, H.; Huber, R. *Trends Biochem. Sci.* **1985**, *10*, 243–248. Zuber, H.; Brunisholz, R.; Sidler, W. In *New Comprehensive Biochemistry: Photosynthesis*; Ames, J., Ed.; Elsevier: Amsterdam, 1987; pp 233–271.

<sup>65</sup> Mathis, P.; Rutherford, A. W. In *New Comprehensive Biochemistry: Photosynthesis*; Ames, J., Ed.; Elsevier: Amsterdam, 1987; pp 63–96.

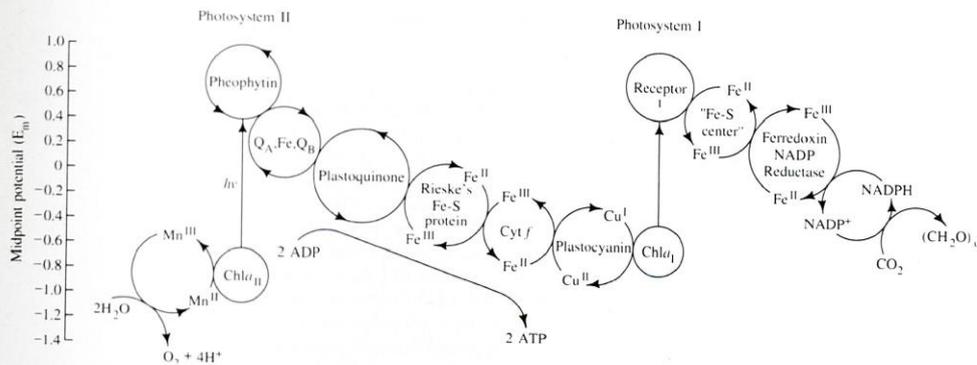
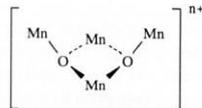


Fig. 19.16 Electron flow in photosystems I and II ("Z-scheme"). Vertical axis gives mid-point redox potential with reducing species (top) and oxidizing species (bottom).

these oxidation states are.<sup>66</sup> In the reduced form the oxidation states may be as low as three Mn(II) and one Mn(III), but they are more likely to be three Mn(III) and one Mn(IV). A suggested scheme for this redox chemistry is shown in Fig. 19.17 in which the active site cycles between a cubane-like and an adamantane-like configuration. There have been several other suggestions concerning these structures, including



"butterfly clusters" and other modifications of the Mn<sub>4</sub> configuration.<sup>67</sup>

### Chlorophyll and the Photosynthetic Reaction Center

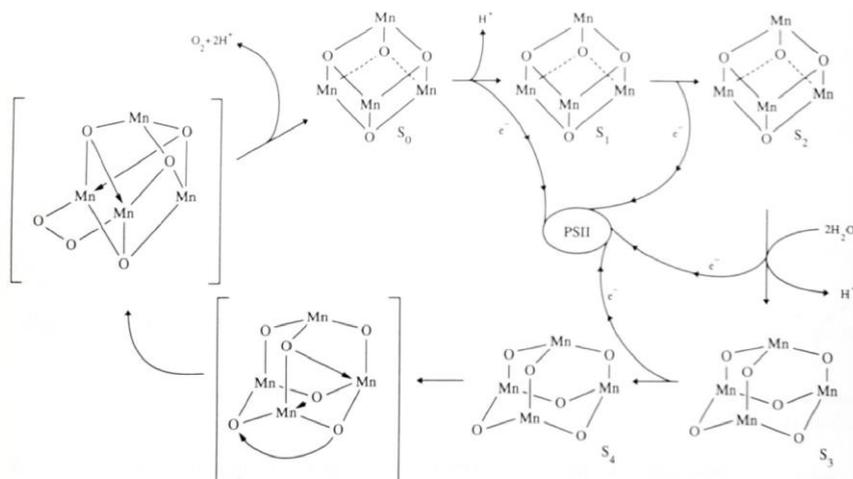
The chlorophyll ring system is a porphyrin in which a double bond in one of the pyrrole rings has been reduced. A fused cyclopentanone ring is also present (Fig. 19.18). Bacteriochlorophyll is similar but has a double bond in a second pyrrole ring reduced, and it has a substituent acetyl group in place of a vinyl group. Chlorophyll absorbs low-energy light in the red region (~600-700 nm). The exact frequency depends on the nature of the substituents.

Based on our knowledge of the structure of chlorophyll as well as the results of studies on the photo behavior of chlorophyll in vitro, it is possible to summarize some of the features of the chlorophyll system which enhance its usefulness as a pigment in photosynthesis.<sup>68</sup> First, there is extensive conjugation of the porphyrin ring. This lowers the energy of the electronic transitions and shifts the absorption maximum into

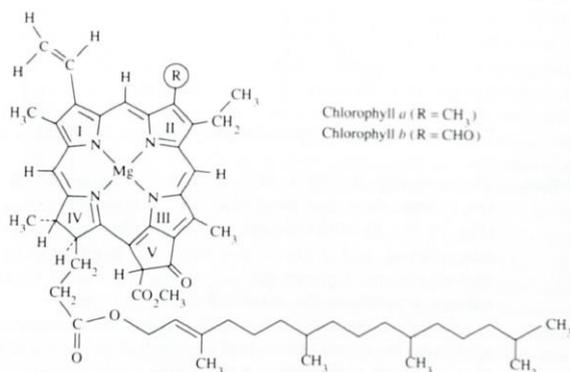
<sup>66</sup> Dismukes, G. C. *Photochem. Photobiol.* **1986**, *43*, 99. Babcock, G. T. In *New Comprehensive Biochemistry: Photosynthesis*; Amesz, J., Ed.; Elsevier: Amsterdam, 1987; pp 125-158. Brudvig, G. W. *J. Bioenerg. Biomembr.* **1987**, *19*, 91.

<sup>67</sup> See Brudvig, G. W.; Crabtree, R. H. *Prog. Inorg. Chem.* **1989**, *37*, 99-142; Que, L., Jr.; True, A. E. *Ibid.* **1990**, *38*, 97-200.

<sup>68</sup> Maggiora, G. M.; Ingraham, L. L. *Struct. Bonding (Berlin)* **1967**, *2*, 126. Hindman, J. C.; Kugel, R.; Svirnickas, A.; Katz, J. J. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 5-9.



**Fig. 19.17** One proposal for the involvement of Mn centers in the photoevolution of dioxygen. [Modified from Brudvig, G. W.; Crabtree, R. H. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *83*, 4586. Reproduced with permission.]



**Fig. 19.18** Structure of chlorophyll. The long alkyl chain at the bottom is the phytol group.

the region of visible light. Conjugation also helps to make the ring rigid, and thus less energy is wasted in internal thermal degradation (via molecular vibrations).

The maximum intensity of light *reaching the earth's surface* is in the visible region; ultraviolet light is absorbed in the earth's atmosphere by species such as dioxygen and ozone (trioxygen), infrared light is absorbed by carbon dioxide, water, etc. The absorption spectra of photosynthetic systems fall nicely within that portion of the sun's spectrum that reaches the earth. Some of the energy of the light not absorbed by the chlorophyll itself is captured by accessory pigments. In the octave of light from

about 350 to 700 nm, one or more photosynthetic pigments absorbs at every frequency. This is the portion of the total spectrum that is of highest intensity and corresponds rather closely to the sensitivity of the human eye, another system adapted to that portion of light that reaches earth.

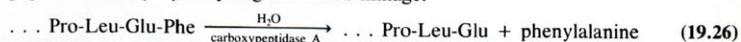
Thanks to careful spectroscopic and crystallographic work we have considerable information about the reaction center in photosynthetic bacteria, and it is probable that the photosynthetic systems in higher plants are modifications, perhaps partial duplications, of the bacterial system.<sup>69</sup> The reaction center is a protein with a molecular weight of about 150,000. The heart of the reaction center is a pair of chlorophyll molecules, often referred to as the "special pair" (Fig. 19.19).<sup>70</sup> The special pair are in contact with each other through the overlap of one of the pyrrole rings in each molecule. In addition, an acetyl group on each molecule coordinates to the magnesium atom of the other. The sixth coordination position on each magnesium atom is occupied by a nitrogen atom from a histidine residue in the protein chain. Associated with the special pair are pheophytin molecules and quinone molecules that accept the electron from the reaction center (Fig. 19.20). Near the quinone molecule is a nonheme iron atom complexed by four histidines and one glutamic acid. The electron appears to be passed through the iron atom to the redox chain. The "hole" (vacancy resulting in a cationic charge) in the reaction center is filled with an electron from a cytochrome molecule (there are four cytochrome-*c*-type centers lying near the special pair). The separation of charge between the electron being passed down the Z-scheme chain and the positive charge residing on the Fe(III)-cytochrome represents potential energy that is utilized in the photosynthesis.

## Enzymes

Enzymes are the catalysts of biological systems. They not only control the rate of reactions but, by favoring certain geometries in the transition state, can lower the activation energy for the formation of one product rather than another. The basic structure of enzymes is built of proteins. Those of interest to the inorganic chemist are composed of a protein structure (called an apoenzyme) and a small *prosthetic group*, which may be either a simple metal ion or a complexed metal ion. For example, heme is the prosthetic group in hemoglobin. A reversibly bound group that combines with an enzyme for a particular reaction and then is released to combine with another is termed a *coenzyme*. Both prosthetic groups and coenzymes are sometimes called cofactors.<sup>71</sup>

## Structure and Function

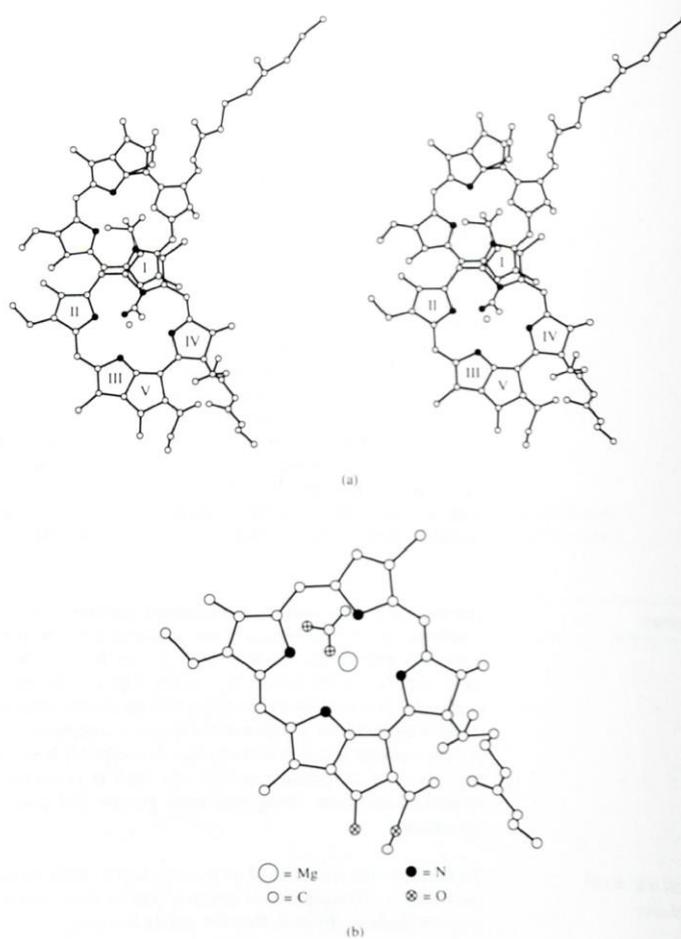
To illustrate the structure of an enzyme and its relation to function, consider carboxypeptidase A. This pancreatic enzyme cleaves the carboxyl terminal amino acid from a peptide chain by hydrolyzing the amide linkage:



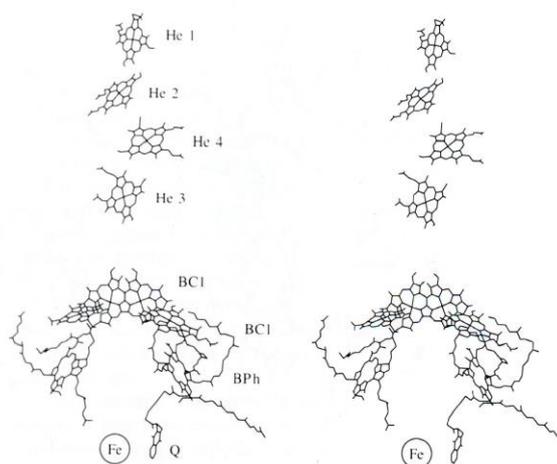
<sup>69</sup> Youvan, D. C.; Marrs, B. L. *Cell* **1984**, *39*, 1; *Sci. Am.* **1987**, *256*(6), 42-48.

<sup>70</sup> Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature (London)* **1985**, *318*, 618. Parson, W. W. In *New Comprehensive Biochemistry: Photosynthesis*; Ames, J., Ed.; Elsevier: Amsterdam, 1987; pp 43-61. Budil, D. E.; Gast, P.; Chang, C-H; Schiffer, M.; Norris, J. R. *Ann. Rev. Phys. Chem.* **1987**, *38*, 561-583.

<sup>71</sup> These terms are not always used in exactly the same way. See Dixon, M.; Webb, E. C. *Enzymes*, 3rd ed.; Academic: New York, 1979; Hammes, G. G. *Enzyme Catalysis and Regulation*; Academic: New York, 1982; Palmer, T. *Understanding Enzymes*, 2nd ed.; Ellis Horwood: Chichester, 1985.

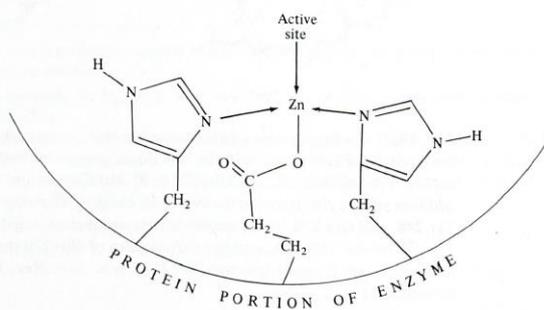


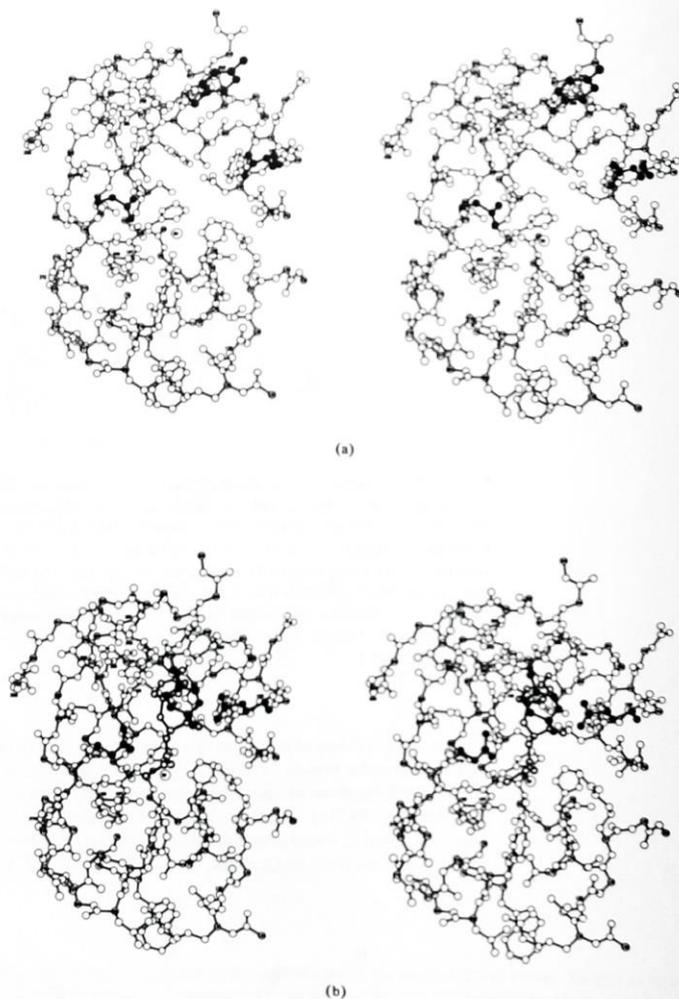
**Fig. 19.19** (a) Stereoview of the special pair in the photoreaction center. Rings I of the chlorophyll molecules are stacked upon each other, and the magnesium atom of each chlorophyll is coordinated by an acetate group from the other molecule. (b) Close-up of the nearer chlorophyll molecule. The unattached acetate group is from the other chlorophyll molecule. [Modified from Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *J. Mol. Biol.* 1984, 180, 385-398. Reproduced with permission.]



**Fig. 19.20** Stereoview of the photosynthetic reaction center. The photoexcited electron is transferred from the special pair to another molecule of bacteriochlorophyll (BChl), then to a molecule of bacteriopheophytin (BPh), then to a bound quinone (Q), all in a period of 250 ps. From the quinone it passes through the nonheme iron (Fe) to an unbound quinone (not shown) in a period of about 100  $\mu$ s. The electron is restored to the "hole" in the special pair via the chain of hemes (He 1, etc.) in four cytochrome molecules, also extremely rapidly ( $\sim$ 270 ps). The special pair here is rotated  $90^\circ$  with respect to Fig. 19.19. [From Deisenhofer, J.; Michel, H.; Huber, R. *Trends Biochem. Sci.* **1985**, 243-248. Reproduced with permission.]

