

Chapter 31

BIOINORGANIC CHEMISTRY

31-1 Overview

Biochemistry is not merely an elaboration of organic chemistry. The chemistry of life involves, in essential and indispensable ways, at least 25 elements. In addition to the “organic” elements C, H, N, and O, there are 9 other elements that are required in relatively large quantities, and called, therefore, **macronutrients**. These elements are Na, K, Mg, Ca, S, P, Cl, Si, and Fe. There are also many other elements, **micronutrients**, that are required in small amounts by at least some forms of life: V, Cr, Mn, Co, Ni, Cu, Zn, Mo, W, Se, F, and I. As research activity intensifies, and as instrumental methods of analysis and detection become more sophisticated and sensitive, it is likely that other elements will be added to the list of micronutrients. The elements Cr, Ni, W, and Se have been added only within the last few years.

The metallic elements play a variety of roles in biochemistry. Several of the most important roles are the following:

1. *Regulatory action* is exercised by Na^+ , K^+ , Mg^{2+} , and Ca^{2+} . The flux of these ions through cell membranes and other boundary layers sends signals that turn metabolic reactions on and off.
2. *The structural role* of calcium in bones and teeth is well known, but many proteins owe their structural integrity to the presence of metal ions that tie together and make rigid certain portions of these large molecules, portions that would otherwise be only loosely linked. Metal ions particularly known to do this are Ca^{2+} and Zn^{2+} .
3. An enormous amount of *electron-transfer* chemistry goes on in biological systems, and nearly all of it critically depends on metal-containing electron-transfer agents. These include cytochromes (Fe), ferredoxins (Fe), and a number of copper-containing “blue proteins,” such as azurin, plastocyanin, and stellacyanin.
4. *Metalloenzymes or metallocoenzymes* are involved in a great deal of enzymatic activity, which depends on the presence of metal ions at the active site of the enzyme or in a key coenzyme. Of the latter, the best known is vitamin B_{12} , which contains Co. Important metalloenzymes include carboxypeptidase (Zn), alcohol dehydrogenase (Zn), superoxide dismutase (Cu, Zn), urease (Ni), and cytochrome P-450 (Fe).
5. All aerobic forms of life depend on *oxygen carriers*, molecules that carry oxygen from the point of intake (such as the lungs) to tissues where O_2 is

used in oxidative processes that generate energy. There are three major types of oxygen carriers, and all of them contain metal ions that provide the actual binding sites for the O_2 molecules. These types are

Hemoglobins (Fe), found in all mammals.

Hemerythrins (Fe), found in various marine invertebrates.

Hemocyanins (Cu), found in arthropods and molluscs.

Each of these roles will be discussed in this chapter.

31-2 The Role of Model Systems

Because of the size and complexity of most biochemical molecules and processes, it is often advantageous to find smaller and simpler models upon which controlled experiments can be more easily performed, and with which hypotheses can be tested. Bioinorganic chemistry has been an especially fruitful area for the use of model systems, particularly where transition metals are involved. Of course it is not always possible to find or develop suitable models, and it can be dangerously misleading should overly simplistic models be used naively. Even in the best of circumstances, a model can give only a partial view of how the real system works. If these limitations are recognized, the model system approach can provide valuable guidance to eventual study of the real systems.

The broad and detailed knowledge that we have of coordination chemistry sets the stage for an understanding of the role of metal ions in biological systems. Fundamental principles and generalizations about the behavior of metal complexes are valid whether the metal is coordinated by some relatively simple set of man-made ligands or by a gigantic protein molecule, where the coordinating groups are often carboxyl oxygen atoms, thiol sulfur atoms, or amine nitrogen atoms. Moreover, the optical spectra, magnetic moments, and EPR spectra of transition metal ions afford the same powerful methods of study as when applied to the simpler complexes. Thus we have methods for checking the models against the real systems.

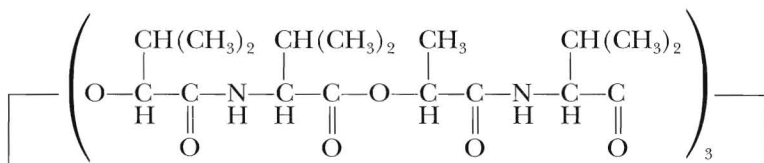
Throughout this chapter we shall frequently refer to model systems that have played a role in understanding real bioinorganic systems. Among these are iron–porphyrin compounds relevant to the understanding of hemoglobin, myoglobin, cytochromes, and enzyme P-450; models for hemerythrin; the cobaloxime model for vitamin B_{12} ; iron–sulfur cluster compounds as models for ferredoxins; and a number of copper complexes that serve as models for a variety of copper-containing enzymes.

31-3 The Alkali and Alkaline Earth Metals

The elements Na, K, Mg, and Ca are ubiquitous in living systems and play an assortment of vital roles. Inorganic chemists who were interested in coordination chemistry used to have a tendency to regard these elements as relatively uninteresting. Nothing could be further from the truth if one is seeking an understanding of life processes.

Sodium and Potassium

Sodium and potassium ion concentrations and the balance (or ratio) of their concentrations in various parts of an organism are controlled by a number of special complexing agents. These generally are **cavitands**, that is, macrocyclic molecules with polar interior groups for binding the ions and nonpolar (hydrophobic) exterior groups that enable the cavitands to carry the Na^+ or K^+ ions across cell boundaries. An example is the cyclic dodecapeptide **valinomycin**, shown as Structure 31-I, and in Chapter 10 as Structure 10-III.



31-I

The Na^+ concentration within animal cells has to be kept about 10 times lower than that in the extracellular fluids, whereas an opposite **gradient** (by a factor of 30) must be maintained for the K^+ ion. The maintenance of these balances requires energy, and when such balances are abruptly changed, electrical potentials responsible for the transmission of nerve impulses are created.

Calcium

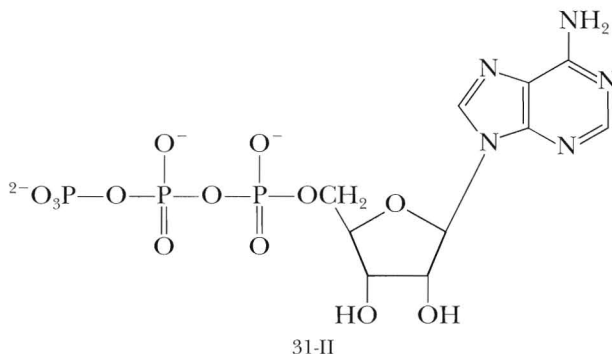
Calcium serves in a staggering variety of roles, the most obvious being in structural materials such as teeth, bones, shells, and a number of other less well-known calcium-rich deposits. It is important to note that none of these calciferous biological materials is an inert "mineral." Bone, for example, though consisting largely of calcium carbonate and phosphate, is continually being deposited and reabsorbed, and it acts as a buffer for body Ca^{2+} and phosphate ions, as controlled by hormonal action. The form of calcium phosphate that occurs in bone and teeth has the same composition as the mineral apatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{X}_2$, where X represents F, Cl, or OH, or a mixture of these.

Calcium is essential to the action of extracellular enzymes, and it participates in many regulatory processes. It is generally complexed by the side-chain carboxyl groups of proteins, with additional bonding sometimes to peptide carbonyl groups and hydroxyl groups.

Magnesium

Magnesium, because of its high charge/radius ratio and consequent strong hydration [as $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$], plays biological roles that are very different from those of calcium. One of its major roles is as a counterion to the negatively charged ROPO_3H^- groups in nucleotides and polynucleotides. Sometimes it approaches the phosphate anions as $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$, but it is also found as $[\text{Mg}(\text{H}_2\text{O})_5]^{2+}$ or $[\text{Mg}(\text{H}_2\text{O})_4]^{2+}$ with one or two phosphate oxygen atoms, respectively, completing its first coordination sphere. The magnesium ion helps to stabilize the three-dimensional structure of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and is thus crucial to the proper functioning of the

genetic machinery of the cell. Also, adenosine diphosphate (ADP) and adenosine triphosphate (ATP, shown in Structure 31-II) exist mainly as 1:1 complexes with the magnesium ion. Magnesium also has a unique role in the plant kingdom as the central atom of chlorophyll, which will be discussed in Section 31-4.



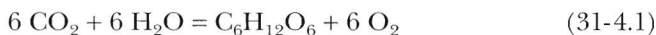
31-4 Metalloporphyrins

One of the most important ways in which metal ions are involved in biochemistry is in complexes with a type of macrocyclic ligand called a *porphyrin*. Porphyrins are derivatives of *porphine*. They differ in the arrangement of substituents around the periphery. The porphine molecule is shown in Fig. 31-1 (*a*), and the two most important metal complexes of porphyrins, *chlorophyll* and *heme*, are shown in Fig. 31-1 (*b*) and (*c*). In these complexes the inner hydrogen atoms have been displaced by the metal ions.

Chlorophyll

There are several very similar but not identical chlorophyll molecules. Green plants contain two and various algae contain others. Notice that in Fig. 31-1 (*b*) the basic porphine system has been modified in two ways. In pyrrole ring IV, one of the double bonds has been trans-hydrogenated, and a cyclopentanone ring has been fused to the side of pyrrole ring III. Nevertheless, the fundamental properties of the porphine system are retained.

Photosynthesis is a complex sequence of processes in which solar energy is first absorbed and ultimately—in a series of redox reactions, some of which proceed in the dark—used to drive the overall endothermic process of combining water and carbon dioxide to give glucose; molecular oxygen is released simultaneously:



The function of the chlorophyll molecules in the chloroplast is to absorb photons in the red part of the visible spectrum (near 700 nm) and pass this energy of excitation on to other species in the reaction chain. The ability to absorb the light is due basically to the conjugated polyene structure of the porphine ring system. The role of the magnesium ion is, at least, twofold. (1) It helps to

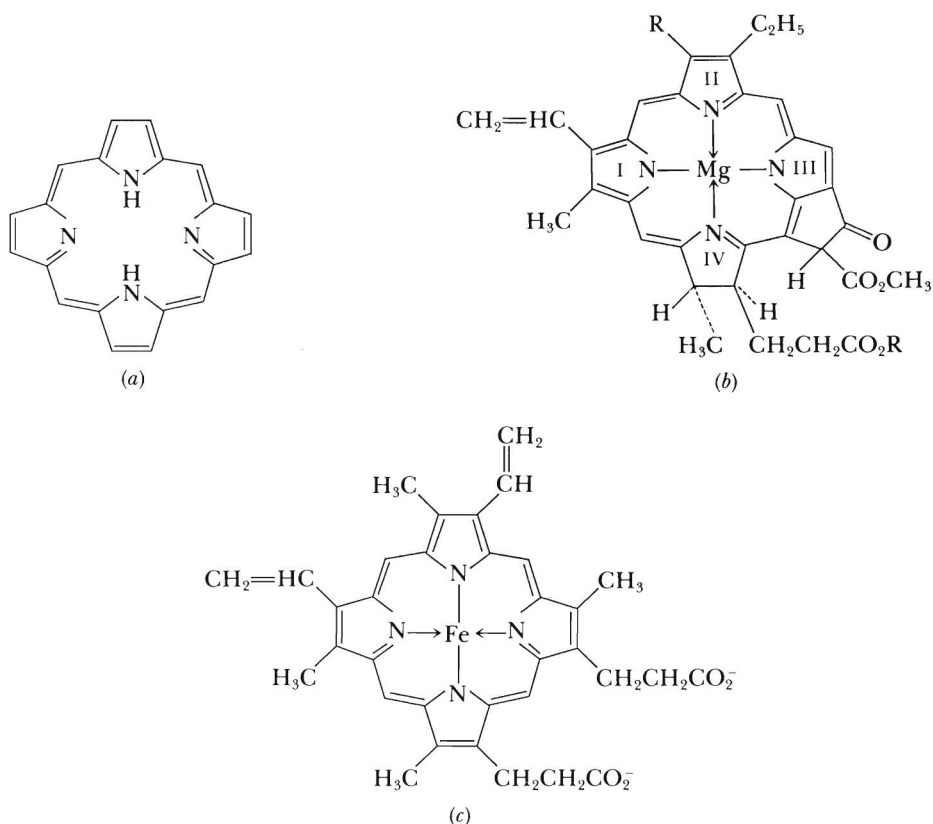


Figure 31-1 (a) The prototype porphine molecule. (b) One of the chlorophyll molecules. (c) The heme group.

make the entire molecule rigid so that energy is not too easily lost thermally, that is, degraded to molecular vibrations. (2) It enhances the rate at which the short-lived singlet excited state initially formed by photon absorption is transformed into the corresponding triplet state, which has a longer lifetime and thus can transfer its excitation energy into the redox chain.

At an early stage of the electron-transfer sequence that leads ultimately to the release of molecular oxygen, a polynuclear manganese complex, of unknown composition, undergoes reversible redox reactions. At still other stages, iron-containing substances, called cytochromes and ferredoxins, and a copper-containing substance, called plastocyanin, also participate. Thus, photosynthesis requires the participation of complexes of no less than four metallic elements.

Heme Proteins

Iron is certainly the most widespread of the transition metals in living systems. Its compounds participate in a variety of activities. The two main functions of iron-containing materials are (1) transport of oxygen, and (2) mediation in electron-transfer chains. So much iron is required for these purposes that there is also a chemical system to store and transport iron. We turn first to compounds in

which the iron is present as heme, the porphyrin complex depicted in Fig. 31-1(c). The heme group functions in all cases in intimate association with a protein molecule. The chief heme proteins are

1. Hemoglobins
2. Myoglobins
3. Cytochromes, including a special type, P-450
4. Enzymes such as catalase and peroxidase

Hemoglobin and Myoglobin

These are closely related. Hemoglobin has a molecular weight of 64,500 and consists of four subunits, each containing one heme group. Myoglobin is very similar to one of the subunits of hemoglobin, one of which is shown in Fig. 31-2. Hemoglobin has two functions. (1) It binds oxygen molecules to its iron atoms and transports them from the lungs to muscles where they are delivered to myoglobin molecules. These store the oxygen until it is required for metabolic ac-

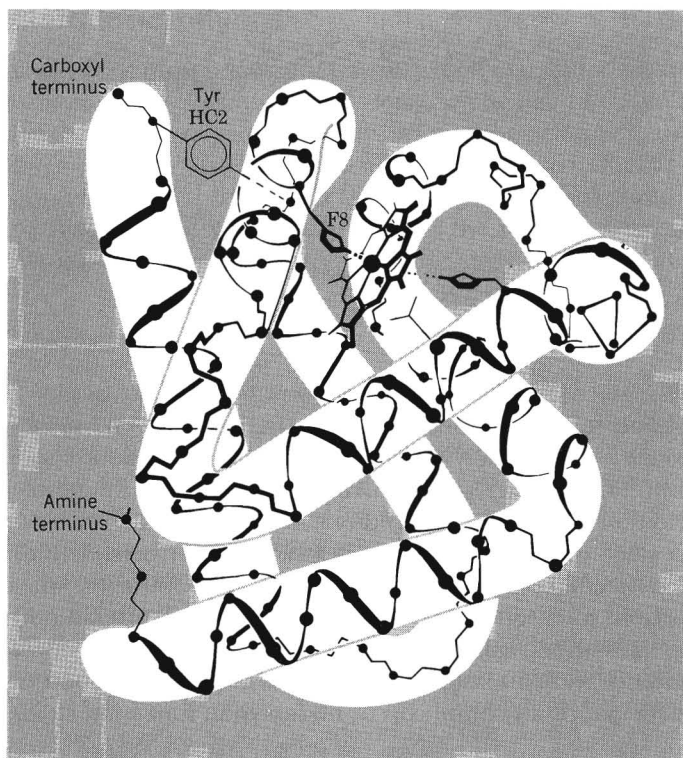
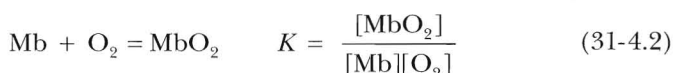


Figure 31-2 A representation of one of the four subunits of hemoglobin. The continuous black band represents the peptide chain and the various sections of the helix. Dots on the helical chain represent α -carbon atoms. The heme group is near the top of the diagram (just to the right of center), with the iron atom represented as a large dot. The coordinated histidine is labeled F8, meaning the 8th residue of the F helix. [This diagram was adapted from one kindly provided by M. Perutz.]

tion. (2) The hemoglobin then uses certain amino groups to bind carbon dioxide and carry it back to the lungs.