The enzyme consists of a protein chain of 307 amino acid residues plus one  $Zn^{2+}$  ion to yield a molecular weight of about 34,600. The molecule is roughly egg-shaped, with a maximum dimension of approximately 5000 pm and a minimum dimension of about 3800 pm (Fig. 19.21a). There is a cleft on one side that contains the zinc ion, the active site. The metal is coordinated approximately tetrahedrally to two nitrogen atoms and an oxygen atom from three amino acids (His 69, Glu 72, His 169) in the protein chain:





**Fig. 19.21** (a) Stereoview of about one quarter of the carboxypeptidase A molecule, showing the cavity, the zinc atom, and the functional groups (shown with black atoms) Arg-145 (right), Tyr-248 (above), and Glu-270 (left). (b) Stereoview of the same region, after the addition of glycy-L-tyrosine (heavy open circles), showing the new positions of Arg-145, Tyr-248, and Glu-270. The guanidinium movement of Arg-145 is 200 pm, the hydroxyl of Tyr-248 moves 1200 pm, and the carboxylate of Glu-270 moves 200 pm when Gly-Tyr bonds to the enzyme. [From Lipscomb, W. N. *Chem. Soc. Rev.* 1972, 1, 319. Reproduced with permission.]

The fourth coordination site is free to accept a pair of electrons from a donor atom in the substrate to be cleaved.<sup>72</sup> The enzyme is thought to act through coordination of the zinc atom to the carbonyl group of the amide linkage. In addition, a nearby hydrophobic pocket envelops the organic group of the amino acid to be cleaved (Fig. 19.22) and those amino acids with aromatic side groups react most readily. Accompanying these events is a change in conformation of the enzyme: The arginine side chain moves about 200 pm closer to the carboxylate group of the substrate, and the phenolic group of the tyrosine comes within hydrogen bonding distance of the imido group of the C-terminal amino acid, a shift of 1200 pm.<sup>73</sup> The hydrogen bonding to the free carboxyl group (by arginine) and the amide linkage (by tyrosine) not only holds the substrate to the enzyme but helps break the N—C bond. Nucleophilic displacement of the amide group by an attacking carboxylate group from a glutamate group could form an anhydride link to the remainder of the peptide chain. Hydrolysis of this anhydride could then complete the cycle and regenerate the original enzyme. More likely, the glutamate acts indirectly by polarizing a water molecule (Fig. 19.22b) that attacks the amide linkage.

This example illustrates the basic key-and-lock theory first proposed by Emil Fischer in which the enzyme and substrate fit each other sterically. However, there is more to enzymatic catalysis than merely bringing reactants together. There is good evidence that the enzyme also encourages the reaction by placing a strain on the bond to be broken. Evidence comes from spectroscopic studies of enzymes containing metal ions that, unlike  $Zn^{2+}$ , show  $d-d$  transitions. The spectrum of the enzyme containing such a metal ion provides information on the microsymmetry of the site of the metal. For example,  $Co^{2+}$  can replace the  $Zn^{2+}$  and the enzyme retains its activity. The spectrum of carboxypeptidase A( $Co^{II}$ ) is "irregular" and has a high absorptivity (extinction coefficient), indicating that a regular tetrahedron is not present.<sup>74</sup> The distortion presumably aids the metal to effect the reaction. It has been suggested that the metal in the enzyme is peculiarly poised for action and that this lowers the energy of the transition state. The term *entatic*<sup>75</sup> has been coined to describe this state of the metal in an enzyme.

The substitution of a different metal into an enzyme provides a very useful method for studying the immediate environment of the metal site. In addition to the use of  $Co^{2+}$  for spectral studies, appropriate substitution allows the use of physical methods such as electron paramagnetic resonance ( $Co^{2+}$ ,  $Cu^{2+}$ ), the Mössbauer effect ( $Fe^{2+}$ ), proton magnetic resonance relaxation techniques ( $Mn^{2+}$ ), or X-ray crystallography (with a heavy metal atom to aid in the structure solution).<sup>76</sup>

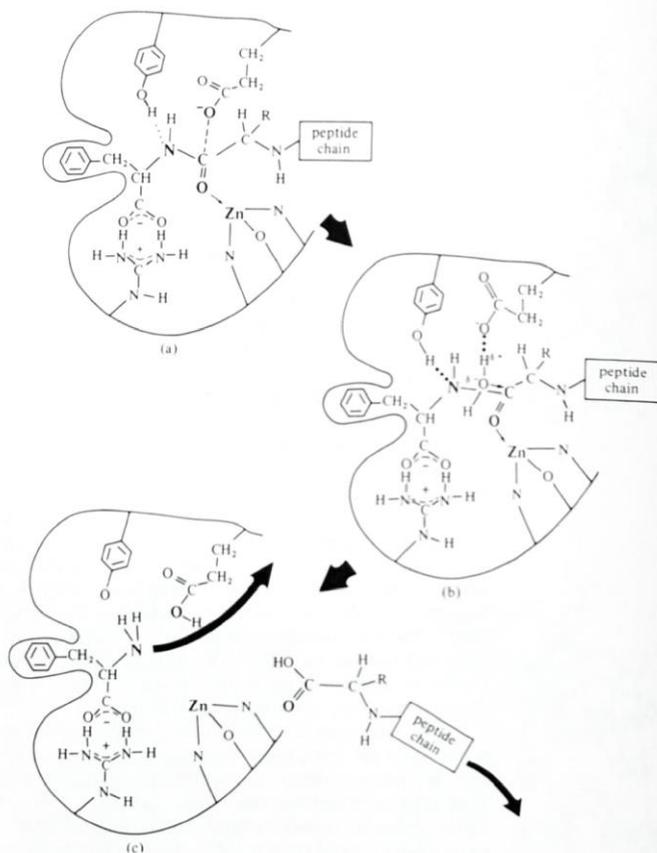
<sup>72</sup> There is probably a loosely bound water molecule at this position when the enzyme is not engaged in active catalysis.

<sup>73</sup> Lipscomb, W. N. *Chem. Soc. Rev.* **1972**, 1, 319; *Tetrahedron* **1974**, 30, 1725; *Acc. Chem. Res.* **1982**, 15, 232.

<sup>74</sup> Consider the relation between absorptivity and symmetry, Chapter 11. Vallee, B. L.; Williams, R. J. P. *Proc. Natl. Acad. Sci. U. S. A.* **1968**, 59, 498. Ulmer, D. D.; Vallee, B. L. *Bioinorganic Chemistry*; Gould, R. F., Ed.; Advances in Chemistry 100; American Chemical Society: Washington, DC, 1971; Chapter 10.

<sup>75</sup> Gr. ἐντετανω, to stretch, strain, or bend.

<sup>76</sup> Williams, R. J. P. *Endeavour* **1967**, 26, 96.

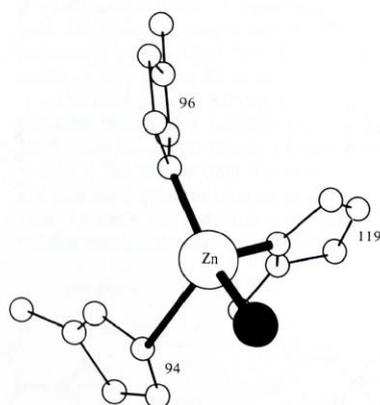


**Fig. 19.22** Suggested mode of action of carboxypeptidase A in the hydrolysis of an amide linkage in a polypeptide. (a) Positioning of the substrate on the enzyme. Interactions are (1) coordinate covalent bond, carbonyl oxygen to zinc; (2) hydrogen bonds, arginine to carboxylate and tyrosine to amide; (3) van der Waals attractions, hydrophobic pocket to aromatic ring; (4) dipole attraction and possible incipient bond formation, carboxylate (glutamate) oxygen to carbonyl group (amide linkage). Drawing is diagrammatic portrayal in two dimensions of the three-dimensional structure. (b) Probable intervention of polarized water molecule in the incipient breaking of the amide (N—C bond) linkage. (c) Completed reaction, removal of the products (amino acid and shortened peptide chain). Original configuration of enzyme returns after proton shift from glutamic acid to tyrosine. For a more detailed discussion of the mechanism of catalysis by carboxypeptidase A, see Breslow, R.; Wernick, D. L. *Proc. Natl. Acad. Sci. U. S. A.* 1977, 74, 1303.

### Inhibition and Poisoning

The study of the factors that enable an apoenzyme to select the appropriate metal ion is of importance to the proper understanding of enzyme action.<sup>77</sup> The factors that favor the formation of certain complexes in the laboratory should also be important in biological systems. For example, the Irving-Williams series and the hard-soft acid-base principles should be helpful guides. Thus we expect to find the really hard metal ions [Group IA (1); Group IIA (2)] preferring ligands with oxygen donor atoms. The somewhat softer metal atoms of the first transition series (Co to Zn) may prefer coordination to nitrogen atoms (cf. Fig. 9.5). The important thiol group,  $-\text{SH}$ , should have a particularly strong affinity for soft metal ions.

The usual structural principles of coordination chemistry such as the chelate effect, the preference for five- and six-membered rings, and the stability of certain ring conformations should hold in biological systems. In addition, however, enzymes present structural effects not observed in simpler complexes. An interesting example is carbonic anhydrase which catalyzes the interconversion of carbon dioxide and carbonates. Like carboxypeptidase, carbonic anhydrase has one zinc atom per molecule (with a molecular weight of  $\sim 30,000$ ), in this case coordinated to three histidine residues (His 94, His 96, His 119)<sup>78</sup> and a water molecule or hydroxide ion. The active site (Fig. 19.23) contains other amino acids that may function through hydrogen bonding, proton transfer, etc. The relative binding power of the zinc ion towards halide ions is reversed in the enzyme ( $\text{I}^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$ ) compared with the free  $\text{Zn}^{2+}$  ions ( $\text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^-$ ). This reversal could be interpreted as some sort of "softening" effect on the zinc by the apoenzyme were it not that the soft ligand  $\text{CN}^-$  is bound equally well by the free ion as by the complex.<sup>79</sup> Furthermore,  $\text{NO}_3^-$ ,  $\text{CNO}^-$ ,



**Fig. 19.23** Active site of carbonic anhydrase. In the resting enzyme a water molecule (O = ●) coordinates to the zinc atom. All hydrogen atoms have been omitted for clarity.

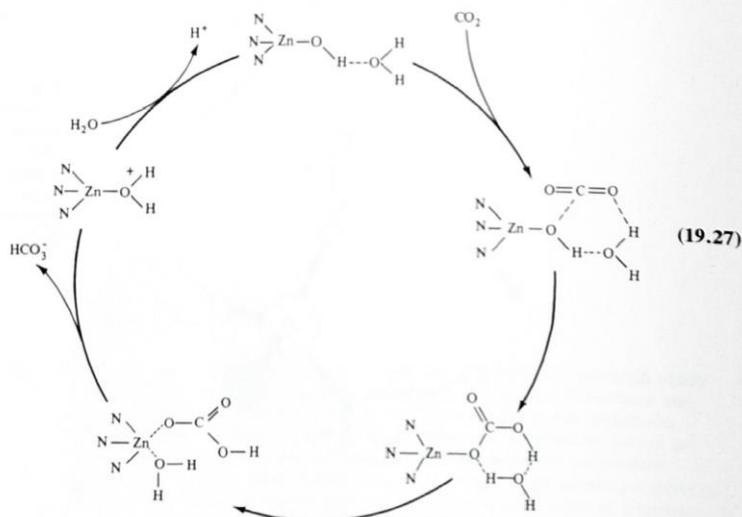
<sup>77</sup> Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201.

<sup>78</sup> There are three human isoenzymes of carbonic anhydrase that differ slightly in amino acid composition, and so the sequencing numbers for the histidines differ among them.

<sup>79</sup> It should be noted that this discussion is based on a *comparison* of the equilibrium constants of enzymic zinc-cyanide complexation versus aqueous zinc-cyanide complexations. Cyanide has a high affinity for the soft zinc ion under *both* conditions (stability constant of  $[\text{Zn}(\text{CN})_4]^{2-} = 7.7 \times 10^{16}$ ); hence it should not be concluded that there is any lack of affinity for cyanide in the enzyme.

and  $N_3^-$ , none of which is known for exceptional softness, are bound with exceptional strength. They are, however, isoelectronic and isostructural with the reactants and products of the enzyme reaction,  $CO_2$ ,  $CO_3^{2-}$ , and  $HCO_3^-$ , respectively. The explanation appears to be a tailoring of the structure of the enzyme molecule to form a pocket about 450 pm long next to the zinc ion, perhaps containing an additional positive center, to stabilize ions of appropriate size.

Although some mechanisms illustrate the carbon dioxide coordinated directly to the zinc atom, this is highly unlikely. The infrared asymmetric stretch for carbon dioxide is found at  $2343.5\text{ cm}^{-1}$  in the bound enzyme compared with  $2321\text{ cm}^{-1}$  for the free molecule, hardly compatible with a strong interaction of one oxygen atom and not the other. The visible spectrum of the  $Co^{2+}$ -substituted enzyme shows very small shifts upon binding  $CO_2$ , again incompatible with strong oxygen-metal interactions. The zinc atom is thought to be considerably more acidic in carbonic anhydrase than in carboxypeptidase A. The substitution of a third, neutral, and less basic histidine in place of the glutamate anion contributes to the greater acidity. In addition, the three histidines are pulled back making the zinc more electronegative and more acidic towards the fourth position (see Problem 19.38). This polarizes an attached water molecule, perhaps to the point of loss of a hydrogen ion to form a coordinated hydroxyl group. The mechanism of the reversible hydration of carbon dioxide to carbonic acid (actually the hydrogen carbonate ion at physiological pHs) is thought to follow the pathway shown in Eq. 19.27. Like all truly catalytic processes, it is a closed loop:<sup>80</sup>



It may operate either clockwise (as drawn) to hydrate carbon dioxide, or counterclockwise to release carbon dioxide (from the hydrogen carbonate anion) from

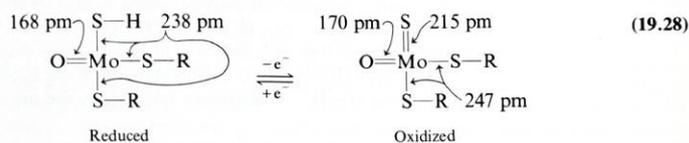
<sup>80</sup> Bertini, I.; Luchinat, C.; Scozzafava, A. *Struct. Bonding (Berlin)* **1982**, *48*, 45-91. Lindskog, S. *Adv. Inorg. Biochem.* **1982**, *4*, 115.

solution (as in the blood in the lungs), depending upon the concentrations of the reactants.

Ligands that can coordinate to an active center in an enzyme and prevent coordination by the substrate will tend to inhibit the action of that enzyme.<sup>81</sup> We have seen that azide can occupy the pocket tailored to fit the carbon dioxide molecule. This prevents the latter from approaching the active site. Furthermore, the infrared evidence indicates that the azide ion *actually does* bind the zinc atom: The asymmetric stretching mode of the azide ion is strongly shifted with respect to the free ion absorption. Thus the zinc is inhibited from acting as a Lewis acid towards water with the formation of a coordinated hydroxide ion. Other inhibitors also bind to the metal atom. As little as  $4 \times 10^{-6}$  M cyanide or hydrogen sulfide inhibits the enzymatic activity by 85%.

Inhibition may also be effected by metal ions. Most prosthetic groups involve metals of the first transition series (molybdenum seems to be the sole exception). Coordination of the apoenzyme to a heavier metal ion may destroy the enzymatic activity. Particularly poisonous in this regard are metal ions such as  $\text{Hg}^{2+}$ . The latter has a special affinity for sulfur (see HSAB, Chapter 9) and thus tends to form extremely stable complexes with amino acids containing sulfur such as cysteine, cystine, and methionine. The inhibition of an enzyme by  $\text{Hg}^{2+}$  has been taken as an indication of the presence of thiol groups but is not infallible. (For example,  $\text{Hg}^{2+}$  completely abolishes the activity of carboxypeptidase A in hydrolyzing amide linkages.) Nevertheless, the affinity of sulfur and mercury is responsible for many of the poisonous effects of mercury in biological systems. Often these effects may be reversed by addition of sulfur-containing compounds such as cysteine or glutathione. Another sulfur donor, 2,3-dimercaptopropanol, has a strong affinity for soft metal ions. Developed during World War I as an antidote for the organoarsenic war gas, lewisite, it was dubbed British antilewisite (BAL). It has proved to be extremely useful as an antidote for arsenic, cadmium, and mercury poisoning.

The inhibition of enzyme systems does not necessarily cause unwanted effects. Consider the enzyme xanthine oxidase. It contains two atoms of molybdenum, four  $\text{Fe}_2\text{S}_2$ , and two FAD (flavin adenine dinucleotide) moieties, and it has a molecular weight of 275,000–300,000. There is no evidence that the two units ( $\text{Mo}/2\text{Fe}_2\text{S}_2/\text{FAD}$ ) are near each other or interact in any way. It is believed that the immediate environment of each molybdenum atom consists of one oxygen and three sulfur atoms (additional ligands may be present):<sup>82</sup>

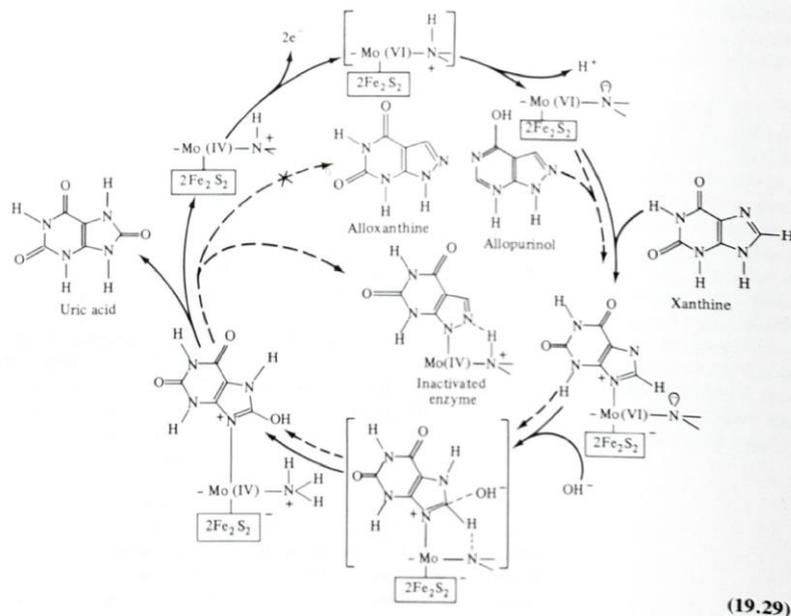


The determination of a single oxygen atom at 168–170 pm (and therefore doubly bonded) and three sulfur atoms at 238 pm (single-bonded HS— and RS—) in the reduced form, or two at 247 pm (RS—) and one at 215 pm (S=) in the oxidized form, can be made from the EXAFS spectrum.