The heme group is attached to the protein in both hemoglobin and myoglobin through a coordinated histidine–nitrogen atom (F8), shown in Fig. 31-2. The position trans to the histidine–nitrogen atom is occupied by a water molecule in the deoxy species or O_2 in the oxygenated species. The structure of the Fe– O_2 grouping is still uncertain, but changes in the oxidation state of iron and the introduction of O_2 (and other ligands) cause important changes in the structure of heme, as we describe here.

Hemoglobin is not simply a passive container for oxygen but an intricate molecular machine. This may be appreciated by comparing its affinity for O_2 to that of myoglobin. For myoglobin (Mb) we have the following simple equilibrium:



If f represents the fraction of myoglobin molecules bearing oxygen and P represents the equilibrium partial pressure of oxygen, then

$$K = \frac{f}{(1-f)P} \quad \text{and} \quad f = \frac{KP}{1+KP} \quad (31-4.3)$$

This is the equation for the hyperbolic curve labeled Mb in Fig. 31-3. Hemoglobin with its four subunits has more complex behavior; it approximately follows the equation

$$f = \frac{KP^n}{1+KP^n} \quad n \approx 2.8 \quad (31-4.4)$$

where the exact value of n depends on pH. Thus, for hemoglobin (Hb) the oxygen-binding curves are sigmoidal, as is shown in Fig. 31-3. The fact that n exceeds unity can be ascribed physically to the fact that attachment of O_2 to one heme group increases the binding constant for the next O_2 , which in turn increases the constant for the next one, and so on.

Although Hb is about as good an O_2 binder as Mb at high O_2 pressure, it is much poorer at the lower pressures prevailing in muscle and, hence, passes on its oxygen to the Mb as required. Moreover, the need for O_2 will be greatest in tissues that have already consumed oxygen and simultaneously have produced CO_2 . The CO_2 lowers the pH, thus causing the Hb to release even more oxygen to the Mb. The pH-sensitivity (called the Bohr effect), as well as the progressive increase of the O_2 binding constants in Hb, is due to interactions between the subunits; Mb behaves more simply because it consists of only one unit. It is clear that each of the two is essential in the complete oxygen-transport process. Carbon monoxide, PF_3 , and a few other substances are toxic because they become bound to the iron atoms of Hb more strongly than O_2 ; their effect is one of competitive inhibition.

The way in which interactions between the four subunits in Hb give rise to both the cooperativity in oxygen binding and to the Bohr effect (pH dependence), both of which are so essential to the role played by Hb, is now partly un-

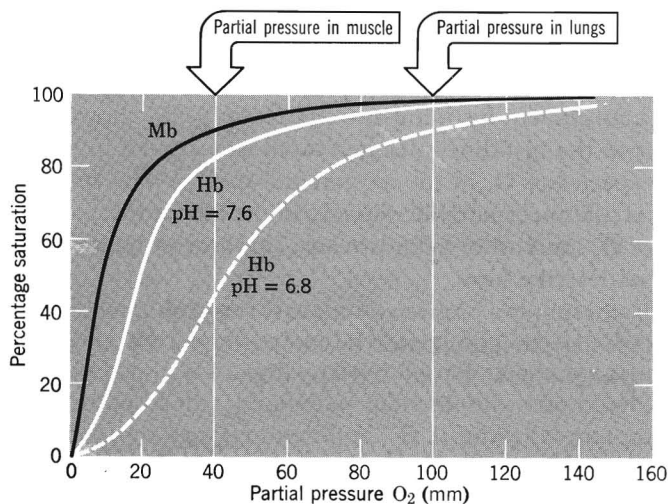


Figure 31-3 The oxygen-binding curves for myoglobin (Mb) and hemoglobin (Hb), showing also the pH dependence for the latter.

derstood. The mechanism is very intricate, but one essential feature depends directly on the coordination chemistry involved. Deoxyhemoglobin has a high-spin distribution of electrons, with one electron occupying the $d_{x^2-y^2}$ orbital that points directly toward the four porphyrin nitrogen atoms. The presence of this electron in effect increases the radius of the iron atom in these directions by repelling the lone-pair electrons of the nitrogen atoms. The result is that the iron atom actually lies about 0.7–0.8 Å out of the plane of these nitrogen atoms, in order that it not be in too close contact with them. The iron atom is also coordinated by a nitrogen atom on the imidazole ring of the amino acid histidine, labeled F8 in Fig. 31-2. Thus the iron atom in deoxyhemoglobin has square pyramidal coordination, as is shown in Fig. 31-4(a).

When an oxygen molecule becomes bound to the iron atom, it occupies a position opposite to the imidazole–nitrogen atom. The presence of this sixth ligand alters the strength of the ligand field, and the iron atom goes into a low-spin state, in which the six d electrons occupy the d_{xy} , d_{yz} , and d_{zx} orbitals. The $d_{x^2-y^2}$ orbital is then empty and the previous effect of an electron occupying this orbital in repelling the porphyrin nitrogen atoms vanishes. The iron atom is thus able to slip into the center of an approximately planar porphyrin ring and an essentially octahedral complex is formed, as shown in Fig. 31-4(b).

As the iron atom moves, it pulls the imidazole side chain of histidine F8 with it, thus moving that ring about 0.75 Å. This shift is then transmitted to other parts of the protein chain to which F8 belongs and, in particular, a large movement of the phenolic side chain of tyrosine HC2 is produced. From here various shifts of atoms in the neighboring subunit are caused, and these shifts influence the oxygen-binding capability of the heme group in that subunit. Thus the movement of the iron atom of the heme group in one subunit of hemoglobin acts as a kind of “trigger,” which sets into motion extensive structural changes in other subunits.

One of the interesting problems about oxygen binding by hemoglobin concerns the structure of the Fe–O₂ grouping. Three possibilities are shown in Fig.

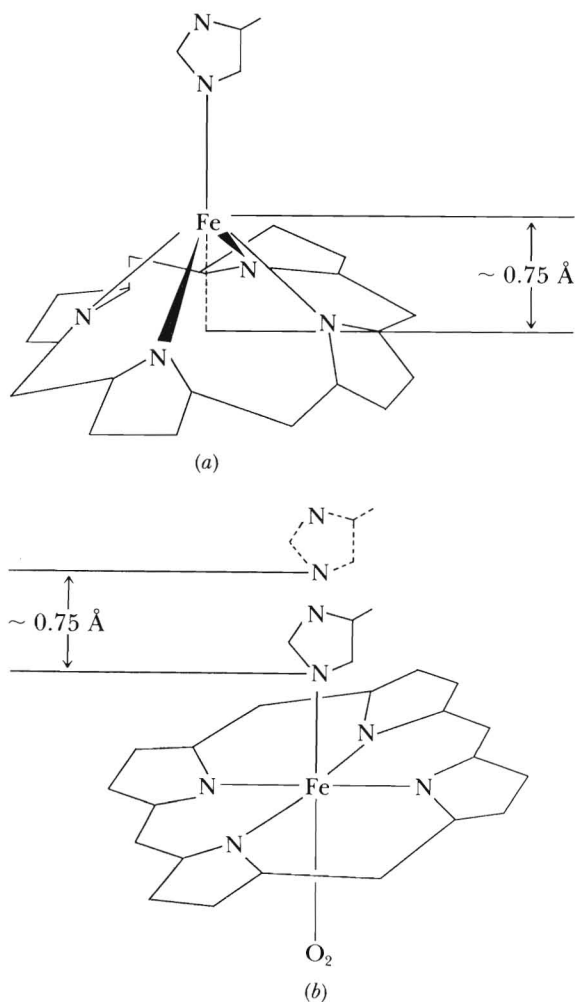


Figure 31-4 (a) The five-coordinate, high-spin Fe^{II} in deoxyhemoglobin. (b) The six-coordinate, low-spin iron in oxyhemoglobin, showing the distance that the side-chain histidine residue F8 has moved upon oxygenation.

31-5. The linear geometry has no precedent and is least probable. The side-on arrangement is found in some simple O_2 complexes involving other metals, such as $(\text{PPh}_3)_2\text{ClIrO}_2$, but is very unlikely for hemoglobin. The bent chain appears most probable, since O_2 is isoelectronic with NO^+ , and since the latter forms complexes with bent $\text{Co}^{\text{III}}-\text{N}-\text{O}$ chains. Also, there is one fairly good model compound, an iron(II)porphyrin complex of O_2 , in which the bent arrangement has been found.

Recent X-ray studies on both $\text{Mb}(\text{O}_2)$ and $\text{Hb}(\text{O}_2)_4$ indicated that O_2 binds in the bent end-on fashion, with an $\text{Fe}-\text{O}-\text{O}$ angle of approximately 130° .

Hemoglobin Modeling

The ability of the heme in hemoglobin or myoglobin to bind an O_2 molecule and later release it without the iron atom becoming permanently oxidized

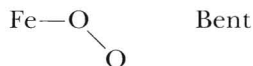


Figure 31-5 Three conceivable O_2 -iron bonding geometries for hemoglobin or myoglobin.

to the iron(III) state is obviously essential to the functioning of these oxygen carriers. This remarkable ability has been taken for granted in the preceding discussion, but it merits further discussion. It is the reversibility of the hemoglobin and myoglobin reactions with O_2 that must be matched by any useful model.

Early attempts to employ simple Fe^{II} -porphyrin complexes, or even free heme itself, plus an aromatic amine molecule (to take the place of the histidine F8) were not successful. On exposing such a “model” to O_2 , oxidation (rather than oxygenation) occurred promptly and irreversibly. Oxygen was absorbed, but not released. The reason for this is now understood: Dioxygen reacts to produce an O-bridged dinuclear complex of iron(III), as in Reaction 31-4.5.



In hemoglobin and myoglobin, the bulk of the protein surrounding the heme unit assures that each heme unit remains isolated. To have an effective model, something must be added to the simple iron-porphyrin to accomplish this same degree of bulk. The two ways in which this has been done are represented schematically in Fig. 31-6 and then more realistically in Fig. 31-7. Model compounds such as those shown in Fig. 31-7 do engage in reversible oxygen binding, quite similar to the behavior of myoglobin. In the two examples shown in Fig. 31-7, a suitable amine (such as pyridine) is bound on the unprotected side, and the O_2 molecule then enters either between the “pickets” or under the “cap,” where it is bound end-on to the iron atom.

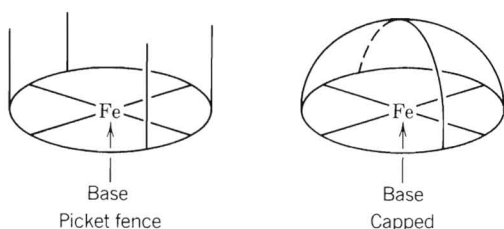


Figure 31-6 Schematic representations of two ways in which hemelike models may be modified to preclude dimerization via μ -O bridging.

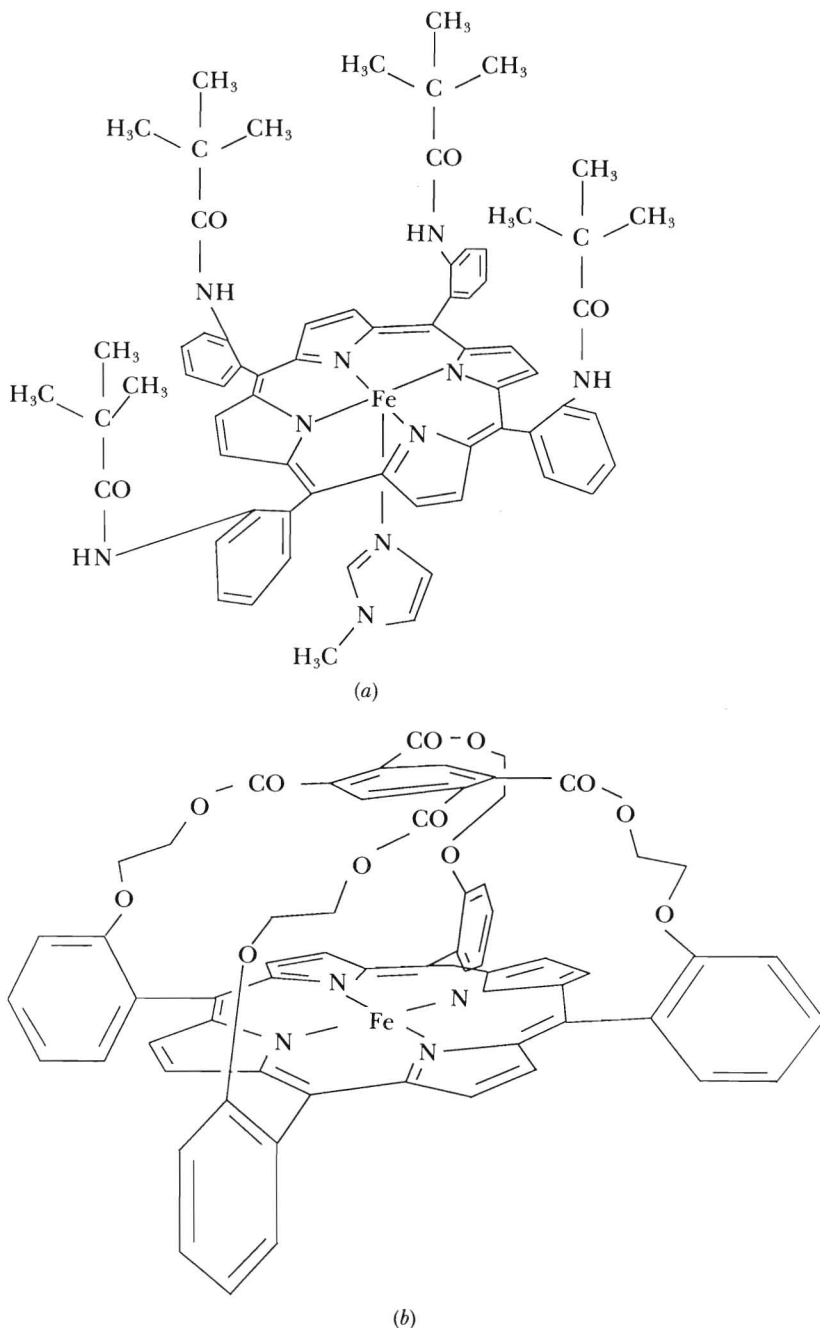


Figure 31-7 Actual examples of (a) the “picket fence” and (b) the “capped” types of heme models.

Other Heme Proteins

It is a fascinating fact that heme, the iron–porphyrin complex shown in Fig. 31-1(c), functions in Nature for a number of other tasks in addition to carrying oxygen. We shall not go into any of these in detail, but they should at least be mentioned since inorganic chemists have contributed to our understanding of all of them, both through research on the natural materials themselves, and through fabrication and study of model systems.

Cytochromes. Cytochromes are found in both plants and animals and serve as electron carriers. They accept an electron from a slightly better reducing agent and pass it on to a slightly better oxidizing agent. In the cytochromes, the heme iron is coordinated by a nitrogen atom of an imidazole ring on one side of the porphyrin plane, and it is coordinated on the other side of the porphyrin plane by the sulfur atom of a methionine residue from a different part of the protein backbone. Thus the potential oxygen-carrying capacity of the heme in cytochromes is blocked.

Cytochrome P-450 Enzymes. These enzymes are heme-containing oxygenases that catalyze the introduction of oxygen atoms into substrates. Of the many possible substrates, the most important are molecules in which C—H groups are converted into C—OH groups. The catalytic cycle entails a substance in which the iron atom attains a high (IV or V) oxidation state. The coordination sphere of the iron atom includes, in addition to the porphyrin ring, one sulfur atom, but whether the sixth coordination position is occupied by a water ligand or is vacant in the resting enzyme is uncertain.

Peroxidases and Catalases. Peroxidases catalyze the oxidation of a variety of substances by peroxides, mainly H_2O_2 . Catalases catalyze decomposition of H_2O_2 (and some other peroxides) to H_2O and O_2 . They have many similarities both in structure and in aspects of their mechanisms. They both have high-spin ferric heme groups lodged deeply in large protein molecules, with a histidine nitrogen atom occupying the fifth coordination site. The sixth coordination position may be occupied by a water ligand in the resting enzyme. There is growing evidence that a porphyrin— $\text{Fe}^{\text{IV}}=\text{O}$ substance is the key intermediate in the function of peroxidases and catalases.

31-5 Iron-Sulfur Proteins

Iron–sulfur proteins contain strongly bound, functional iron atoms, but not porphyrins. The iron atoms are bound by sulfur atoms. These proteins all participate in electron-transfer sequences.