<sup>81</sup> Compare the action of carbon monoxide on hemoglobin, page 896.

<sup>82</sup> Cramer, S. P.; Wahl, R.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1981**, *103*, 7721–7727.

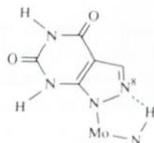
This enzyme catalyzes the oxidation of xanthine to uric acid:



The electron flow may be represented as:



Uric acid is the chief end product of purine metabolism in primates, birds, lizards, and snakes. An inborn metabolic error in humans results in increased levels of uric acid and its deposition as painful crystals in the joints. This condition (gout) may be treated by the drug allopurinol which is also oxidized by xanthine oxidase to alloxanthine (dashed line in Eq. 19.29). However, alloxanthine binds so tightly to the molybdenum that the enzyme is inactivated, the catalytic cycle broken, and uric acid formation is inhibited. The extra stability of the alloxanthine complex may be a result of strong N—H···N hydrogen bonding by the nitrogen in the 8-position:



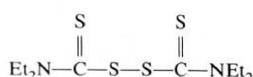
This structure resembles the hydrogen bonded transition state for the nucleophilic attack of hydroxide ion (Eq. 19.29) where the hydrogen bond promotes the attack on

the carbon. With a nitrogen atom at the 8-position there is no way for the alloxanthine to leave.<sup>83</sup>

A closely related enzyme is aldehyde oxidase. It also contains two (Mo/2Fe<sub>2</sub>S<sub>2</sub>/FAD) units with a molecular weight of about 300,000. It converts acetaldehyde to acetic acid via electron flow:



When ethanol is consumed, the initial metabolic product is the extremely poisonous acetaldehyde, which is kept in low concentration by the oxidase-catalyzed conversion to harmless acetic acid. The drug Antabuse, used for treating alcoholism, is a sulfur-containing ligand, disulfiram:

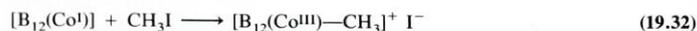


In the body, Antabuse inhibits acetaldehyde oxidase, presumably via the soft-soft molybdenum-sulfur interaction.<sup>84</sup> Any alcohol ingested will be converted to acetaldehyde which, in the absence of a pathway to destroy it, will build up with severely unpleasant effects, discouraging further consumption.

### Vitamin B<sub>12</sub> and the B<sub>12</sub> Coenzymes<sup>85</sup>

In 1948 an "anti-pernicious anemia factor" was isolated, crystallized, and named vitamin B<sub>12</sub> or cyanocobalamin. The molecule is built around a corrin ring containing a cobalt(III) atom. The corrin ring is a modified porphyrin ring in which one of the =CH— bridges between two of the pyrrole-type rings is missing, contracting the ring. The fifth and sixth coordination sites on the cobalt are filled by a nitrogen atom from an imidazole ring and a cyanide ion. The latter is an artifact of the isolation procedure and is not present in the biological system, where the sixth position appears to hold a loosely bound water molecule.

Vitamin B<sub>12</sub> may be reduced by one electron ("vitamin B<sub>12r</sub>") or two electrons ("vitamin B<sub>12s</sub>") to form the Co(II) and Co(I) complexes, respectively.<sup>86</sup> The latter is strongly nucleophilic and readily undergoes alkylation via oxidative addition:<sup>87</sup>



<sup>83</sup> Stiefel, E. I. *Prog. Inorg. Chem.* **1977**, *22*, 1-223. The 1988 Nobel Prize in Physiology and Medicine was awarded for "rational synthesis," i. e., the tailoring of drugs for specific sites and actions. Elion, G. B. *In Vitro Cell. Dev. Biol.* **1989**, *25*, 321-330.

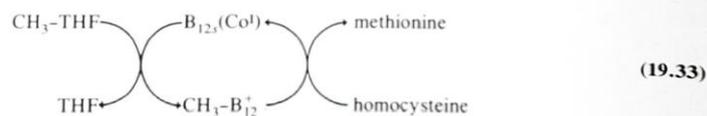
<sup>84</sup> The above surmise is based on the known chemistry between molybdenum and sulfur-containing ligands. It has been suggested that disulfiram inhibits the enzyme by oxidizing essential sulfhydryl groups to form internal S—S bonds (see Vallari, R. C.; Pietruszko, R. *Science* **1982**, *216*, 637). Disulfiram is also used to prevent renal toxicity from platinum when *cis*-diamminedichloroplatinum (II) (see page 958) is used to treat neoplasms and trypanosomiasis (see Wyszor, M. S.; Zwelling, L. A.; Sanders, J. E.; Grenan, M. M. *Science* **1982**, *217*, 454-456). The complexing agent is thought to be diethyldithiocarbamate, a metabolite of disulfiram.

<sup>85</sup> Ochiai, E.-I. *General Principles of Biochemistry of the Elements*; Plenum: New York, 1987; pp 217-221. Crabtree, R. H. *The Organometallic Chemistry of the Transition Metals*; Wiley: New York, 1988; pp 388-393.

<sup>86</sup> The *r* stands for "reduced" and the *s* for "super-reduced." The latter may seem to be something of an exaggeration until one recalls that the predominant coordination chemistry of cobalt is that of Co(III), with less for Co(II), and very little for other oxidation states.

<sup>87</sup> See Chapter 15 with respect to the basicity of metals in low oxidation states and oxidative addition reactions.

In biological systems the two-electron reduction may be accomplished by NADH and flavin adenine dinucleotide (FAD). The methyl donor is N<sup>5</sup>-methyltetrahydrofolate (CH<sub>3</sub>-THF). The Co(III) corrinoid (methylcobalamin) can then partake in biomethylation reactions:

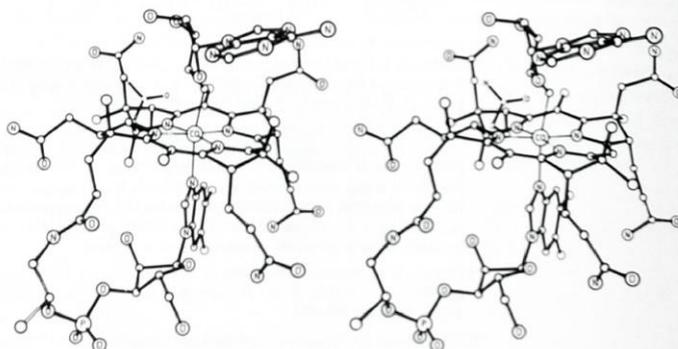


Certain bacteria can methylate not only sulfur in organic compounds but also various heavy metals such as Hg, As, Tl, Pb, Sn, Au, Pd, and Pt in anaerobic sludges. Thus methylmercury cation, CH<sub>3</sub>Hg<sup>+</sup>, may be formed from inorganic mercury compounds resulting in environmental health problems (see page 947). A related reaction posed a serious problem in the 19th century when Paris green, approximately Cu<sub>3</sub>(AsO<sub>3</sub>)<sub>2</sub> with copper acetate present to enhance the color, was used as a pigment in wallpaper. Under humid conditions certain molds would grow on the wallpaper and form volatile trimethylarsine causing arsenic poisoning to those living in the premises.

When vitamin B<sub>12s</sub> reacts with adenosine triphosphate (ATP), alkylation takes place as in Eq. 19.33 with the formation of a direct carbon-cobalt bond between adenosyl and the metal, forming B<sub>12</sub> coenzyme (Fig. 19.24). It acts in concert with several other enzymes to effect 1,2-shifts of the general type:

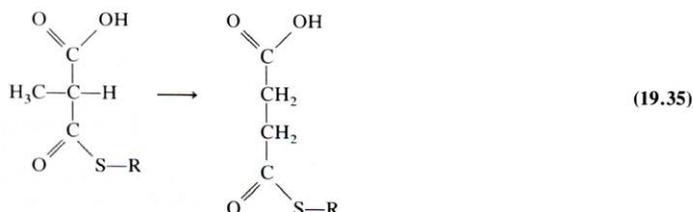


One of the indicators of pernicious anemia, a disease caused by inability to absorb B<sub>12</sub> through the gut wall, is an increase in excretion of methylmalonic acid as the body fails to convert it to succinic acid.



**Fig. 19.24** A stereoview of the molecular structure of vitamin B<sub>12</sub>. [From Lenhart, P. G., *Proc. Roy. Soc.* 1968, A303, 45. Reproduced with permission.]

Until the proposed mechanism is examined, some of these rearrangements appear unusual:



It is believed that the reaction starts with homolytic cleavage of the cobalt-carbon bond (at a cost of perhaps  $100 \text{ kJ mol}^{-1}$ )<sup>88</sup> to yield a Co(II) atom and a 5'-deoxyadenosyl radical. This radical then abstracts a hydrogen atom (in Eq. 19.35 from the methyl group). Migration of the  $-\text{C}(\text{O})\text{SR}$  group takes place, followed by return of the hydrogen atom from 5'-deoxyadenosine to the substrate. This regenerates the 5'-deoxyadenosyl radical, which can recombine with the Co(II) atom to form the coenzyme.

A third type of reaction employing  $\text{B}_{12}$  coenzyme is the reduction of  $-\text{CH}(\text{OH})-$  groups to  $-\text{CH}_2-$  groups, as in the reduction of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA).

$\text{B}_{12}$  is unusual in several ways. The ability to form a metal-carbon bond in a biological system appears to be unique: These are nature's only organometallic compounds.<sup>89</sup> It is the only vitamin known to contain a metal. It appears to be synthesized exclusively by bacteria. It is not found in higher plants, and although it is essential for all higher animals, it must be obtained from food sources, hence its designation as a "vitamin."

The fitness of cobalamin to serve its biochemical functions has been variously ascribed to different factors by different authors.<sup>90</sup> Certainly, the existence of three oxidation states,  $\text{Co}^{\text{I}}$ ,  $\text{Co}^{\text{II}}$ , and  $\text{Co}^{\text{III}}$ , stable in aqueous (and hence biological) media is necessary. This, in itself, may eliminate the earlier transition metals (without accessible +1 oxidation states) and copper ( $\text{Cu}^{\text{II}}$  is strongly oxidizing). In addition, we have seen that  $d^8/d^6$  ( $16e^-/18e^-$ ) systems are ideal for oxidative addition/reductive elimination reactions. It has also been suggested that the flexibility of the corrin ring allows changes in conformation that may be beneficial. In this regard it may be noted that the cobalt porphyrin analogues of  $\text{B}_{12}$  cannot be reduced to Co(I) in aqueous solution. Hence the corrin ring was selected in place of porphyrin in the evolution of the  $\text{B}_{12}$  cobalt complexes.

<sup>88</sup> Halpern, J.; *Acc. Chem. Res.* **1982**, *15*, 238; Kim, S.-H.; Leung, T.W. *J. Am. Chem. Soc.* **1984**, *106*, 8317-8319.

<sup>89</sup> This truism of yesterday is currently being challenged—there is increasing evidence of other biological metal-carbon bonds, but, unlike  $\text{B}_{12}$ , none of the suspected compounds has been isolated and characterized.

<sup>90</sup> Schrauzer, G. N. *Angew. Chem. Int. Ed. Engl.* **1976**, *15*, 417. Ochiai, E.-I. *J. Chem. Educ.* **1978**, *55*, 631; *General Principles of Biochemistry of the Elements*; Plenum: New York, 1987; p 221.

**Metallothioneins<sup>91</sup>**

We have seen that heavy metals can replace essential metals in enzymes and destroy the enzymatic activity. In addition, by coordinating to sulfur-bearing amino acids in the protein chain they might cause an enzyme to be "bent out of shape" and lose its activity. Protection of enzymes from toxic metals is thus requisite for their proper function. Serving this purpose is a group of proteins that have the following characteristics: (1) The molecular weights are about 6000 with 61 to 62 amino acids. (2) One-third (20) of these amino acids are cysteine [ $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ ] residues, grouped in Cys—Cys and Cys—X—Cys groups (X = a separating amino acid). (3) None of the cysteines are linked by S—S bridges (cystine). (4) There are few or no histidines or amino acids with aromatic side chains. (5) With such a high percentage of amino acids bearing thiol groups and "clumped" along the protein chain, the thioneins are able to bind several metal ions per molecule, preferentially the softer metals such as  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ , etc. Metallothioneins containing  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  might possibly be important in the transport of these essential elements, but the evidence is mostly negative. On the other hand, the binding of heavy metals such as cadmium and mercury suggests a protective function against these toxic metals. Indeed, increased amounts of thioneins are found in the liver, kidney, and spleen after exposure to them. Furthermore, it can be demonstrated that cell lines that fail to produce thioneins are extremely sensitive to cadmium poisoning while "over-producers" have enhanced protection. It has been suggested that the binding of thioneins to cadmium and other heavy metals, with extremely high stability constants, is one of protection alone; perhaps the reduced binding of copper, an essential metal but one toxic in high concentrations, serves a "buffering function" of providing copper for enzymes but not at levels sufficiently high to be toxic. The question of whether the weaker binding of the less toxic zinc serves a similar function, or "just happens," is moot.

For +2 cations such as zinc(II) and cadmium(II) each metallothionein molecule contains up to seven metal atoms. X-ray studies indicate that the metal atoms are in approximately tetrahedral sites bound to the cysteine sulfur atoms. The soft mercury(II) ion has a higher affinity for sulfur and will displace cadmium from metallothionein. At first the mercury ions occupy tetrahedral sites but as the number increases, the geometries of the metal sites and protein change until about nine Hg(II) atoms are bound in a linear (S—Hg—S) fashion.<sup>92</sup> Up to twelve +1 cations such as copper(I) and silver(I) can bind per molecule, indicating a coordination number lower than four, probably three (see Problem 12.34).

An intriguing problem about which we know very little is the mechanism of metal identification by the body that triggers its response, as in the case of the build-up of metallothioneins upon exposure to toxic metals. Perhaps the best understood of the metalloregulatory proteins is MerR that protects bacteria from mercurial toxicity. It is extremely sensitive to  $\text{Hg}^{2+}$ , and distinguishes it from its congeners  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$ . There is good evidence that the mercury receptor forms three-coordinate mercury(II) complexes (see Fig. 12.1c), making possible this specificity.<sup>93</sup>

<sup>91</sup> Hamer, D. H. *Ann. Rev. Biochem.* **1986**, *55*, 913. Dalgarno, D. C.; Armitage, I. M. *Adv. Inorg. Biochem.* **1984**, *6*, 113. Kojima, Y.; Kägi, J. H. R. *Trends Biochem. Sci.* **1978**, *3*, 90.

<sup>92</sup> Johnson, B. A.; Armitage, I. A. *Inorg. Chem.* **1987**, *26*, 3139-3144.

<sup>93</sup> Wright, J. G.; Natan, M. J.; MacDonnell, F. M.; Ralston, D. M.; O'Halloran, T. V. *Prog. Inorg. Chem.* **1990**, *38*, 323-412.

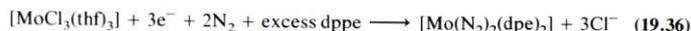
### Nitrogen Fixation

An enzyme system of particular importance is that which promotes the fixation of atmospheric dinitrogen. This is of considerable interest for a variety of reasons. It is a very important step in the nitrogen cycle, providing available nitrogen for plant nutrition. It is an intriguing process since it occurs readily in various bacteria, blue-green algae, yeasts, and in symbiotic bacteria-legume associations under mild conditions. However, nitrogen stubbornly resists ordinary chemical attack, even under stringent conditions.

Molecular nitrogen,  $N_2$ , is so unresponsive to ordinary chemical reactions that it has been characterized as "almost as inert as a noble gas."<sup>94</sup> The very large triple bond energy ( $945 \text{ kJ mol}^{-1}$ ) tends to make the activation energy prohibitively large. Thus, in spite of the fact that the overall enthalpy of formation of ammonia is exothermic by about  $50 \text{ kJ mol}^{-1}$ , the common Haber process requires about 20 MPa pressure and 500 °C temperature to proceed, even in the presence of the best Haber catalyst. In addition to the purely pragmatic task of furnishing the huge supply of nitrogen compounds necessary for industrial and agricultural uses as cheaply as possible, the chemist is intrigued by the possibility of discovering processes that will work under less drastic conditions. We know they exist: We can watch a clover plant growing at 100 kPa and 25 °C!

### In Vitro Nitrogen Fixation

The discovery that dinitrogen was capable of forming stable complexes with transition metals (Chapter 15) led to extensive investigation of the possibility of fixation via such complexes. An important development was the discovery that certain phosphine complexes of molybdenum and tungsten containing dinitrogen readily yield ammonia in acidic media:<sup>95</sup>



where thf = tetrahydrofuran and dppe = 1,2-bis(diphenylphosphino)ethane,  $\text{Ph}_2\text{PCH}_2\text{-CH}_2\text{PPh}_2$ . Both reactions take place at room temperature and atmospheric pressure. The reducing agent is a Grignard reagent. This reaction sequence is important because it models the in vivo nitrogenase systems that appear to employ molybdenum.

We should not conclude, however, that ambient temperature and pressure reactions are likely to replace the Haber-Bosch process. Despite the fact that the latter requires high temperature and pressure, it is efficient and well entrenched, and it can produce large volumes of product in short time periods. With respect to the former processes, it is certain that the chemist will not be able to keep pace with the lively imagination of the journalist. As an interesting aside on the inherent inability of the scientist to match ever increasing expectations, the reader is directed to the following selection of titles and headlines. The first is the title of the initial research report by Chatt's group in England and the remainder are headlines of various reports of it in the popular press:<sup>95</sup>

<sup>94</sup> Jolly, W. L. *The Chemistry of the Non-Metals*; Prentice-Hall: Englewood Cliffs, NJ, 1966; p 72.

<sup>95</sup> Chatt, J.; Pearman, A. J.; Richards, R. L. *Nature (London)* **1975**, 253, 39–40. For a very readable account of the early work, including the headlines on page 934, see Chatt, J. *Proc. Roy. Instn. Great Br.* **1976**, 49, 281. For a current overview, see Leigh, G. J. *Acc. Chem. Res.* **1992**, 25, 177–181. For catalytic reduction in aqueous solution see Shilov, A. E. In *Perspectives in Coordination Chemistry*; Williams, A. F.; Floriana, C.; Merbach, A. E., Eds.; VCH: New York, 1992; pp 233–244.

The reduction of mono-co-ordinated molecular nitrogen to ammonia in a protic environment	<i>Nature (London)</i> (Jan. 3, 1975)
Fuel-saving way to make fertiliser	<i>The Times</i> (Jan. 3, 1975)
Fuel break-through	<i>The Guardian</i> (Jan. 3, 1975)
More progress in nitrogen fixation	<i>New Scientist</i> (Jan. 9, 1975)
Cheaper nitrogen by 1990	<i>Farmer's Weekly</i> (Jan. 10, 1975)
Basic life process created in UK lab	<i>The Province</i> (British Columbia, Jan. 15, 1975)

With each retelling the story grew, until by the time it reached British Columbia, it appeared that the press was *almost* able in 12 days to duplicate what is recorded as a 6-day event in Genesis! The resultant disappointment when scientists are not able to meet expectations benefits neither them nor the public (but that, too, is good copy for the popular press!).

### In Vivo Nitrogen Fixation

There are several bacteria and blue-green algae that can fix molecular nitrogen *in vivo*. Both free-living species and symbiotic species are involved. There are the strictly anaerobic *Clostridium pasteurianum*,<sup>96</sup> facultative aerobes like *Klebsiella pneumoniae*, and strict aerobes like *Azotobacter vinelandii*. Even in the aerobic forms it appears that the nitrogen fixation takes place under essentially anaerobic conditions (see below). The most important nitrogen-fixing species are the mutualistic species of *Rhizobium* living in root nodules of various species of legumes (clover, alfalfa, beans, peas, etc.).

The active enzyme in nitrogen fixation is nitrogenase. It is not a unique enzyme but appears to differ somewhat from species to species. Nevertheless the various enzymes are very similar. Two proteins are involved. The smaller has a molecular weight of 57,000–73,000. It contains an Fe<sub>4</sub>S<sub>4</sub> cluster. The larger protein is an  $\alpha_2\beta_2$  tetramer with a molecular weight of 220,000–240,000 containing two molybdenum atoms, about 30 iron atoms, and about 30 labile sulfide ions.<sup>97</sup> The iron–sulfur clusters probably act as redox centers. It is possible to isolate a soluble protein-free cofactor containing molybdenum and iron (ca. 1 Mo, 7–8 Fe, and 4–6 S<sup>2-</sup>). Recombination of the cofactor with inactive nitrogenase restores the activity.

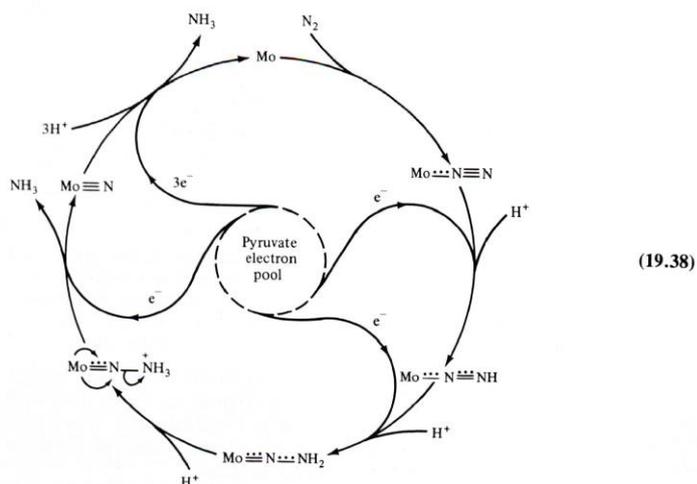
It seems likely that the active site for dinitrogen binding involves the molybdenum atom. It has been established by EXAFS<sup>98</sup> that the coordination sphere consists of several sulfur atoms at distances of about 235 pm. An Mo=O double bond, so common in complexes of Mo(IV) and Mo(VI), is *not* present. There are other heavy atoms, perhaps iron, nearby (~270 pm). The ultimate source of reductive capacity is pyruvate, and the electrons are transferred via ferredoxin (see page 911) to nitro-

<sup>96</sup> *Clostridium* includes, in addition to the useful nitrogen-fixing *C. pasteurianum*, the dangerous anaerobic species *C. tetani* (causes tetanus, "lockjaw"), *C. botulinum* (causes botulism), and *C. welchi* (causes "gas gangrene").

<sup>97</sup> Stiefel, E. I. *Prog. Inorg. Chem.* **1977**, *22*, 1–223. Nelson, M. J.; Lindahl, P. A.; Orme-Johnson, W. H. *Adv. Inorg. Biochem.* **1982**, *4*, 1–40. Burgmayer, S. J. N.; Stiefel, E. I. *J. Chem. Educ.* **1985**, *62*, 943. *Note added in proof:* The structure of nitrogenase has now been determined. Georgiadis, M. M.; Komiya, H.; Chakrabarti, P.; Woo, D.; Kornuc, J. J.; Rees, D. C. *Science* **1992**, *257*, 1653. Kim, J.; Rees, D. C. *Science* **1992**, *257*, 1677; *Nature* **1992**, *360*, 553.

<sup>98</sup> Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398–3407. Conradson, S. D.; Burgess B. K.; Newton, W. E.; Mortensen, L. E.; Hodgson, K. O. *J. Am. Chem. Soc.* **1987**, *109*, 7507–7515.

genase. There is some evidence, not strong, that Mo(III) is involved. Two Mo(III) atoms cycling through Mo(VI) would provide the six electrons necessary for reduction of dinitrogen. Alternatively, since the enzyme is rich in ferredoxin-type clusters, there should be a ready flow of electrons, and the molybdenum may stay in the one or two oxidation states that most readily bind dinitrogen and its intermediate reductants. The overall catalytic cycle may resemble that shown in Eq. 19.38.<sup>99</sup>



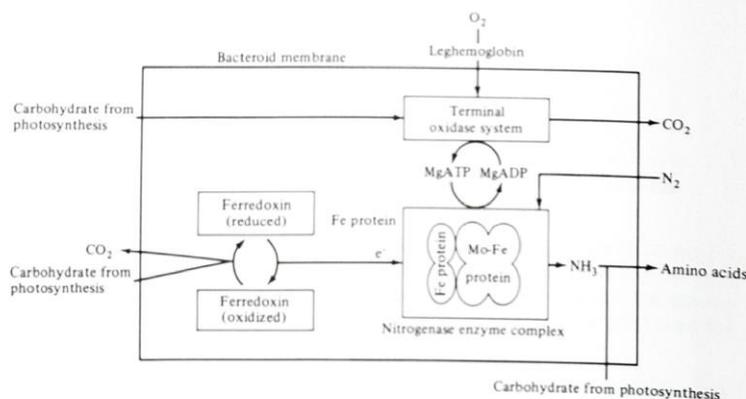
A schematic diagram for the production of fixed nitrogen compounds, including the sources of materials and energy, and the overall reactions, is given in Fig. 19.25. Note the presence of leghemoglobin. This is a monomeric, oxygen-binding molecule rather closely resembling myoglobin. It is felt that the leghemoglobin binds any oxygen that is present very tightly and thus protects the nitrogenase, which cannot operate in the presence of oxygen. On the other hand, it allows a reservoir of oxygen for respiration to supply energy to keep the fixation process going.

### The Biochemistry of Iron<sup>100</sup>

It is impossible to cover adequately the chemistry of various elements in biological systems in a single chapter. Before discussing the salient points of other essential and trace elements, the biochemistry of iron will be discussed briefly. Iron is the most

<sup>99</sup> Note added in proof: The structure of the Fe-Mo cofactor cited in Footnote 97 has led those authors to suggest that the molybdenum does *not* directly participate in binding the dinitrogen molecule. The Mo is already six-coordinate with three S atoms, two O atoms from a homocitrate anion, and one N atom from a histidine in the protein chain. Therefore, in Eq. 19.38 the N<sub>2</sub> is probably bound to an Fe-S cluster in place of Mo.

<sup>100</sup> Crichton, R. R. *Inorganic Biochemistry of Iron Metabolism*; Ellis Horwood: New York, 1991.



**Fig. 19.25** Schematic diagram of nitrogenase activity in a bacterial cell. Carbohydrate provides reducing capacity (ferredoxin), energy (MgATP), and organic precursors for the manufacture of amino acids. [From Skinner, K. J. *Chem. Eng. News* 1976, 54(41), 22-35. Reproduced with permission.]

abundant transition element and serves more biological roles than any other metal. It can therefore serve to illustrate the possibilities available for the absorption, storage, handling, and use of an essential metal. Iron has received much study, and similar results can be expected for other metals as studies of the chemistry of trace elements in biological systems advance.

### Availability of Iron

Although iron is the fourth most abundant element in the earth's crust, it is not always readily available for use. Both  $\text{Fe}(\text{OH})_2$  and  $\text{Fe}(\text{OH})_3$  have very low solubilities, the latter especially so ( $K_{\text{sp}}^{\text{II}} = 2 \times 10^{-15}$ ;  $K_{\text{sp}}^{\text{III}} = 2 \times 10^{-39}$ ). An extreme example is iron deficiency in pineapples grown on rust-red soil on Oahu Island containing over 20% Fe, but none of it available because it is kept in the +3 oxidation state by the presence of manganese dioxide and the absence of organic reducing agents.<sup>101</sup> Similarly under alkaline conditions in the soil (e.g., in geographic regions where the principal rocks are limestone and dolomite) even iron(II) is not readily available to plants. The stress is especially severe on those species such as rhododendron and azalea that naturally live in soils of low pH. Under these circumstances gardeners and farmers often resort to the use of "iron chelate," an edta complex. The latter is soluble and makes iron available to the plant for the manufacture of cytochromes, ferredoxins, etc. The clever application of coordination chemistry by the chemical agronomist was predated by some hundreds of millions of years by certain higher plants. Some, such as wheat and oats, adapted to grow on alkaline soils, have evolved the ability to exude various polyamino-acid chelating agents through the root tips to solubilize the iron so that it may be absorbed.<sup>102</sup>

<sup>101</sup> Brasted, R. C. *J. Chem. Educ.* 1970, 47, 634.

<sup>102</sup> Sugiura, Y.; Nomoto, K. *Struct. Bonding (Berlin)* 1984, 58, 107.

The presence of organic chelates of iron in surface waters has been related to the "red tide," an explosive "bloom" of algae (*Gymnodium breve*) that results in mass mortality of fish. It is possible to correlate the occurrence of these outbreaks with the volume of stream flow and the concentrations of iron and humic acid.<sup>103</sup> At least one of the dinoflagellates in the red tide possesses an iron-binding siderophore (see below).<sup>104</sup>

Within the organism a variety of complexing agents are used to transport the iron. In higher animals it is carried in the bloodstream by the *transferrins*. These iron-binding proteins are responsible for the transport of iron to the site of synthesis of other iron-containing compounds (such as hemoglobin and the cytochromes) and its insertion via enzymes into the porphyrin ring.<sup>105</sup> The iron is present in the +3 oxidation state ( $\text{Fe}^{2+}$  does not bind) and is coordinated to two or three tyrosyl residues, a couple of histidyl residues, and perhaps a tryptophanyl residue in a protein chain of molecular weight about 80,000.<sup>106</sup> There are two iron-binding sites per molecule.

Most aerobic microorganisms have analogous compounds, called siderophores, which solubilize and transport iron(III). They have relatively low molecular weights (500–1000) and, depending upon their molecular structure and means of chelating iron, are classified into several groups such as the ferrichromes, ferrioxamines, and enterobactins. Some examples are shown in Fig. 19.26. It is obvious that these molecules are polydentate ligands with many potential ligating atoms to form chelates. They readily form extremely stable octahedral complexes with high spin Fe(III). Although the complexes are very stable, which is extremely important to their biological function (see below), they are labile, which allows the iron to be transported and transferred within the bacteria.<sup>107</sup> The ferrichromes and ferrioxamines are trihydroxamic acids which form neutral trischelates from three bidentate hydroxamate monoanions. Enterobactin contains a different chelating functional group, *o*-dihydroxybenzene ("catechol"). Each catechol group in enterobactin behaves as a dianion for a total charge of -6 for the ligand. A characteristic of all of these is that, in addition to the natural tendency of trischelates to form globular complexes, the remainder of the siderophore molecule consists of a symmetric, hydrophilic portion that presumably aids in transport across the cell membrane (Fig. 19.27).

It is interesting that biologically functioning iron compounds such as hemoglobin, myoglobin, cytochromes, and ferredoxins employ iron(II) compounds, but the siderophores and transferrins coordinate iron(III). The reduced iron compounds *within* biological systems may reflect an evolutionary history from a primitive reducing atmosphere on earth (see page 951), whereas the siderophores are the response to the need to deal currently with iron(III) in an oxidized *external* environment.

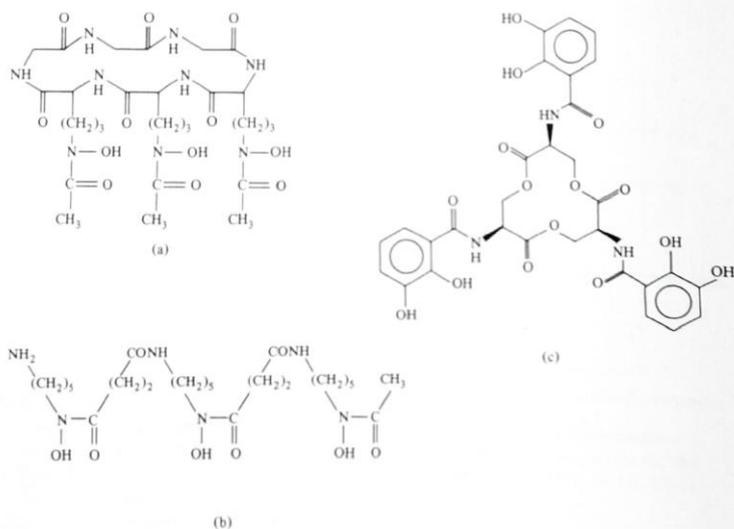
<sup>103</sup> Martin, D. F.; Martin, B. B. *J. Chem Educ.* **1976**, *53*, 614.

<sup>104</sup> Trick, C. G.; Andersen, R. J.; Gillam, A.; Harrison, P. J. *Science* **1983**, *219*, 306–308.

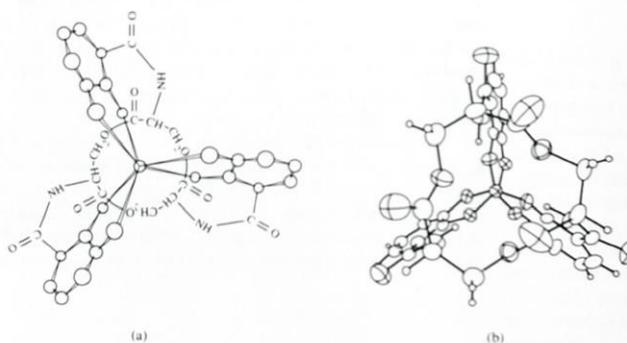
<sup>105</sup> *Biochemistry of Nonheme Iron*; Bezkorovainy, A., Ed.; Plenum: New York, 1980; Chapter 4. Kochan, I. In *Bioinorganic Chemistry-II*; Raymond, K. N., Ed.; American Chemical Society: Washington, DC, 1977.

<sup>106</sup> Llinás, M. *Struct. Bonding (Berlin)* **1973**, *17*, 135–220.

<sup>107</sup> Raymond, K. N.; Müller, G.; Matzanke, B. F. *Top. Curr. Chem.* **1984**, *123*, 49. Enterobactin is the most powerful iron(III) chelator known with an overall stability constant of  $K_f \approx 10^{49}$  (Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 906).



**Fig. 19.26** Three types of bacterial siderophores: (a) desferrichrome; (b) desferrioxamine B; (c) enterobactin.



**Fig. 19.27** The  $\Delta$ -cis isomers of metal enterobactins. The metal lies at the center of a distorted octahedron of the six oxygen atoms of the three catechol ligands with approximate  $C_3$  symmetry. (a) The structure of iron(III) enterobactin as determined by CD spectra. (b) ORTEP plot of the structure of V(IV) enterobactin as determined crystallographically. Note that although both structures are viewed down the approximate threefold axis and the atoms (except Fe/V) are the same in (b) as in (a), the views are  $180^\circ$  apart. [From Isied, S. S.; Kuo, G.; Raymond, K. N. *J. Am. Chem. Soc.* **1976**, *98*, 1763; Karpishin, T. B.; Raymond, K. N. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 466–468. Reproduced with permission.]