Rubredoxins

These are found in anaerobic bacteria where they are believed to participate in biological redox reactions. They are relatively low-molecular weight proteins (~ 6000), and usually contain only one iron atom. In the best characterized rubredoxin, from the bacterium *Clostridium pasteurianum*, the iron atom, which is normally in the III oxidation state, is surrounded by a distorted tetrahedron of cysteinyl sulfur atoms. The Fe—S distances range from 2.24 to 2.33 Å, and the S—Fe—S angles from 104 to 114°. A schematic representation of this is given in Fig. 31-8. When

the Fe^{III} is reduced to Fe^{II} , there is a slight ($0.05 \text{ \AA}$) increase in the $\text{Fe}-\text{S}$ distances, but the essentially tetrahedral coordination is maintained. Mössbauer spectroscopy has shown that the iron is in the high-spin condition in both oxidation states. Inorganic chemists have prepared and studied $[\text{Fe}(\text{SR})_4]^{2-}$ and $[\text{Fe}(\text{SR})_4]^-$ complexes as models to help understand the properties of the rubredoxins.

Ferredoxins

Ferredoxins are also relatively small proteins (6000–12,000) that contain iron–sulfur redox centers that are held in place by bonds from cysteine sulfur atoms to iron. The difference from rubredoxin is that here the redox centers are clusters of two, three, or four iron atoms, together with several sulfur atoms (so-called inorganic sulfur). In each case, an approximate tetrahedron of sulfur atoms is completed about each iron atom by the sulfur atoms of cysteine residues of the peptide. These systems are generally called ferredoxins and are often abbreviated Fd.

The two-iron Fd's, complete with their attached cysteine sulfur atoms, can be described as two tetrahedral FeS_4 units sharing an edge. In a convenient notation, the two-iron clusters can be represented as $[2\text{Fe}-2\text{S}]^{n+}$. They have relatively simple behavior. Their normal state is $[2\text{Fe}-2\text{S}]^{2+}$ [meaning that both iron atoms are iron(III)], but they can be reduced at potentials similar to that of the standard H^+/H_2 electrode (i.e., -0.4 V on the hydrogen scale) to $[2\text{Fe}-2\text{S}]^+$.

Several kinds of spectroscopic evidence indicate that in the reduced $[2\text{Fe}-2\text{S}]^+$ cluster, the added electron is localized on one iron atom, so that one Fe^{II} and one Fe^{III} atom are present. In the $[2\text{Fe}-2\text{S}]^{2+}$ cluster, the $\text{Fe}-\text{Fe}$ distance is only $2.72 \text{ \AA}$, and the two formally high-spin (d^5) iron atoms have their magnetic moments so strongly coupled antiferromagnetically that the cluster is diamagnetic. Upon reduction to give $[2\text{Fe}-2\text{S}]^+$, this coupling persists, and the $[2\text{Fe}-2\text{S}]^+$ cluster has only one unpaired electron. This has been very helpful, since it means that ESR detection of the reduced cluster is quite easy.

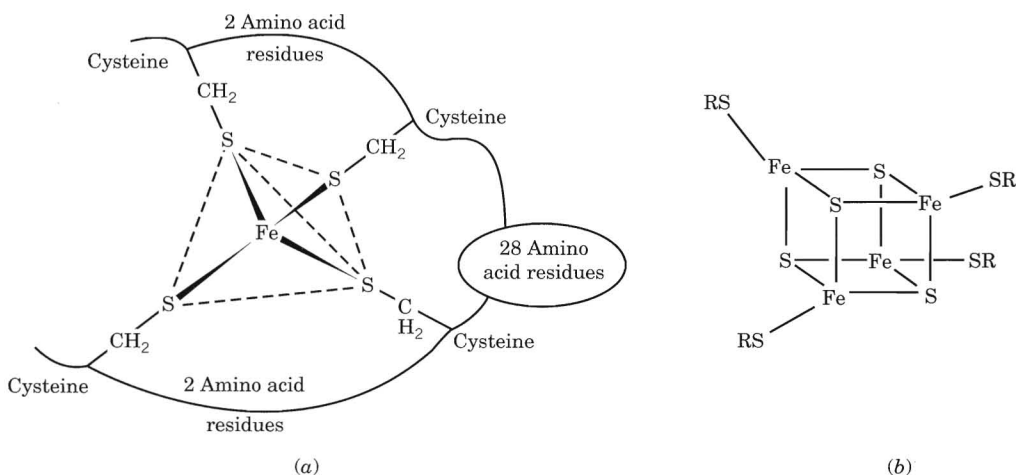
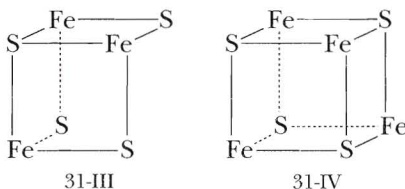


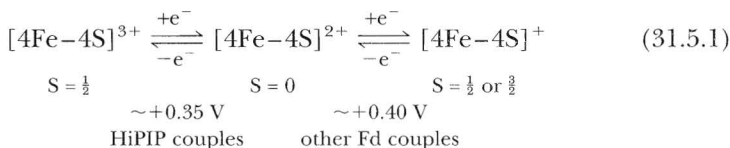
Figure 31-8 (a) The environment of the iron atom in the rubredoxin molecule. (b) The Fe_4S_4 cluster found in the four-iron ferredoxins and HiPiPs. The thiolate side-chains of cysteines are represented by RS.

In recent years it has become known that there are important Fd's that contain three-iron clusters, which have the general Structure 31-III. The structure is a fragment of the four-iron cluster structure (31-IV) and the $[3\text{Fe}-4\text{S}]^{n+}$ unit can have oxidation states corresponding to $n = +1, 0$, and -1 .



The four-iron Fd's, which contain $[4\text{Fe}-4\text{S}]^{n+}$ clusters appear to be more common than the two-iron or three-iron ones, and they have quite complex behavior. In biological systems they have three oxidation levels, giving the charges of $+3$, $+2$, or $+1$. In any given system, though, only one pair of these charge types is employed. For many of these the normally isolated substance contains a diamagnetic $[4\text{Fe}-4\text{S}]^{2+}$ cluster; this can be reversibly reduced at about -0.4 V (vs. the hydrogen electrode) to give $[4\text{Fe}-4\text{S}]^+$, which has one unpaired electron.

One particularly important class of four-iron Fd's are sometimes called *high-potential iron-sulfur proteins* (abbreviated HiPIP). Here the operative redox couple, at about $+0.75$ V is between the clusters $[4\text{Fe}-4\text{S}]^{3+}$ and $[4\text{Fe}-4\text{S}]^{2+}$. The redox behavior of both HiPIPs and other Fd's is summarized in Reaction 31-5.1,



where the redox potentials are given in volts (V) against the standard hydrogen electrode.

Let us now emphasize a very important point, for which there is not yet a generally accepted explanation. Both HiPIP and the other Fd's are normally isolated with the $[4\text{Fe}-4\text{S}]^{2+}$ cluster. For the latter, a reversible, one electron reduction occurs at about -0.40 V, but reversible oxidation to the $3+$ level has never been accomplished. Conversely, reversible one-electron oxidation readily occurs for HiPIPs, but reduction can be achieved only under forcing conditions having no relevance to the biological situation. Unquestionably, however, the $[4\text{Fe}-4\text{S}]^{2+}$ clusters in the two types of compounds are the same. What, then, causes the marked difference in their redox behavior?

Two hypotheses are under consideration. One focuses on the number of hydrogen bonds from surrounding protein NH groups to cysteinyl sulfur atoms. There appear to be about twice as many of these for the usual Fd than for a HiPIP; thus reduction (the introduction of negative charge) would be preferred for a usual Fd. A second hypothesis is that oxidation and reduction of the $[4\text{Fe}-4\text{S}]^{2+}$ cluster lead to different sorts of structural deformations, and that the protein conformations about the cluster in usual Fd's and HiPIPs differ so as to favor the reductively induced changes in the Fd case and the oxidatively induced ones in the HiPIP case. This is a fascinating question which will, no doubt,

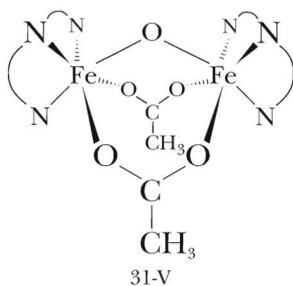
be resolved as better structural data are obtained and, perhaps, as model systems become better characterized.