**Competition for Iron**

In addition to the transport of iron, the transferrins of higher animals and the siderophores of bacteria show another interesting parallel. It can most readily be shown by ovotransferrin (called conalbumin in the older literature) of egg white, though we shall see other examples. There is a large amount, up to 16%, in the protein of egg white, although it has been impossible to find an iron-transporting function for it there. In fact, in some 200 species for which ovotransferrin has been studied, 99% were completely devoid of iron binding to the protein! Ovotransferrin and other transferrins, in general, have larger stability constants towards iron(III) than do the various siderophores. It is thus quite likely that they act as antibacterial agents. In the presence of excess ovotransferrin, bacteria would be iron deficient since the siderophore cannot compete successfully for the iron.<sup>105,108</sup>

Lactoferrin, found in mother's milk, appears to be the most potent antibacterial transferrin and seems to play a role in the protection of breast-fed infants from certain infectious diseases. It has been claimed that milk proteins remain intact in the infant's stomach for up to 90 minutes and then pass into the small intestine unchanged, thus retaining their iron-binding capacity. In guinea pigs, addition of hematin to the diet abolishes the protective effects of the mother's milk.<sup>109</sup>

The question of iron chelation as an antibacterial defense is receiving increasing attention. It appears to be far more general than had previously been supposed.<sup>110</sup> An interesting sidelight is that the fever that often accompanies infection enhances the bacteriostatic action of the body's transferrins.

An interesting side effect of the presence of ovotransferrin in egg whites is the custom, long established before any rational explanation, of beating egg whites in copper bowls to stabilize the foam (as in meringues, etc.) The copper complex of ovotransferrin stabilizes the protein of egg white against denaturation and thus stabilizes the foam.<sup>111</sup>

Another interesting example of this sort is the competition between bacteria and the roots of higher plants. Both use chelators to win iron from the soil. However, higher plants have one more mechanism with which to compete: The Fe(III) is reduced and absorbed by the roots in the uncomplexed Fe(II) form. When edta and other chelating agents are used to correct chlorosis in plants due to iron deficiency, the action is merely one of solubilizing the Fe(III) and making it physically accessible to the roots—the chelates are not absorbed intact. Indeed, chelates that strongly bind Fe(II) may actually inhibit iron uptake from the root medium.<sup>112</sup>

Exactly the opposite problem may occur for plants whose roots are growing in anaerobic media. In flooded soils the roots may be exposed to high levels of iron(II), posing potential problems of iron toxicity. Rice plants and water lilies with roots in anaerobic soils transport dioxygen (from the air or photosynthesis, or both) to the periphery of the roots where it oxidizes the iron(II) to iron(III). In this case the insolubility of iron(III) hydroxide is utilized to protect the plant from iron poisoning.<sup>113</sup> A similar problem from *too much* iron occurs in parts of sub-Saharan Africa.

<sup>108</sup> Webb, J.; van Bockxmeer, F. M. *J. Chem. Educ.* **1980**, *57*, 639.

<sup>109</sup> *Biochemistry of Nonheme Iron*; Bezkorovainy, A., Ed.; Plenum: New York, 1980; pp 336–337.

<sup>110</sup> Weinberg, E. D. *Microbiol. Rev.* **1978**, *42*, 45. Hider, R. C. *Struct. Bonding (Berlin)* **1984**, *58*, 25. *Iron and Infection*; Bullen, J. J.; Griffiths, E., Eds.; Wiley: New York, 1987.

<sup>111</sup> McGee, H. J.; Long, S. R.; Briggs, W. R. *Nature (London)* **1984**, *308*, 667.

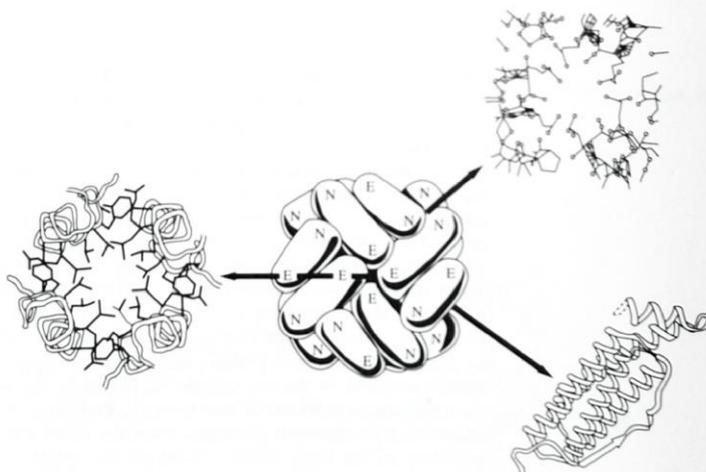
<sup>112</sup> Olsen, R. A.; Clark, R. B.; Bennett, J. H. *Am. Sci.* **1981**, *69*, 378–384.

<sup>113</sup> Dacey, J. W. H. *Science* **1980**, *210*, 1017.

The excess dietary iron is derived from a traditional fermented maize beverage that is home-brewed in steel drums.<sup>114</sup> It should be noted in this connection that the body has no mechanism for the excretion of iron, and except for women in the child-bearing years, the dietary requirement for iron is extremely low.

The absorption of iron in the gut, preferentially in the +2 oxidation state, was once thought to be a result of special physiological mechanisms, but now is generally agreed to be merely another aspect of the differential solubility of  $\text{Fe}(\text{OH})_2$  and  $\text{Fe}(\text{OH})_3$ . However, there is a significant differential in the absorption of heme versus nonheme iron: Heme iron is absorbed 5–10 times more readily than nonheme iron.<sup>115</sup> Since meat contains large quantities of hemoglobin, myoglobin, and cytochromes, this difference could be nutritionally significant.

It is conceivable that iron could be stored in the form of a complex such as transferrin or even hemoglobin, and in lower organisms ferrichrome apparently serves this purpose. Such storage is wasteful, however, and higher animals have evolved a simpler method of storing iron as *ferritin*. If iron(III) nitrate is allowed to hydrolyze in a solution made slightly basic by the hydrogen carbonate ion ( $\text{HCO}_3^-$ ), it spontaneously forms spheres of "FeOOH" of about 7000 pm in diameter. The core of a ferritin particle is similar and contains up to 4500 iron atoms and apparently some



**Fig. 19.28** Structural features of apoferritin. The gross quaternary structure of the assembled molecule is shown in the center and more details on the fourfold channels (left), the threefold channels (upper right) and the subunits (lower right) are also illustrated. [From Harrison, P. M.; Treffry, A.; Lilley, T. H. J. *Inorg. Biochem.* **1986**, *27*, 287–293. Reproduced with permission.]

<sup>114</sup> Rollinson, C. L.; Enig, M. G. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; Grayson, M., Ed.; Wiley: New York, 1981; Vol. 15, pp 570–603. Gordeuk, V. R.; Bacon, B. R.; Brittenham, G. M. *Ann. Rev. Nutr.* **1987**, *7*, 485–508.

<sup>115</sup> Narins, D. In *Biochemistry of Nonheme Iron*; Bezkorovainy, A., Ed.; Plenum: New York, 1980; Chapter 3. Hallberg, L. *Ann. Rev. Nutr.* **1981**, *1*, 123–147.

phosphate as well as oxo and hydroxo ligands. This core is surrounded by a protein covering (called *apoferritin*) that allows controlled access to the core through eight hydrophilic channels (along threefold axes) and six hydrophobic channels (along fourfold axes) (see Fig. 19.28). It is thought that the iron(III) enters via the hydrophilic channels and leaves via the hydrophobic channels, but the mechanism of iron transfer is obscure. In any event, ferritin provides high-density storage of inorganic iron combined with ready availability.<sup>116</sup>

### Essential and Trace Elements in Biological Systems

The discussion of metalloporphyrins and metalloenzymes systems has indicated the importance of certain metals in chemical reactions within living organisms. Certain elements are *essential* in that they are absolutely necessary (perhaps in large, perhaps in small quantities) for life processes. Other elements are nonessential since they play no positive role in biological systems. Obviously, determining the essentiality of an element is difficult. The term "trace element" although widely used is not precisely defined. For example, molybdenum averages about 1–2 ppm in rocks, soils, plants, and marine animals and even lower in land animals. Yet it is an essential trace metal. At the other extreme, iron, which averages about 5% in rocks and soils and 0.02–0.04% in plants and animals, might or might not be considered a "trace" metal.

Although the role of iron in various heme derivatives and zinc in carboxypeptidase and carbonic anhydrase is clear, there are many instances in which little is known of the function of the trace metal. For example, it has been known for some time that ascidians ("sea squirts") concentrate vanadium from sea water by a factor of a millionfold, but a satisfactory explanation for its role in these animals remains elusive.<sup>117</sup> There are many elements that are known to be useful but for which no specific function has yet been proved. The list of known functions is expanding rapidly, however.

The problem of toxicity is difficult to quantify. There are so many synergistic effects between various components of biological systems that it is almost impossible to define the limits of beneficial and detrimental concentrations. There is also endless variation among organisms. Truly, "one man's meat is another man's poison." The phenomenon of an essential element becoming toxic at higher than normal concentrations is not rare. Selenium is an essential element in mammals yet one of the most vexing problems is the poisoning of livestock from eating plants that concentrate this element (page 951).

The importance of trace elements is manifold and, unfortunately, previously hampered by relatively insensitive analytical methods. Good methods for determining concentrations of 1 ppm or less have been available for relatively few elements; yet these may be the optimum concentrations for a particular trace element. When the responses of living organisms are more sensitive than the laboratory "black boxes," the chemist naturally develops an inferiority complex. Fortunately, the recent development of analytical techniques capable of determining *parts per billion* has opened new vistas for the study of these problems. Some of these techniques are atomic absorption, atomic fluorescence, activation analysis, and X-ray fluorescence.

<sup>116</sup> Theil, E. C. *Ann. Rev. Biochem.* **1987**, *56*, 289. Lippard, S. J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 344.

<sup>117</sup> Kustin, K.; McLeod, G. C.; Gilbert, T. R.; Briggs, L. R., IV *Struct. Bonding (Berlin)* **1983**, *53*, 139–160. Boyd, D. W.; Kustin, K. *Adv. Inorg. Biochem.* **1984**, *6*, 312–365. Wever, R.; Kustin, K. *Adv. Inorg. Chem.* **1990**, *35*, 81–115.

### Periodic Survey of Essential and Trace Elements

The biochemistry of iron has just been discussed in some detail including the biochemical species involved, bioaccumulation, transport, storage, and toxicity. Space does not permit an extensive discussion of other elements of importance. However, a brief discussion will be presented here with a table summarizing what is currently known.

The number of elements that are known to be biologically important comprises a relatively small fraction of the 109 known elements. Natural abundance limits the availability of the elements for such use. Molybdenum ( $Z = 42$ ) is the heaviest metal, and iodine ( $Z = 53$ ) is the heaviest nonmetal of known biological importance. The metals of importance in enzymes are principally those of the first transition series, and the other elements of importance are relatively light: sodium, potassium, magnesium, calcium, carbon, nitrogen, phosphorus, oxygen, chlorine, and, of course, hydrogen.

Table 19.3 lists elements that have been found to be essential or poisonous, together with notes on biological functions and leading references that may be followed by the interested reader.<sup>118</sup> It is certain that the information in this list will be expanded as the present techniques and theory are improved.

### Biological Importance, Biological Fitness, and Relative Abundance

There are at least two ways, maybe more, of looking at the fitness of particular elements to serve particular biological functions. The more "chemical" approach is to suggest that iron functions well in cytochromes and ferredoxins because the  $\text{Fe}^{3+}/\text{Fe}^{2+}$  couple has a reduction potential in the appropriate range for life processes and, conversely, that mercury is poisonous because it binds irreversibly with enzymes, destroying their activity. Basically, a given element cannot function in a biological role unless it has specific properties. Yet chemical properties are fixed, biological systems are not, and there is the "biological" perspective of deciding how those biological systems adapted to the working materials available to them: the "fitness of the organism" to exploit fixed chemical starting materials. From this point of view, one is immediately attracted to the question: "What are the starting materials?" It is then useful to attempt to correlate biological activity with the crustal abundance of a given element.<sup>119</sup> If we look at some typical essential transition elements, we find in addition to Fe, Co, Zn, Cu, and Mo mentioned previously, V, Cr, Mn, and Ni. Representative essential metals are Na, K, Mg, and Ca, and essential nonmetals are C, N, O, P, S, and Cl (see page 953). All of these elements except Mo are relatively abundant in the earth's crust (Table 19.4).<sup>120</sup> When we look for abundant elements that are *not* essential elements, we find only three—Al, Ti, and Zr—all of which form extremely insoluble oxides at biologically reasonable pH values. No common element is toxic at levels normally encountered, though almost anything can be harmful at too high levels (cf. toxicity of the sodium chloride in sea water to freshwater plants and animals). When we consider the elements that are currently causing problems in the environment, we find that they are all extremely rare in their

<sup>118</sup> Two books have been written devoted to this general subject: Ochiai, E.-I. *General Principles of Biochemistry of the Elements*; Plenum: New York, 1987. Bowen, H. J. M. *Environmental Chemistry of the Elements*; Academic: New York, 1979.

<sup>119</sup> Huheey, J. E. In *REACTS 1973. Proceedings of the Regional Annual Chemistry Teaching Symposium*; Egolf, K.; Rodez, M. A.; Won, A. J. K.; Zidick, C., Eds.; University of Maryland: College Park, 1973; pp 52-78.

<sup>120</sup> Because almost all of the earth's crust is silicon dioxide or silicates, silicon and oxygen make up over 95% of the crust and mantle. Only about two dozen elements occur with a frequency of one atom per 10,000 atoms of silicon; these are considered "abundant" or "relatively abundant."

Table 19.3  
Function and toxicity of the elements in biological systems

Atomic number	Element	Biological functions	Toxicity <sup>a</sup>	Comments
1	Hydrogen	Molecular hydrogen metabolized by some bacteria.		Constituent of water and all organic molecules. D <sub>2</sub> O is toxic to mammals. Bacterial hydrogenases are nickel-containing enzymes. <sup>b</sup>
2	Helium	None known.		Used to replace nitrogen as an O <sub>2</sub> diluent in breathing mixtures to prevent the "bends" in high-pressure work.
3	Lithium	None known.	Slightly toxic.	Used pharmacologically to treat manic-depressive patients. <sup>c</sup>
4	Beryllium	None known.	Very toxic.	Pollution occurs from industrial smokes. There are some fears concerning poisoning from camping lantern mantles. <sup>d</sup>
5	Boron	Unknown, but essential for green algae and higher plants; probably essential ultratrace element in animals. <sup>e</sup>	Moderately toxic to plants; slightly toxic to mammals.	
6	Carbon	Synthesis of all organic molecules and of biogenetic carbonates.	Carbon monoxide is slightly toxic to plants and very toxic to mammals; CN <sup>-</sup> is very toxic to all organisms.	Carbon dioxide and CO are global pollutants from burning fossil fuels; CN <sup>-</sup> is a local pollutant of rivers near mines.
7	Nitrogen	Synthesis of proteins, nucleic acids, etc. Steps in the nitrogen cycle (organic N → NH <sub>3</sub> → NO <sub>2</sub> <sup>-</sup> → NO <sub>3</sub> <sup>-</sup> → N <sub>2</sub> → organic N) are important activities of certain microorganisms.	Ammonia is toxic at high concentrations.	Leaching of nitrogenous fertilizers from agricultural land and nitrogenous materials in sewage cause serious water pollution. Nitrogen oxides are widespread source of acid rain. <sup>f</sup>
8	Oxygen	Structural atom of water and most organic molecules in biological systems; required for respiration by most organisms.	Induces convulsions at high P <sub>O<sub>2</sub></sub> ; very toxic as ozone, superoxide, peroxide, and hydroxyl radicals. <sup>g</sup>	
9	Fluorine	Probably essential element; <sup>h</sup> used as CaF <sub>2</sub> by some mollusks.	Moderately toxic, may cause mottled teeth.	Pollution by fluoride present in superphosphate fertilizers. Ca. 1 ppm in water provides cariostatic action; <sup>i</sup> beneficial in the treatment of osteoporosis.

Table 19.3 (Continued)

Atomic number	Element	Biological functions	Toxicity <sup>o</sup>	Comments
10	Neon	None known.		
11	Sodium	Important in nerve functioning in animals. Major cation of extracellular fluid in animals.	Relatively harmless except in excessive amounts (lethal dose ca. 3 g kg <sup>-1</sup> ). Associated with some forms of hypertension.	Tolerance of and/or dependence upon sodium chloride can be an important consideration in the survival of plants and aquatic animals. This depends upon osmotic regulation rather than sodium specificity.
12	Magnesium	Essential to all organisms. Present in all chlorophylls. Has other electrochemical and enzyme-activating functions. U.S. population may be marginally deficient. <sup>l</sup>		May cause deficiencies of other elements (e.g., Fe) by the effect of the alkalinity of dolomite.
13	Aluminum	May activate succinic dehydrogenase and δ-aminolevulinic dehydrase. <sup>k</sup> The latter is involved in porphyrin synthesis. <sup>l</sup>	Moderately toxic to most plants; slightly toxic to mammals. Suggested as involved in the etiology of Alzheimer's disease and other neurologic diseases. <sup>m</sup>	Relatively inaccessible except in acidic media as a result of insolubility of Al(OH) <sub>3</sub> . Soils and waters high in Al <sup>3+</sup> and low in Mg <sup>2+</sup> and Ca <sup>2+</sup> implicated in neurologic diseases. <sup>n</sup>
14	Silicon	Essential element for growth and skeletal development in chicks and rats; <sup>o</sup> probably essential in higher plants. Used in the form of silicon dioxide for structural purposes in diatoms, some protozoa, some sponges, limpets, and one family of plants. <sup>p</sup>	Not chemically toxic, but large amounts of finely divided silicates or silica are injurious to the mammalian lung.	Long-term exposure to finely divided asbestos from construction work poses a health problem. Some evidence for a negative correlation between silicon content of drinking water and heart disease. <sup>o</sup>
15	Phosphorus	Important constituent of DNA, RNA, bones, teeth, some shells, membrane phospholipids, ADP and ATP, and metabolic intermediates.	Inorganic phosphates are relatively harmless; P <sub>4</sub> and PH <sub>3</sub> are very toxic in mammals and fish. Phosphate esters are used as insecticides (nerve poisons).	Leached from fertilizers applied to agricultural land; present in detergents and other sewage sources.
16	Sulfur	Essential element in most proteins; important in tertiary structure (through S—S links) of proteins; involved in vitamins, fat metabolism, and detoxification processes. <sup>f</sup> H <sub>2</sub> SO <sub>4</sub> in digestive fluid in ascidians ("sea squirts"); H <sub>2</sub> S replaces H <sub>2</sub> O in photosynthesis of some bacteria; H <sub>2</sub> S and S <sub>8</sub> are oxidized by other bacteria.	Elemental sulfur is highly toxic to most bacteria and fungi, relatively harmless to higher organisms. H <sub>2</sub> S is highly toxic to mammals; SO <sub>2</sub> is highly toxic.	Sulfur dioxide is a serious atmospheric pollutant, especially serious when it settles in undisturbed pockets; oxidized to H <sub>2</sub> SO <sub>4</sub> ; widespread cause of acid rain. <sup>f</sup> Sulfide minerals cause acid mine drainage.

Table 19.3 (Continued)

Atomic number	Element	Biological functions	Toxicity <sup>a</sup>	Comments
17	Chlorine	Essential for higher plants and mammals. NaCl electrolyte; HCl in digestive juices; impaired growth in infants has been linked to chloride deficiency.	Relatively harmless as Cl <sup>-</sup> . Highly toxic in oxidizing forms: Cl <sub>2</sub> , ClO <sup>-</sup> , ClO <sub>3</sub> .	
18	Argon	None known.		
19	Potassium	Essential to all organisms with the possible exception of blue-green algae; major cation in intracellular fluid in animals; essential for transmission of nerve impulse and cardiac function.	Extremely toxic to mammals when injected intravenously; emesis prevents oral toxicity.	Pollution problem possible from leaching of fertilizers from agricultural land.
20	Calcium	Essential for all organisms; used in cell walls, bones, and some shells as structural component; important electrochemically and involved in blood clotting.	Relatively harmless.	May cause deficiencies of other elements (e.g., Fe) by effect of alkalinity of limestone.
21	Scandium	None known.	Scarcely toxic.	
22	Titanium	None known, but it tends to be accumulated in siliceous tissues.	Relatively harmless.	Relatively unavailable because of insolubility of TiO <sub>2</sub> .
23	Vanadium	Essential to ascidians ("sea squirts"), which concentrate in a millionfold from sea water. Essential to chicks and rats. Deficiencies cause reduced growth, impaired reproduction and survival of young, impaired tooth and bone metabolism and feather development. <sup>9</sup> May be a factor in manic-depressive illness. <sup>7</sup>	Highly toxic to mammals if injected intravenously.	Possible pollutant from industrial smokes—may cause lung disease.
24	Chromium	Essential; involved in glucose metabolism and diabetes; potentiates effect of insulin. <sup>9</sup> Presence in glucose tolerance factor from brewer's yeast questioned. <sup>8</sup>	Highly toxic as Cr(VI); carcinogenic; moderately toxic as Cr(III).	Potential pollutant since amount used industrially is large compared with normal biological levels; normally relatively unavailable because of low solubility. Cr(VI) used in comfort cooling towers, environmental hazard.
25	Manganese	Essential to all organisms; activates numerous enzymes; deficiencies in soils lead to infertility in mammals, bone malformation in growing chicks.	Moderately toxic.	

Table 19.3 (Continued)

Atomic number	Element	Biological functions	Toxicity <sup>a</sup>	Comments
26	Iron	Essential to all organisms. See text.	Normally only slight toxicity, but excessive intake can cause siderosis and damage to organs through excessive iron storage (hemo-chromatosis). <sup>f</sup>	A very abundant element (5% of earth's crust); may not be available at high pHs.
27	Cobalt	Essential for many organisms including mammals; activates a number of enzymes; vitamin B <sub>12</sub> .	Very toxic to plants and moderately so when injected intravenously in mammals.	Extensive areas are known where low soil cobalt affects the health of grazing animals. <sup>g</sup>
28	Nickel	Essential trace element. Chicks and rats raised on deficient diet show impaired liver function and morphology; <sup>h</sup> stabilizes coiled ribosomes. Active metal in several hydrogenases and plant ureases. <sup>g</sup>	Very toxic to most plants, moderately so to mammals; carcinogenic.	Local industrial pollutant of air and water.
29	Copper	Essential to all organisms; constituent of redox enzymes and hemocyanin. <sup>h</sup>	Very toxic to most plants; highly toxic to invertebrates, moderately so to mammals.	Pollution from industrial smoke and possibly from agricultural use. Wilson's disease, genetic recessive, results in toxic increase in copper storage.
30	Zinc	Essential to all organisms; used in >70 enzymes; stabilizes coiled ribosomes. Plays a role in sexual maturation and reproduction. U.S. population marginally deficient.	Moderately to slightly toxic; orally causes vomiting and diarrhea. <sup>f</sup>	Pollution from industrial smoke may cause lung disease: use of zinc promotes cadmium pollution. Certain areas (e.g., Iran and Egypt) are zinc deficient. <sup>g</sup>
<i>Most of the heavier elements are comparatively unimportant biologically. Some of the exceptions are:</i>				
33	Arsenic	Essential ultratrace element in red algae, chick, rat, pig, goat, and probably humans. Deficiency results in depressed growth and increased mortality.	Moderately toxic to plants, highly toxic to mammals.	Serious pollution problems in some areas: sources include mining, burning coal, impure sulfuric acid, insecticides, and herbicides.
34	Selenium	Essential to mammals and some higher plants. Component of glutathione peroxidase, protects against free-radical oxidant stressors; protects against heavy ("soft") metal ions. <sup>e,h,i</sup>	Moderately toxic to plants, highly toxic to mammals.	Livestock grown on soils high in selenium are poisoned by eating <i>Astragalus</i> ("loco-weed"), which concentrates it; sheep grown on land deficient in selenium develop "white muscle disease." Deficiency of selenium involved in Keshan disease in China. <sup>v</sup>
35	Bromine	May be essential in red algae and mammals.	Nontoxic except in oxidizing forms, e.g., Br <sub>2</sub> .	Function unknown, but found in the molluscan pigment, royal purple.

Table 19.3 (Continued)

Atomic number	Element	Biological functions	Toxicity <sup>a</sup>	Comments
37	Rubidium	None known.		Suppresses depressive phase of manic-depressive illness. <sup>c,w</sup>
42	Molybdenum	Essential to all organisms with the possible exception of green algae; used in enzymes connected with nitrogen fixation and nitrate reduction.	Moderately toxic and antagonistic to copper—molybdenum excesses in pasturage can cause copper deficiency. <sup>f</sup> Excessive exposure in parts of U.S.S.R. associated with a gout-like syndrome. <sup>o</sup>	Pollution from industrial smoke may be linked with lung disease.
48	Cadmium	Weak evidence for ultratrace essentiality in rats. <sup>e</sup>	Moderately toxic to all organisms; a cumulative poison in mammals, causing renal failure; possibly linked with hypertension in man.	Has caused serious disease ("itai itai") in Japan from pollution. May also pose pollution problem associated with industrial use of zinc, e.g., galvanization.
50	Tin	Weak evidence for ultratrace essentiality in rats. <sup>e</sup>	Organotin compounds used as bacteriostats and fungistats; its use in anti-foulant boat paints now discouraged because of danger to estuarine and marine life.	
53	Iodine	Essential in many organisms; thyroxine important in metabolism and growth regulation, amphibian metamorphosis.	Scarcely toxic as the iodide; low iodide availability in certain areas increases the incidence of goiter, largely eliminated by the use of iodized salt. Elemental iodine is toxic like Cl <sub>2</sub> and Br <sub>2</sub> .	Concentrated up to 2.5 ppt by some marine algae.
74	Tungsten	Rare.	Molybdenum antagonist.	Found in enzymes in thermophilic (thermal vent) bacteria. <sup>x</sup>
78	Platinum	None known.	Moderately toxic to mammals by intravenous injection.	<i>cis</i> -Diamminedichloroplatinum(II) used as an anti-cancer drug. <sup>y</sup>
79	Gold	None known.	Scarcely toxic.	Some use in the treatment of arthritis. <sup>y,z</sup> Concentrated up to 10% of dry weight by certain algae. <sup>aa</sup>
80	Mercury	None known.	Very toxic to fungi and green plants, and to mammals if in soluble form; a cumulative poison in mammals.	Serious pollution problems from use of organomercurials as fungicides and from industrial uses of mercury.

Table 19.3 (Continued)

Atomic number	Element	Biological functions	Toxicity <sup>a</sup>	Comments
82	Lead	None known.	Very toxic to most plants; cumulative poison in mammals. Inhibits $\delta$ -aminolevulinic dehydrase and thus hemoglobin synthesis in mammals (see A1). One of the symptoms of lead poisoning is anemia. Toxic to central nervous system.	Worldwide pollutant of the atmosphere, concentrated in urban areas from the combustion of tetraethyl lead in gasoline; local pollutant from mines; some poisoning from lead-based paint pigments.
88-103	Radium and Actinides	None known.	May be concentrated in organisms and toxic as a result of radioactivity.	Potential pollutants from use of nuclear fuel as energy source.
92	Uranium	None known.	U <sup>VI</sup> is reduced to U <sup>IV</sup> by iron-reducing bacteria. May be important in the biogeochemical deposit of U. <sup>bb</sup>	Iron-reducing bacteria might be of use in decontaminating (precipitating) uranium-polluted water. <sup>bb</sup>

<sup>a</sup> Toxic effects often are exhibited only at concentrations above those occurring naturally in the environment. See p 952.

<sup>b</sup> Crabtree, R. H. *The Organometallic Chemistry of the Transition Metals*; Wiley: New York, 1988; pp 400-404.

<sup>c</sup> Fieve, R. R.; Jamison, K. R.; Goodnick, P. J. In *Metal Ions in Neurology and Psychiatry*; Gabay, S.; Harris, J.; Ho, B. T., Eds.; Alan R. Liss: New York, 1985; pp 107-120.

<sup>d</sup> Griggs, K. *Science* **1973**, *181*, 842.

<sup>e</sup> Nielsen, F. H. *Ann. Rev. Nutr.* **1984**, *4*, 21.

<sup>f</sup> Mohnen, V. A. *Sci. Amer.* **1988**, *259*(2), 30.

<sup>g</sup> Fridovich, I. *Am. Sci.* **1975**, *63*, 54. *Biological and Clinical Aspects of Superoxide and Superoxide Dismutase*; Bannister, W. H.; Bannister, J. V., Eds.; Elsevier-North Holland: New York, 1980.

<sup>h</sup> Schwartz, K. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974**, *33*, 1748.

<sup>i</sup> Schamschula, R. G.; Barmes, D. E. *Ann. Rev. Nutr.* **1981**, *1*, 427.

<sup>j</sup> Raloff, J. *Sci. News* **1988**, *133*, 356.

<sup>k</sup> Bowen, H. J. M. *Environmental Chemistry of the Elements*; Academic: New York, 1979.

<sup>l</sup> Harper, H. A. *Review of Physiological Chemistry*; Lange: Los Altos, CA, 1971.

<sup>m</sup> Eichorn, G. L.; Butzow, J. J.; Clark, P.; von Hahn, H. P.; Rao, G.; Heim, J. M.; Tarian, E.; Crapper, D. R.; Karlik, S. J. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; ACS Symposium Series 140; American Chemical Society: Washington, DC, 1980; Chapter 4. Wurtman, R. J. *Sci. Amer.* **1985**, *252*(1), 62. MacDonald, T. L.; Humphreys, W. G.; Martin, R. B. *Science* **1987**, *236*, 183.

<sup>n</sup> Gadjusek, D. C. *N. Engl. J. Med.* **1985**, *312*, 714.

<sup>o</sup> Mertz, W. *Science* **1981**, *213*, 1332.

<sup>p</sup> Volcani, B. E. In *Silicon and Siliceous Structures in Biological Systems*; Simpson, T. L.; Volcani, B. E., Eds.; Springer-Verlag: Berlin, 1981.

<sup>q</sup> Nielsen, F. H.; Mertz, W. In *Present Knowledge in Nutrition*, 5th ed.; Olson, R. E.; Vroquist, H. P.; Chichester, C. O.; Darby, W. J.; Kolbye, A. C., Jr.; Stalvey, R. M., Eds.; Nutrition Foundation: Washington, DC, 1984; Chapter 42.

<sup>r</sup> Naylor, G. J. In *Metal Ions in Neurology and Psychiatry*; Gabay, S.; Harris, J.; Ho, B. T., Eds.; Alan R. Liss: New York, 1985; pp 91-105.

<sup>s</sup> Haylock, S. J.; Buckley, P. D.; Blackwell, L. F. *J. Inorg. Biochem.* **1983**, *19*, 105.

<sup>t</sup> Rollinson, C. L.; Enig, M. G. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; Grayson, M., Ed.; Wiley: New York, 1981; Vol. 15, pp 570-603.

<sup>u</sup> *Copper in Animals and Man*; Howell, J. M.; Gawthorne, J. M., Eds.; CRC: Boca Raton, FL, 1987. Linder, M. C. *Biochemistry of Copper*; Plenum: New York, 1991.

<sup>v</sup> Levander, O. A. *Ann. Rev. Nutr.* **1987**, *7*, 227. Odum, J. D. *Struct. Bonding (Berlin)* **1983**, *54*, 1.

<sup>w</sup> Fieve, R. R.; Jamison, K. R. *Mod. Probl. Pharmacopsychiatry* **1982**, *18*, 145.

<sup>x</sup> George, G. N.; Prince, R. C.; Mukund, S.; Adams, M. W. W. *J. Am. Chem. Soc.* **1992**, *114*, 3521-3523. Adams, M. W. W. *Adv. Inorg. Chem.* **1992**, *38*, 341-396.

<sup>y</sup> *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1985.

<sup>z</sup> Corey, E. J.; Mehrotra, M. M.; Khan, A. U. *Science* **1987**, *236*, 68.

<sup>aa</sup> Watkins, J. W., II; Elder, R. C.; Greene, B.; Darnall, D. W. *Inorg. Chem.* **1987**, *26*, 1147.

<sup>bb</sup> Lovley, D. R.; Phillips, E. J. P.; Gorby, Y. A.; Landa, E. R. *Nature* **1991**, *350*, 413-416.

**Table 19.4**  
Abundances of the elements  
in the earth's crust, rivers,  
and sea water<sup>a</sup>

Element	Earth's crust		River water mg L <sup>-1</sup>	Ocean water mg L <sup>-1</sup>
	g kg <sup>-1</sup>	atoms/10 <sup>4</sup> atoms Si		
Hydrogen			1.119 × 10 <sup>5</sup>	1.078 × 10 <sup>5</sup>
Helium				7.2 × 10 <sup>-6</sup>
Lithium	0.02	3	0.003	0.18
Beryllium	0.028	3	< 1 × 10 <sup>-4</sup>	6 × 10 <sup>-7</sup>
Boron	0.01	1	0.01	4.5
Carbon <sup>b</sup>			1.2	28
Nitrogen			0.25 <sup>c</sup>	0.5 <sup>c,d</sup>
Oxygen	474 <sup>e</sup>	9600	8.8 × 10 <sup>5</sup>	8.56 × 10 <sup>5</sup>
Fluorine	0.625	32.7	0.1	1.4
Neon				0.00012
Sodium	24	1040	9	1.105 × 10 <sup>4</sup>
Magnesium	20	820	4.1	1.326 × 10 <sup>3</sup>
Aluminum	82	3020	0.4	0.005 <sup>d</sup>
Silicon	282	10,000	4	1 <sup>d</sup>
Phosphorus	1	32	0.02	0.07 <sup>d</sup>
Sulfur	0.26	8.1	3.7	928
Chlorine	0.13	3.6	8	1.987 × 10 <sup>4</sup>
Argon				0.45
Potassium	24	610	2.3	416
Calcium	42	1040	1.5	4.22
Scandium	0.022	0.48	4 × 10 <sup>-6</sup>	1.5 × 10 <sup>-6</sup>
Titanium	5.7	120	0.003	0.001
Vanadium	0.135	2.64	0.001	0.0015
Chromium	0.1	2	0.001	0.0006 <sup>d</sup>
Manganese	0.95	17	~0.005	0.002 <sup>d</sup>
Iron	56	1000	0.67	0.003 <sup>d</sup>
Cobalt	0.025	0.42	0.0002	8 × 10 <sup>-5d</sup>
Nickel	0.075	1.3	0.0003	0.002
Copper	0.055	0.86	0.005	0.003 <sup>d</sup>
Zinc	0.070	1.1	0.01	0.005
Gallium	0.015	0.21	1 × 10 <sup>-4</sup>	3 × 10 <sup>-5</sup>
Germanium	0.0015	0.021		6 × 10 <sup>-5</sup>
Arsenic	0.0018	0.024	~0.001	0.0023
Selenium	5 × 10 <sup>-5</sup>	6 × 10 <sup>-4</sup>	0.0002	0.00045
Bromine	0.0025	0.031	~0.02	68
Krypton				0.00021
Rubidium	0.09	1	0.001	0.12
Strontium	0.375	4.26	0.050	8.5
Yttrium	0.033	0.37	0.04	1.3 × 10 <sup>-5</sup>
Zirconium	0.165	1.80	0.003	2.6 × 10 <sup>-6</sup>
Niobium	0.02	0.2		1 × 10 <sup>-6</sup>
Molybdenum	0.0015	0.016	0.001	0.01
Technetium				
Ruthenium	1 × 10 <sup>-6e</sup>	1 × 10 <sup>-5e</sup>		7 × 10 <sup>-7</sup>
Rhodium	2 × 10 <sup>-7e</sup>	2 × 10 <sup>-6e</sup>		
Palladium	8 × 10 <sup>-7e</sup>	8 × 10 <sup>-6e</sup>		
Silver	7 × 10 <sup>-5</sup>	6 × 10 <sup>-4</sup>	0.0003	0.0001
Cadmium	0.0002	0.0018		5 × 10 <sup>-5</sup>
Indium	0.0001	9 × 10 <sup>-4</sup>		1 × 10 <sup>-7</sup>