The study of ferredoxin biochemistry provides a classic example of how inorganic chemists can use model systems to investigate complex biological processes. It has been possible to synthesize compounds containing $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{x-}$ anions that are very similar in many aspects (especially structural ones) to the $[\text{4Fe-4S}]^{n+}$ clusters that are bonded to the four cysteinyl sulfur atoms of the peptide chain. By treating ferredoxins with solutions of mercaptides, RS^- , it is even possible to extract the $[\text{4Fe-4S}]^{2+}$ clusters from the protein and capture them as $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ anions.

31-6 Hemerythrin

In a number of marine worms, there exists a different solution to the oxygen carrying problem. Again, the active metal is iron, but the rest of the picture is quite different: no porphyrin ligand is involved, and two iron atoms are required to bind one molecule of O_2 . The full details of how the active site of a hemerythrin actually works are still incomplete, but there is good evidence (not conclusive, however) that the process goes according to the scheme shown in Fig. 31-9.

The two-iron active site has the iron atoms connected by three bridging groups, two of which are carboxyl anions from the side chains of glutamic and aspartic acid residues. The other bridging ligand is either O^{2-} or OH^- , but probably OH^- . All of the remaining ligands (which complete an octahedron about one iron atom and a type of five-coordination about the other iron atom) are imidazole nitrogen atoms from histidine residues. The possibility that a sixth ligand (very weakly held) may be present at the second iron atom cannot be entirely ruled out.



Spectroscopic evidence shows that the oxygen is definitely bound in a peroxo form, with the two oxygen atoms not equivalent. It is virtually certain that it occupies the position shown in Fig. 31-9(b), but the finer details, such as the $\text{OO-H} \cdots \text{O}$ hydrogen bond, are speculative.

To develop a better understanding of the interactions between the two iron atoms in the active site of hemerythrins, several model systems, of the type shown in structure 31-V, have been synthesized. In these models, the bridging carboxyl groups are derived from acetic acid, and the nitrogen atoms are supplied by tridentate triamines whose conformations cause them naturally to occupy three mutually *cis* positions.

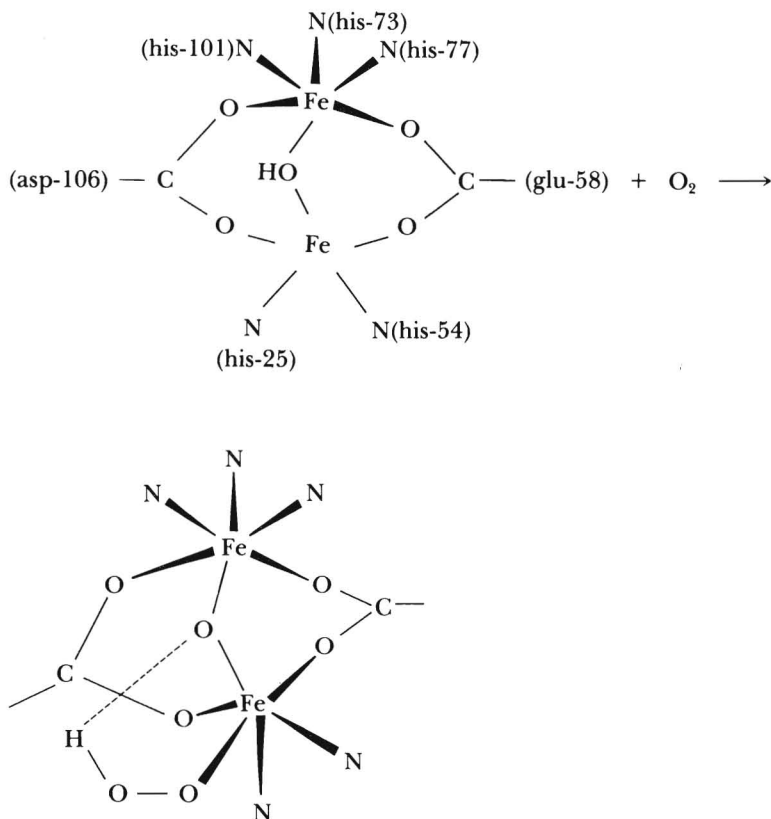


Figure 31-9 A possible description of the mode of oxygen binding by hemerythrin.

31-7 Iron Supply and Transport

Iron metabolism requires provision for storing and transporting iron. In humans and in many other higher animals the storage materials are *ferritin* and *hemosiderin*. These are present in liver, spleen, and bone marrow. Ferritin is a water soluble, crystalline substance consisting of a shell, or sheath, of protein surrounding a spherical core that contains the iron. The diameter of the core varies from 40 to 88 Å, and may contain up to 4500 iron atoms, having a composition closely approximating $(\text{FeOOH})_8 \cdot \text{FeO} \cdot \text{H}_2\text{PO}_4$. The diffraction pattern of this core is similar to that of the substance ferrihydrite, $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$, which is formed when NH_4OH is added slowly to a solution of ferric nitrate at 80–90 °C. The phosphate is not a part of the bulk structure of the core, but appears to play some role in covering the iron particles of the core and perhaps attaching them to each other and to the protein sheath. Up to 23% of the dry weight may be iron. The protein portion alone, called *apoferritin*, is stable, forms crystals suitable for X-ray diffraction, and has a molecular weight of about 45,000. *Hemosiderin* contains larger proportions of hydrous metal oxide, but is rather variable in composition and properties. It is poorly understood compared to ferritin.

The manner in which the iron enters and leaves ferritin is not well understood. The core can be formed only from aqueous iron(II), so that oxidation to give the correct proportion of iron(III) must accompany, or follow, incorporation in the core. Iron release is controlled by the protein sheath and can occur very rapidly when necessary.

Transferrin is a protein that binds iron(III) very strongly, and transports it from the stomach to the iron metabolic processes of the body. As iron passes from the stomach (which is acidic) into the blood ($\text{pH} \cong 7.4$), it is oxidized to Fe^{III} in a process catalyzed by the copper metalloenzyme ceruloplasmin, after which it is picked up by transferrin molecules. These are proteins with a molecular weight of about 80,000, and they contain two similar but not identical sites that bind iron tightly but reversibly in the presence of certain anions such as CO_3^{2-} and HCO_3^- . The binding constant is approximately 10^{26} , making transferrin an extremely efficient scavenger of iron. Eventually transferrin becomes bound to the cell wall of an immature red cell, which utilizes the iron. Transferrin also carries iron to ferritin, the process of iron(II) transfer being a complex one requiring ATP and ascorbic acid.

In microorganisms, iron is transported by substances called *ferrichromes* and *ferrioxamines*. The former are trihydroxamic acids in which the three hydroxamate groups are on three side chains of a cyclic hexapeptide. The latter have the three hydroxamate groups as part of the peptide chain, which may be cyclic or acyclic. Typical structures are shown in Fig. 31-10.

The importance of these compounds derives from their exceptional ability to chelate iron(III) and then pass through cell membranes, thus carrying iron from inorganic sources, such as $\text{Fe}_2\text{O}_3 \cdot x \text{H}_2\text{O}$, to points of need in the cells.

31-8 The Bioinorganic Chemistry of Cobalt: Vitamin B₁₂

The best-known biological function of cobalt is its intimate involvement in the coenzymes related to vitamin B₁₂, the structure of which is shown in Fig. 31-11. This structure is not as overwhelming as it might seem at first glance. It consists of four principal components:

1. A cobalt atom.
2. A macrocyclic ligand called the *corrin* ring, which bears various substituents. The essential corrin ring system is shown in bold lines. It resembles the porphine ring, but differs in various ways, notably in the absence of one methine ($=\text{CH}-$) bridge between a pair of pyrrole rings.
3. A complex organic portion consisting of a phosphate group, a sugar, and an organic base, the latter being coordinated to the cobalt atom.
4. A sixth ligand may be coordinated to the cobalt atom. This ligand can be varied, and when the cobalt atom is reduced to the oxidation state +1, it is evidently absent.

The entire entity shown in Fig. 31-11, but neglecting the ligand X, is called cobalamin.