Table 19.4 (Continued)

Abundances of the elements in the earth's crust, rivers, and sea water<sup>a</sup>

Element	Earth's crust		River water mg L <sup>-1</sup>	Ocean water mg L <sup>-1</sup>
	g kg <sup>-1</sup>	atoms/10 <sup>4</sup> atoms Si		
Tin	0.002	0.02	4 × 10 <sup>-5</sup>	1 × 10 <sup>-5</sup>
Antimony	0.0002	0.002	0.001	0.0002
Tellurium	4 × 10 <sup>-6c</sup>	4 × 10 <sup>-5c</sup>		
Iodine	0.0005	0.004	~0.005	0.06 <sup>c</sup>
Xenon				5 × 10 <sup>-6</sup>
Cesium	0.003	0.02	5 × 10 <sup>-5</sup>	0.0005
Barium	0.425	3.08	0.01	0.03
Lanthanum	0.03	0.2	0.0002	3.4 × 10 <sup>-6</sup>
Cerium	0.06	0.4		1.2 × 10 <sup>-6</sup>
Praseodymium	0.0082	0.058		6 × 10 <sup>-7</sup>
Neodymium	0.028	0.19		2.8 × 10 <sup>-6</sup>
Promethium				
Samarium	0.006	0.04		4.5 × 10 <sup>-7</sup>
Europium	0.0012	0.08		1.3 × 10 <sup>-7</sup>
Gadolinium	0.0054	0.034		7 × 10 <sup>-7</sup>
Terbium	0.0009	0.006		1.4 × 10 <sup>-7</sup>
Dysprosium	0.003	0.02		9.1 × 10 <sup>-7</sup>
Holmium	0.0012	0.007		2 × 10 <sup>-7</sup>
Erbium	0.0028	0.017		9 × 10 <sup>-7</sup>
Thulium	0.0005	0.003		2 × 10 <sup>-7</sup>
Ytterbium	0.003	0.02		8 × 10 <sup>-7</sup>
Hafnium	0.003	0.02		
Tantalum	0.002	0.01		2 × 10 <sup>-5</sup>
Tungsten	0.0015	0.008	3 × 10 <sup>-5</sup>	0.00012
Rhenium	5 × 10 <sup>-6</sup>	3 × 10 <sup>-5</sup>		1 × 10 <sup>-6</sup>
Osmium	1 × 10 <sup>-8e</sup>	5 × 10 <sup>-8e</sup>		
Iridium	1 × 10 <sup>-8e</sup>	5 × 10 <sup>-8e</sup>		
Gold	4 × 10 <sup>-6</sup>	2 × 10 <sup>-5</sup>	2 × 10 <sup>-6</sup>	5 × 10 <sup>-5d</sup>
Mercury	8 × 10 <sup>-5</sup>	4 × 10 <sup>-4</sup>	7 × 10 <sup>-5</sup>	5 × 10 <sup>-5d</sup>
Thallium	0.00045	0.0022		1 × 10 <sup>-6</sup>
Lead	0.0125	0.06	0.003	3 × 10 <sup>-5d</sup>
Bismuth	0.00014	7 × 10 <sup>-4</sup>		2 × 10 <sup>-5</sup>
Polonium				2 × 10 <sup>-14</sup>
Astatine				
Radon			2 × 10 <sup>-16</sup>	6 × 10 <sup>-16d</sup>
Francium				
Radium			4 × 10 <sup>-10</sup>	1 × 10 <sup>-10d</sup>
Actinium				
Thorium	0.0096	0.041	0.0001	4 × 10 <sup>-8d</sup>
Protactinium				2 × 10 <sup>-19d</sup>
Uranium	0.0027	0.011	4 × 10 <sup>-5</sup>	0.0033

<sup>a</sup> Riley, J. P.; Chester, R. *Introduction to Marine Chemistry*; Academic: New York, 1971, except as noted.<sup>b</sup> Inorganic carbon.<sup>c</sup> Combined nitrogen; about 15 mg L<sup>-1</sup> dissolved N<sub>2</sub>.<sup>d</sup> Considerable variation occurs.<sup>e</sup> Bowen, H. J. M. *Environmental Chemistry of the Elements*; Academic: New York, 1979; Chapter 13.

crustal abundances: Pb (0.08), Cd (0.0018), and Hg ( $4 \times 10^{-5}$ ).<sup>121</sup> The conclusion is inescapable: *Life evolved utilizing those elements that were abundant and available to it and became dependent upon them.* Those elements that are rare were not used by living systems because they were not available; neither did these systems evolve mechanisms to cope with them.

A closely related corollary of this thesis is that many elements that are *essential* when occurring at ambient concentrations are *toxic* at higher concentrations (and, of course, cause deficiency symptoms at lower concentrations). Interesting examples are copper, selenium, and even sodium—all oceanic organisms are adapted to live in 0.6 M NaCl and our blood has been described as a sample of the primeval seas. Yet too high concentrations of NaCl are toxic through simple hypertonicity, i.e., osmotic dehydration. Selenium is a problem when it is either too rare or too abundant in the environment: Livestock grown on selenium-deficient pasture suffer from "white muscle disease"; when grazing plants (*Astragalus*, "locoweed") that concentrate selenium from the soil, they suffer central nervous system toxinoses. Copper is essential to many of the redox enzymes necessary to both plants and animals; yet too much copper is severely toxic to most green plants.

There is an interesting group of trace elements, called *ultratrace elements* because they are needed, if at all, at not more than 1 ppm in food, probably less than 50 ppb. These ultratrace elements include arsenic and nickel, certainly essential at these low concentrations, and cadmium and lead, probably *not* essential. Many of these elements (e.g., Ni, As, Cd, and Pb) are quite toxic at any concentration much above an ultratrace level. Naturally, determination of the essentiality of an ultratrace element is even more difficult than for ordinary trace elements.<sup>122</sup> Life used and adapted to those elements and those concentrations available to it (see next section). When humans started mining, using, and releasing these elements into the environment, the ecosystem was faced with hazards it had never before encountered, and to which it had, therefore, never adapted.

A slightly different view of this idea has been presented by Egami,<sup>123</sup> who has pointed out that the three enzyme systems in the most primitive bacterium, *Clostridium*, are involved in electron transfer (e.g., ferredoxin), reduction of small molecules (e.g., nitrogenase), and hydrolysis (e.g., carboxypeptidase and carbonic anhydrase), and employ, respectively, iron, molybdenum, and zinc, the three most common transition elements in sea water. It is postulated that these enzyme systems arose from protoenzymes that utilized these most common metals in primitive seas. One puzzle is copper, which is fairly abundant in sea water, and although it has been thought to be essential for all organisms, apparently no requirement for it has been found in strict anaerobes. Egami postulates that copper, with a positive standard reduction potential, was incorporated into living systems only when the atmosphere shifted from reducing ( $\text{CH}_4$ ,  $\text{H}_2$ ,  $\text{NH}_3$ ) to oxidizing ( $\text{O}_2$ ).<sup>124</sup> This indicates the importance of considering changes that have occurred with time (including the advent of

<sup>121</sup> All figures in atoms per 10,000 atoms silicon. For a discussion of Pb, Cd, and Hg in the environment, in diet, and their toxicity, see Choudury, B. A.; Chandra, R. K. *Prog. Food Nutr. Sci.* **1987**, *11*, 55.

<sup>122</sup> Nielsen, F. H. *Ann. Rev. Nutr.* **1984**, *4*, 21.

<sup>123</sup> Egami, F. *J. Mol. Evol.* **1974**, *4*, 113; *J. Biochem.* **1975**, *77*, 1165.

<sup>124</sup> See Broda, E. *J. Mol. Evol.* **1975**, *7*, 87, for a discussion of this and related questions concerning the primitive biosphere.

### Adaptations to Natural Abundances<sup>125</sup>

terrestrialism) and perhaps considering the microabundance of the various elements in different habitats.

When the abundance of an element is unusually high or unusually low, organisms develop mechanisms to handle the stress. The first documented examples were the presence of "indicator species" (plants) that grow where soils contain an unusually high concentration of a metal. For example, the sea pink, *Armeria maritima*, has been used in North Wales as an indicator of copper deposits. In one extreme case, the drainage from the copper deposits has concentrated in a bog to an extent of 20,000–30,000 ppm, and the sea pink flourishes. Closely related is the adaptation of various plants to exceptionally high concentrations of various heavy metals in mine dumps and tailings. Not only have some species adapted to extremely high concentrations of normally toxic metals, but they have also evolved a high level of self-fertilization to prevent pollination and gene exchange with nearby populations that are not metal tolerant.

Some of the chemolithotropic bacteria discussed earlier in this chapter illustrate these ideas. In undisturbed situations their habitat is extremely restricted. During the process of mining, however, large surfaces of the appropriate metal sulfide are created and oxidized both in the mine and in the tailings with resultant leaching. This creates a favorable habitat for exploitation by the bacteria. One unfavorable result is the lowering of the pH and the solubilization of metals, usually toxic, into the drainage system. On the other hand, the isolation and selection of productive strains from such sites, and their controlled application, may lead to useful biometallurgical methods of extraction of metals from low-grade ores (see Chapter 10).<sup>126</sup>

The hydrothermal vents discussed previously provide a parallel, *natural* environment with unusually large amounts of various metals—iron, copper, zinc—dissolved from the crustal rocks by the superheated water. It will be of interest to learn how the animals in the hydrothermal ecosystem have developed mechanisms to avoid toxicity from these metals. Another source of possible toxicity, hydrogen sulfide, is somewhat better understood. Hydrogen sulfide is comparable to the cyanide ion in its toxicity towards respiration. The vent organisms have evolved a variety of mechanisms to prevent sulfide toxicity. One of the more interesting is that of the tube worm, *Riftia pachyptila*. Its hemoglobin has a molecular weight of about two million, with an extremely high affinity for dioxygen (recall that the vent waters are anoxic) and a second, high-affinity site to bind sulfide. This second site serves the dual purpose of protecting the tube worm's cytochrome *c* oxidase from sulfide poisoning and protecting the sulfide from premature oxidation. Instead, both the dioxygen and sulfide are transported to symbiotic bacteria that metabolize them to drive the synthesis of ATP and carbohydrates.<sup>127</sup>

At the other extreme are adaptations to very low concentrations of a particular element. We have already seen mechanisms directed towards the sequestration of iron when it is present in small amounts. The ability to *detect* extremely small amounts of an element can be a useful adaptation for an animal if that element is important to it. For example, hermit crabs recognize shells suitable for occupation not only by tactile

<sup>125</sup> Farago, M. E. In *Frontiers in Bioinorganic Chemistry*; Xavier, A. V., Ed.; VCH: Weinheim, 1986; pp 106–122. Ochiai, E.-I. *General Principles of Biochemistry of the Elements*; Plenum: New York, 1987; pp 379–395.

<sup>126</sup> Rossi, G. *Biohydrometallurgy*; McGraw-Hill: New York, 1990.

<sup>127</sup> Childress, J. J.; Felbeck, H.; Somero, G. N. *Sci. Am.* 1987, 256(5), 115–120.

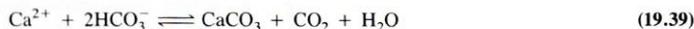
stimuli but apparently also by the minute amount of calcium carbonate that is dissolved in the water around a shell. They can readily distinguish natural shells ( $\text{CaCO}_3$ ), calcium-bearing replicas ( $\text{CaSO}_4$ ), and naturally containing calcium minerals (calcite, aragonite, and gypsum) from non-calcium minerals (celestite,  $\text{SrSO}_4$ ; rhodochrosite,  $\text{MnCO}_3$ ; siderite,  $\text{FeCO}_3$ ; and quartz,  $\text{SiO}_2$ ).<sup>128</sup> Inasmuch as the solubility product of calcium carbonate is only  $10^{-8}$ , the concentration of calcium detected by the hermit crab is of the order of 4 ppm or less. Almost nothing is known about the chemical mechanisms used by organisms in detecting various elements.

### Biochemistry of the Nonmetals

#### Structural Uses of Nonmetals<sup>129</sup>

Many of the nonmetals such as hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, chlorine, and iodine are essential elements, and most are used in quantities far beyond the trace levels. Nevertheless, most of the chemistry of these elements in biological systems is more closely associated with organic chemistry than with inorganic chemistry.

There are three important minerals used by organisms to form hard tissues such as bones and shells. The most widespread of these is calcium carbonate, an important structural component in animals ranging from Protozoa to Mollusca and Echinodermata. It is also a minor component of vertebrate bones. Its widespread use is probably related to the generally uniform distribution of dissolved calcium bicarbonate. Animals employing calcium carbonate are most abundant in fresh waters containing large amounts of calcium and magnesium ("hard water") and in warm, shallow seas where the partial pressure of carbon dioxide is low (e.g., the formation of coral reefs by coelenterates). The successful precipitation of calcium carbonate depends upon the equilibrium:



and is favored by high  $[\text{Ca}^{2+}]$  and low  $[\text{CO}_2]$ . Nevertheless, organisms exhibit a remarkable ability to deposit calcium carbonate from hostile environments. A few freshwater clams and snails are able to build reasonably large and thick shells in lakes with a pH of 5.7–6.0 and as little as 1.1 ppm dissolved calcium carbonate.<sup>130</sup>

It is of interest that two thermodynamically unstable forms of calcium carbonate, aragonite and vaterite, are found in living organisms as well as the more stable calcite. There appears to be no simple explanation for the distribution of the different forms in the various species.

Tissues containing silica are found in the primitive algal phyla Pyrrhophyta (dinoflagellates) and Chrysophyta (diatoms and silicoflagellates). One family of higher plants, the Equisetaceae, or horsetails, contains gritty deposits of silica—hence their

<sup>128</sup> Mesce, K. A. *Science* **1982**, *215*, 993.

<sup>129</sup> Vincent, J. F. *Structural Biomaterials*; Wiley: New York, 1982. Williams, R. J. P. In *Frontiers in Bioinorganic Chemistry*; Xavier, A. V., Ed.; VCH: Weinheim, 1985; pp 431–440. Webb, J.; St. Pierre, T. G.; Dickson, D. P. E.; Mann, S.; Williams, R. J. P.; Perry, C. C.; Grime, C. C.; Watt, F.; Runham, N. W. *Ibid.* pp 441–452.

<sup>130</sup> For a discussion of this point as well as other examples of organisms living on limited concentrations of nutrients, see Allee, A. C.; Emerson, E. E.; Park, O.; Park, T.; Schmidt, K. P. *Principles of Animal Ecology*; W. B. Saunders: Philadelphia, 1949; pp 164–167; pp 189–206. Pennak, R. W. *Freshwater Invertebrates of the United States*; Ronald: New York, 1953; p 681; p 705f.

name "scouring rushes." Some Protozoa (radiolarians), Gastropoda (limpets), and Porifera (glass sponges) employ silica as a structural component. Silicon is an essential trace element in chicks and rats<sup>131</sup> and is probably necessary for proper bone growth in all higher animals.

The third type of compound used extensively as a structural component is apatite,  $\text{Ca}_5(\text{PO}_4)_3\text{X}$ . Hydroxyapatite ( $\text{X} = \text{OH}$ ) is the major component of bone tissue in the vertebrate skeleton. It is also the principal strengthening material in teeth. Partial formation of fluorapatite ( $\text{X} = \text{F}$ ) from application of fluorides strengthens the structure and causes it to be less soluble in the acid formed from fermenting organic material, hence a reduction of caries. Fluorapatite is also used structurally in certain Brachiopod shells.

### Medicinal Chemistry

#### Antibiotics and Related Compounds

The suggested antibiotic action of transferrin is typical of the possible action of several antibiotics in tying up essential metal ions. Streptomycin, aspergillid acid, usnic acid, the tetracyclines, and other antibiotics are known to have chelating properties. Presumably some antibiotics are delicately balanced so as to be able to compete successfully with the metal-binding agents of the bacteria while not disturbing the metal processing by the host. There is evidence that at least some bacteria have developed resistance to antibiotics through the development of altered enzyme systems that can compete successfully with the antibiotic.<sup>132</sup> The action of the antibiotic need not be a simple competitive one. The chelating properties of the antibiotic may be used in metal transport across membranes or to attach the antibiotic to a specific site from which it can interfere with the growth of bacteria.