The term vitamin B₁₂ refers to cyanocobalamin, which has cobalt in the +3 oxidation state and CN^- as the ligand X. The cyanide ligand is introduced dur-

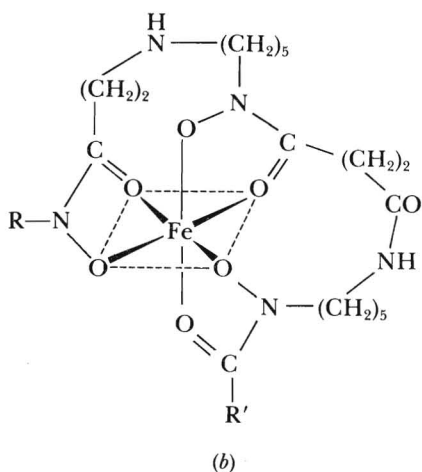
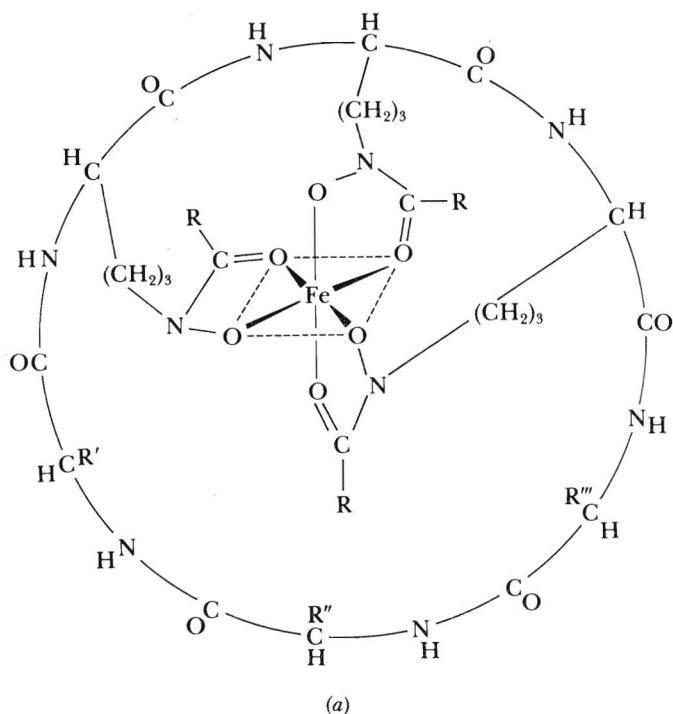


Figure 31-10 (a) A typical ferrichrome. (b) Typical structure of an acyclic ferrioxamine.

ing the isolation procedure and is not present in any active form of the vitamin. In the biological system, the ligand X is likely to be H_2O much of the time, but another possibility, which has been identified by actual isolation of the complex, is the 5'-deoxyadenosyl radical, as shown in Fig. 31-12. The particular coenzyme in which this is found was the first organometallic compound to be observed in a living system.

The B_{12} coenzymes act in concert with a number of enzymes, but the best studied systems involve the dioldehydrases, where reactions such as 31-8.1 are catalyzed.

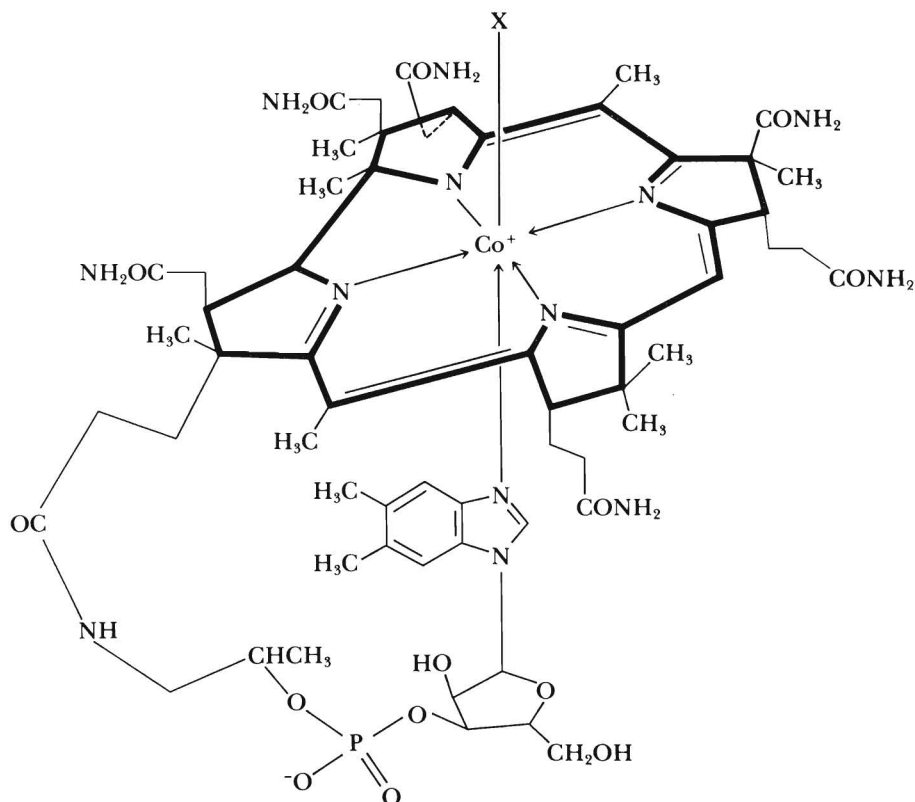
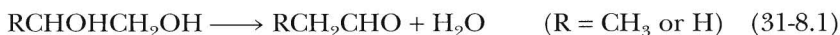


Figure 31-11 The structure of cobalamin. The corrin ring is shown in heavy lines.



From studies of the nonenzymic chemistry of B₁₂ coenzymes and of model systems noted below, a body of knowledge about fundamental B₁₂ chemistry has been built up. Some of this chemistry undoubtedly plays a role in its activities as a coenzyme. The cobalamins can be reduced in neutral or alkaline solution to give cobalt(II) and cobalt(I) species, often called B_{12r} and B_{12s} respectively. The latter is a powerful reducing agent, decomposing water to give hydrogen and B_{12r}. These reductions can apparently be carried out *in vivo* by reduced ferredoxin. When cyano- or hydroxocobalamin is reduced, the ligand (CN⁻ or OH⁻) is lost, and the resulting five-coordinate cobalt(I) species reacts with ATP in the presence of a suitable enzyme to generate the B₁₂ coenzyme.

In nonenzymic systems, rapid reaction of B_{12s} occurs with alkyl halides, alkynes, and the like, as shown in Reactions 31-8.2 to 31-8.4, where [Cb] represents the cobalamin group. Methylcobalamin has an extensive chemistry, some of which is involved in the metabolism of methane-producing bacteria. It transfers CH₃ groups to Hg^{II}, Tl^{III}, Pt^{II}, and Au^I. It is, evidently, in this way that certain bacteria accomplish their unfortunate feat of converting relatively harmless elemental mercury, which collects in sea or lake bottoms, into the exceeding toxic methylmercury ion CH₃Hg⁺.

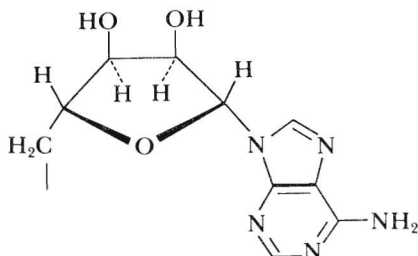
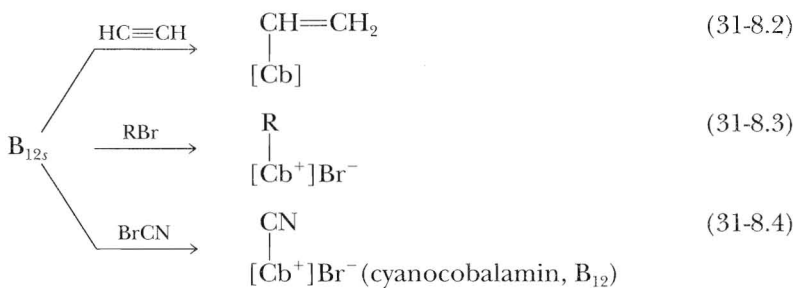


Figure 31-12 The 5'-deoxyadenosyl group that may constitute the ligand X in Fig. 31-11.



A number of models for vitamin B_{12} have been synthesized and studied. The best known are the bis(dimethylglyoximate) complexes, an example of which is shown in Fig. 31-13. This and other models have as their essential feature a planar tetradentate ligand with amido-type nitrogen atoms. Many of these quite successfully model the reducibility to the cobalt(I) state, as well as the formation and reactions of the key cobalt–carbon bonds.