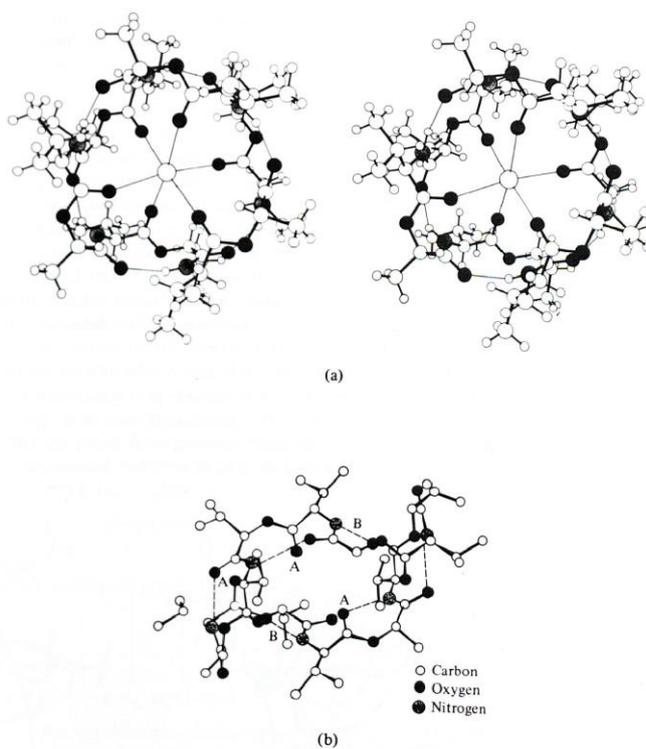
The behavior of valinomycin is typical of a group known as "ionophore antibiotics."<sup>133</sup> These compounds resemble the crown ethers and cryptates (Chapter 12) by having several oxygen or nitrogen atoms spaced along a chain or ring that can wrap around a metal ion (Fig. 19.29a). These antibiotics are useless in humans because they are toxic to mammalian cells, but some of them find use in treating coccidiosis in chickens. The toxicity arises from the ion-transporting ability. Cells become "leaky" with respect to potassium, which is transported across the cell membrane by valinomycin. In the absence of a metal ion, valinomycin has a quite different conformation (Fig. 19.29b), one stabilized by hydrogen bonds between amide and carbonyl groups. It has been postulated<sup>134</sup> that the potassium ion can initially coordinate to the four free carbonyl groups (A) and that this can provide sufficient stabilization to break two of the weaker hydrogen bonds (B). This provides two additional carbonyl groups to coordinate and complete the change in conformation to that shown in Fig. 19.29a. Such a stepwise mechanism would indicate that the whole system is a balanced one and that the reverse process can be readily triggered by a change in environment such as at a membrane surface or if there is a change in hydrogen bonding competition.

<sup>131</sup> Carlisle, E. M. *Science* **1972**, *178*, 619; *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974**, *33*, 1758.

<sup>132</sup> Woodruff, H. B.; Miller, I. M. In *Metabolic Inhibitors*; Hochster, R. M.; Quastel, J. H., Eds.; Academic: New York, 1963; Vol. II, Chapter 17.

<sup>133</sup> Ochiai, E-I. *General Principles of Biochemistry of the Elements*; Plenum, New York, 1987; pp 254-265.

<sup>134</sup> Smith, G. D.; Duax, W. L.; Langs, D. A.; DeTitta, G. T.; Edmonds, J. W.; Rohrer, D. C.; Weeks, C. M. *J. Am. Chem. Soc.* **1976**, *97*, 7242.



**Fig. 19.29** (a) Molecular structure of valinomycin coordinated to the  $K^+$  ion. (b) Molecular structure of the free valinomycin molecule. The carbonyl groups marked A are free to coordinate to  $K^+$ . Hydrogen bonding is shown by dashed lines, with those marked B thought to be most susceptible to breaking. [From Neupert-Laves, K.; Dobler, M. *Helv. Chim. Acta* 1975, 58, 432; Smith G. D.; Duax, W. L.; Langs, D. A.; DeTitta, G. T.; Edmonds, J. W.; Rohrer, D. C.; Weeks, C. M. *J. Am. Chem. Soc.* 1976, 97, 7242. Reproduced with permission.]

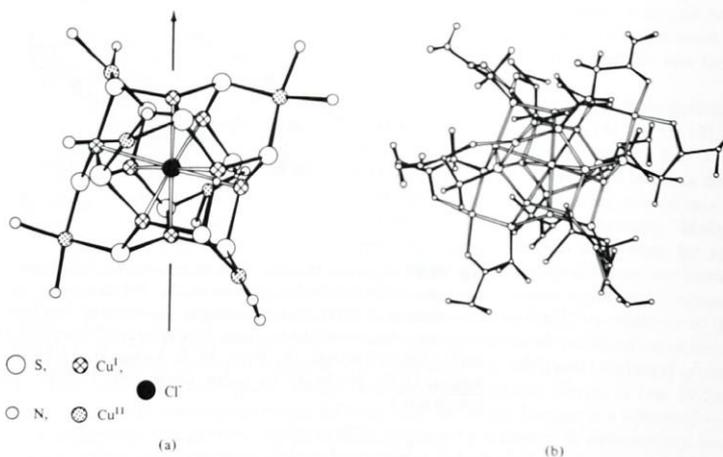
The tetracyclines form an important group of antibiotics. The activity appears to result from their ability to chelate metal ions since the extent of antibacterial activity parallels the ability to form stable chelates. The metal in question appears to be magnesium or calcium since the addition of large amounts of magnesium can inhibit the antibiotic effects. In addition, it is known that in blood plasma the tetracyclines exist as calcium and magnesium complexes.<sup>135</sup>

<sup>135</sup> Lambs, L.; Decock-Le Révérend, B.; Kozłowski, H.; Berthon, G. *Inorg. Chem.* 1988, 27, 3001.

### Chelate Therapy

We have seen previously that chelating agents can be used therapeutically to treat problems caused by the presence of toxic elements. We have also seen that an essential element can be toxic if present in too great a quantity. This is the case in Wilson's disease (hepatolenticular degeneration), a genetic disease involving the buildup of excessive quantities of copper in the body. Many chelating agents have been used to remove the excess copper, but one of the best is D-penicillamine,  $\text{HSC}(\text{CH}_3)_2\text{CH}(\text{NH}_2)\text{COOH}$ . This chelating agent forms a complex with copper ions that is colored an intense purple and, surprisingly, has a molecular weight of 2600. Another surprising finding is that the complex will not form unless chloride or bromide ions are present and the isolated complex always contains a small amount of halide. These puzzling facts were explained when the X-ray crystal structure was done.<sup>136</sup> The structure (Fig. 19.30) consists of a central halide ion surrounded by eight copper(I) atoms bridged by sulfur ligands. These are in turn coordinated to six copper(II) atoms. Finally, the chelating amino groups of the penicillamine complete the coordination sphere of the copper(II) atoms.

As we have seen, the body has essentially no means of eliminating iron, so an excessive intake of iron causes various problems known as siderosis. Chelating agents are used to treat the excessive buildup of iron. In many cases the chelates resemble or are identical to the analogous compounds used by bacteria to chelate iron. Thus desferrioxamine B is the drug of choice for African siderosis.<sup>137</sup> The ideal chelating



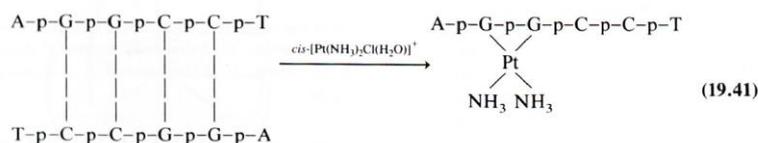
**Fig. 19.30** Molecular structure of copper complex of D-penicillamine. The  $[\text{Cu}_8\text{Cu}_6(\text{penicillamate})_2\text{Cl}]$  ion: (a) the central cluster of Cu and ligating atoms only; (b) the entire ion with the central cluster oriented as in (a). [From Birker, P. J. M. W. L.; Freeman, H. C. *Chem. Commun.* **1976**, 312. Reproduced with permission.]

<sup>136</sup> Birker, P. J. M. W. L.; Freeman, H. C.; *Chem. Commun.* **1976**, 312.

<sup>137</sup> Andersen, W. F. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; ACS Symposium Series 140; American Chemical Society: Washington, DC, 1980; Chapter 15. Gordeuk, V. R.; Bacon, B. R.; Brittenham, G. M. *Ann. Rev. Nutr.* **1987**, 7, 485.

agent will be specific for the metal to be detoxified since a more general chelating agent is apt to cause problems by altering the balance of other essential metals. The concepts of hard and soft metal ions and ligands can be used to aid in this process of designing therapeutic chelators.<sup>138</sup>

A slightly different mode of therapy involves the use of *cis*-diamminedichloroplatinum(II), Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>, and related bis(amine) complexes in the treatment of cancer. The exact action of the drug is not known, but only the *cis* isomer is active at low concentrations, not the *trans* isomer. It is thought that the platinum binds to DNA, with the chloride ligands first being replaced by water molecules and then by a DNA base such as guanine.<sup>139</sup> Studies *in vitro* with nucleotide bases as well as theoretical calculations<sup>140</sup> indicate that the N7 position of guanine is the favored site for platinum coordination. The *cis*-diammine moiety can bind to groups about 280 pm apart, and *in vitro* studies with di- and polynucleosides, as well as *in vivo* studies on DNA support the hypothesis that the most important interaction is *intrastrand* linking of two adjacent guanine bases on the DNA chain by the platinum atom (see Fig. 19.31).<sup>141</sup> The *trans* isomer can bond to groups about 400 pm apart that approach the platinum atom from opposite directions, and it is chemotherapeutically inactive. The binding of cisplatin to DNA would seriously interfere with the ability of the guanine bases to undergo Watson-Crick base pairing. Thus when a self-complementary oligomer (a portion of a DNA chain) reacts with the *cis* isomer, two adjacent guanines are bound and Watson-Crick base pairing is disrupted.<sup>142</sup>



For *cis*-diamminedichloroplatinum(II) to work according to the proposed mechanism, it must hydrolyze *in the right place*; if it hydrolyzes in the blood before it gets to the chromosomes within the cell, it will be more likely to react with a nontarget species. Fortunately for the stability of the complex, the blood is approximately 0.1 M in chloride ion, forcing the hydrolysis equilibrium (Eq. 19.40) back to the chloro complex. Once the drug crosses the cell membrane into the cytoplasm, it finds a

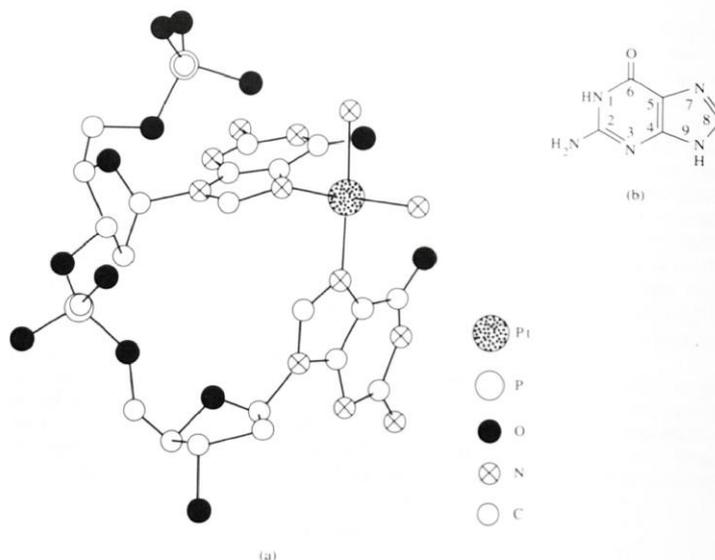
<sup>138</sup> Pitt, C. G.; Martell, A. E. In *Inorganic Chemistry in Biology and Medicine*, Martell, A. E., Ed.; ACS Symposium Series 140; American Chemical Society: Washington, DC, 1980; Chapter 17. Bulman, R. A. *Struct. Bonding (Berlin)* **1987**, 67, 91.

<sup>139</sup> The kinetics of this substitution reaction is discussed in Chapter 13.

<sup>140</sup> Mansy, S.; Chu, G. Y. H.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, 100, 607. Basch, H.; Krauss, M.; Stevens, W. J.; Cohen, D. *Inorg. Chem.* **1986**, 25, 684.

<sup>141</sup> Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, 87, 1153. Reedjik, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding (Berlin)* **1987**, 67, 53. Fouts, C. S.; Marzilli, L. G.; Byrd, R. A.; Summers, M. F.; Zon, G.; Shinozuka, K. *Inorg. Chem.* **1988**, 27, 366. Lippert, B. *Prog. Inorg. Chem.* **1989**, 37, 1-97.

<sup>142</sup> Carradonna, J. P.; Lippard, S. J. *Inorg. Chem.* **1988**, 27, 1454. Bruhn, S. L.; Toney, J. H.; Lippard, S. J. *Prog. Inorg. Chem.* **1990**, 38, 477-516. Lippert, B. *Prog. Inorg. Chem.* **1989**, 37, 1-97.



**Fig. 19.31** (a) Structure of the *cis*-(NH<sub>3</sub>)<sub>2</sub>Pt(d(pGpG)) complex, where d(pGpG) = guanine deoxyribose phosphate dinucleotide. (b) Numbering system of guanine to indicate N7. [From Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science* **1985**, *230*, 412-417. Reproduced with permission.]

chloride ion concentration of only 4 mM: Hydrolysis and subsequent reactions with the appropriate biological targets can then readily take place.<sup>143</sup>

An interesting aspect of the chemotherapeutic use of *cis*-diamminedichloroplatinum(II) and related drugs consists of some negative side effects including nephrotoxicity. They are thought to be the result of the inactivation of enzymes by coordination of Pt(II), like Hg(II), to thiol groups. Application of the ideas of HSAB theory would suggest the protection of these thiols by the use of competitive "rescue agents" that have soft sulfur atoms. These include the diethyldithiocarbamate, Et<sub>2</sub>NCS<sub>2</sub><sup>-</sup>, and thiosulfate, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, ions.<sup>144</sup>

### Metal Complexes as Probes of Nucleic Acids

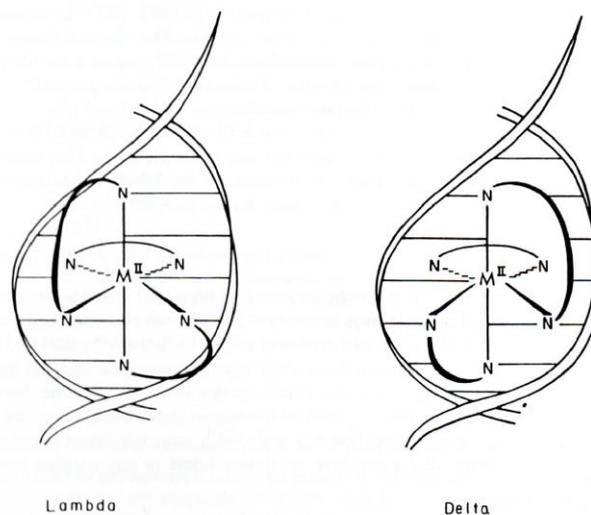
The coordination of *cis*-diammineplatinum(II) to guanine bases in DNA is only one example of a large number of possibilities. The Mg<sup>2+</sup> ion has several important functions with respect to DNA and RNA structure and action. Nature has also anticipated the chemist through the use of "zinc finger" proteins as DNA transcriptional factors. They have a protein chain coordinated tetrahedrally to a zinc atom by

<sup>143</sup> Martin, R. B. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; Chapter 11.

<sup>144</sup> See discussion by Lempers, E. L. M.; Reedijk, J. *Adv. Inorg. Chem.* **1991**, *37*, 175-217.

two cysteines and two histidines and provide specific structural information for site recognition on DNA.

Transition metal complexes may be used to probe specific sites on DNA and RNA chains. Such interactions may yield information concerning the structure at those sites or may induce specific reactions at them. Only one example will be given here.<sup>145</sup> DNA helices are chiral. They would thus be expected to interact with chiral metal complexes in an enantioselective manner. This is illustrated in Fig. 19.32. The intercalation of the  $\Delta$  enantiomer of tris(*o*-phenanthroline)ruthenium(II) into the right-handed helix of B-form DNA<sup>146</sup> is more favorable than that of  $\Lambda$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup>. This is a necessary result of the interaction of the orientation of the "right-handed" ligands with the right-handed helical groove of the DNA. Obviously the chirality of the metal complex is predominant in its interaction with the DNA. We can expect further progress in the use of such enantioselective probes.



**Fig. 19.32** (a)  $\Lambda$ - and  $\Delta$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup>. (b) Illustration of [Ru(phen)<sub>3</sub>]<sup>2+</sup> enantiomers bound by intercalation to B-DNA. The  $\Delta$ -enantiomer (right) fits easily into the right-handed helix, since the ancillary ligands are oriented along the right-handed groove. For the  $\Lambda$ -enantiomer (left), in contrast, steric interference is evident between the ancillary phenanthroline ligands and the phosphate backbone, since for this left-handed enantiomer the ancillary ligands are disposed contrary to the right-handed groove. [From Barton, J. K.; Danishefsky, A. T.; Goldberg, J. M. *J. Am. Chem. Soc.* **1984**, *106*, 2172-2176. Reproduced with permission.]

<sup>145</sup> The reader's attention is drawn to the pioneering work in this area by Jacqueline Barton: Pyle, A. M.; Barton, J. K. *Progr. Inorg. Chem.* **1990**, *38*, 413-475.

<sup>146</sup> A discussion of the structures of A, B, and Z DNA is beyond the scope of this text. See either the reference in Footnote 145 or any modern biochemistry text.

We may thus end this chapter on bioinorganic chemistry and this book on modern inorganic chemistry by noting that a complex that Werner could have synthesized a century ago (and resolved a short time later) is being used to answer questions that neither he nor his contemporary biologists could have conceived.

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**Summary**

It is true that many of the facts in this chapter were gathered by biologists, biochemists, and X-ray crystallographers, not only by inorganic chemists. But the interpretation of these facts and their further exploration falls within the realm of inorganic chemistry. Such factors as (1) alteration of emfs by complexation; (2) stabilization of complexes by ligand field effects; (3) hardness and softness of acids and bases; (4) the thermodynamics and kinetics of both "natural" and "unnatural" (i.e., pollutant) species; (5) catalysis by metal ions; (6) preferred geometry of metal complexes; and (7) energetics of (a) complex formation, (b) redox reactions, and (c) polyanion formation come within the ken of inorganic chemists, and they should be able to contribute fully to the future study of these systems. The effect is already being felt. One need only compare a recent biochemistry text with one of a decade ago to note the emphasis on high spin vs. low spin metal ions, coordination geometry and configuration, and redox reactions and thermodynamics.

The present convergence of physical and analytical techniques combined with inorganic theory makes this one of the most exciting times to be involved in this area of chemistry. One can combine the hard facts and principles of our discipline with the ever elusive yet fascinating mystery of life.