It is interesting that cobalt porphyrins are not very good models for B_{12} since they cannot be reduced to the cobalt(I) state under conditions where vitamin B_{12s} is obtained. This inability of the porphyrin ligand to stabilize the cobalt(I) species may be a reason why the corrin ring system was evolved.

31-9 Metalloenzymes

Enzymes are large protein molecules so built that they can bind at least one reactant (called the substrate) and catalyze an important biochemical reaction. These compounds are extremely efficient as catalysts, typically causing rates to increase 10^6 times or more compared to the uncatalyzed rate. They are also usually highly specific, catalyzing only one, or a few reactions, rather than all those of a given class.

Some enzymes incorporate one or more metal atoms in their normal structure. The metal ion does not merely participate during the time that the enzyme–substrate complex exists, but is a permanent part of the enzyme. The metal atom, or at least one of the metal atoms when two or more are present, occurs at or very near to the active site (the locus of the bound, reacting substrate) and plays a role in the activity of the enzyme. Such enzymes are called *metalloenzymes*, and at least 100 have been identified.

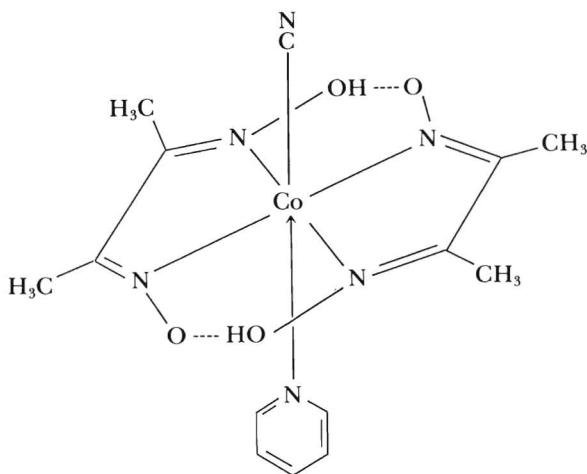


Figure 31-13 A cobaloxime, or bis(dimethylglyoximate)cobalt complex, which is a model for cyanocobalamin, vitamin B₁₂.

The following metals are most often found in metalloenzymes, especially the last three: Mo, Ca, Mn, Fe, Cu, and Zn. Although Co²⁺ can often be made to replace Zn²⁺ in zinc metalloenzymes, with retention or even enhancement of activity, the actual presence of Co²⁺ in the native enzymes is rare.

Zinc Metalloenzymes

No less than 30 zinc metalloenzymes are known. Two of the most important, or at least best studied, are the following:

Carbonic anhydrase (MW = 30,000; 1 Zn):

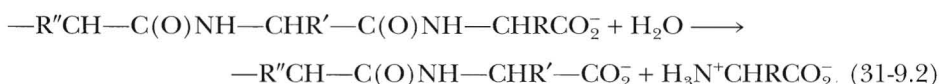
This enzyme occurs in red blood cells and catalyzes the dehydration of the bicarbonate ion and the hydration of CO₂ according to Reaction 31-9.1.



These reactions would otherwise proceed too slowly to be compatible with physiological requirements.

Carboxypeptidase (MW = 34,300; 1 Zn):

This enzyme in the pancreas of mammals catalyzes the hydrolysis of the peptide bound at the carboxyl end of a peptide chain, as in Reaction 31-9.2.



The enzyme has a particular preference for substrates in which the side chain R is aromatic, that is, $-\text{CH}_2\text{C}_6\text{H}_5$ or $-\text{CH}_2\text{C}_6\text{H}_4\text{OH}$.

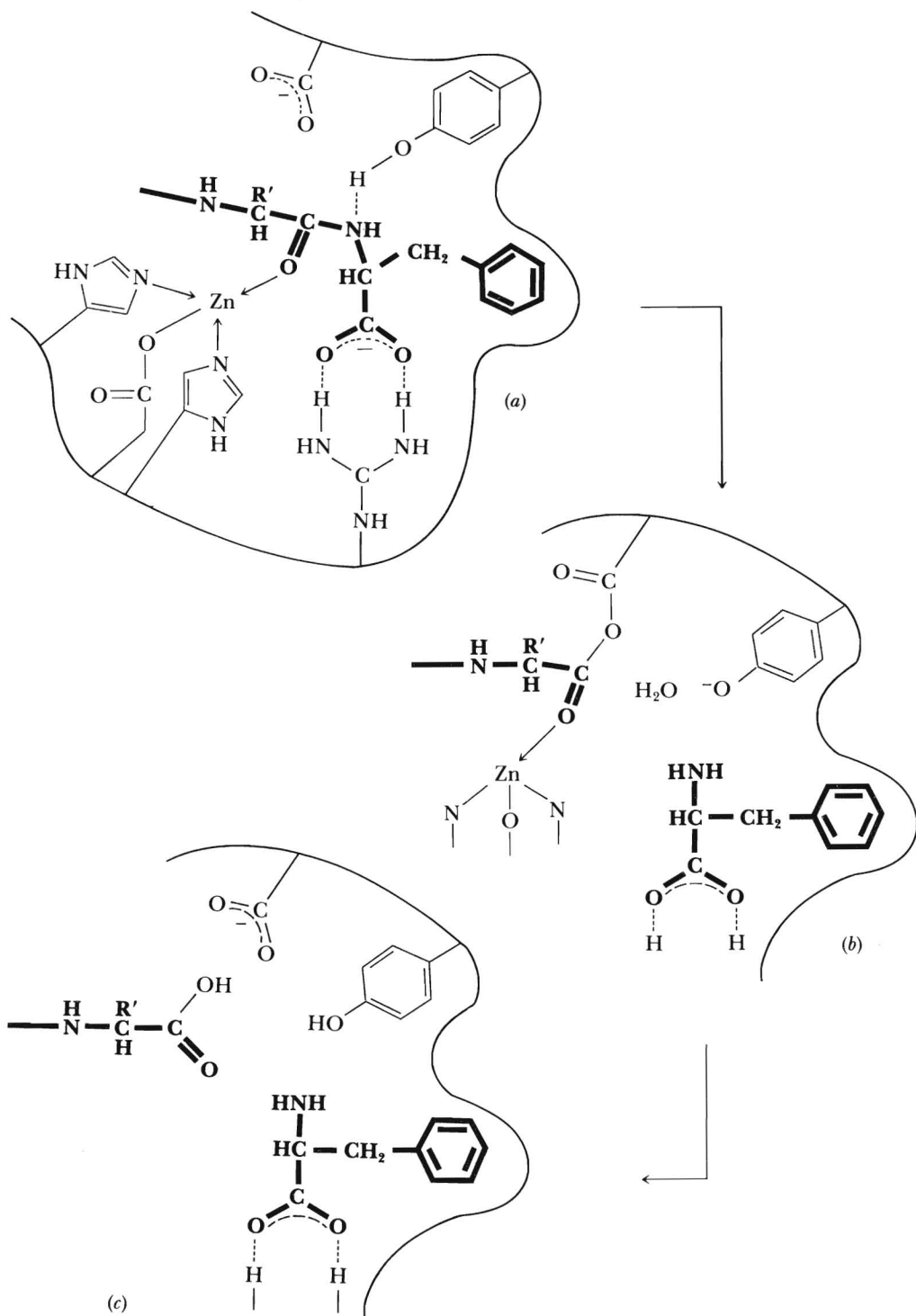


Figure 31-14 (a) A proposed mode of binding of the substrate in carboxypeptidase. The substrate is shown in heavy type and lines. The curved line schematically defines the “surface” of the enzyme molecule. (b) A possible first step in the mechanism, wherein a carboxyl side chain attacks the carbonyl carbon atom, forming an anhydride. (c) Subsequent steps in the proposed mechanism, including hydrolysis of the intermediate anhydride and dissociation of the products from the active site.

The structure and main mechanistic features of carboxypeptidase have been elucidated. The zinc ion is bound in a distorted tetrahedral environment, with two histidine nitrogen atoms, one glutamate carboxyl oxygen atom and a water molecule as ligands. The binding of the substrate probably occurs as shown in Fig. 31-14(a). Notice that the carbonyl oxygen atom of the peptide linkage that is to be broken has replaced the water molecule in the coordination sphere of the zinc ion.

The key step in a possible, but speculative, mechanism is shown in Fig. 31-14(b). Once the peptide bond has been broken with formation of the acid anhydride, rapid hydrolysis of the anhydride would occur, as in Fig. 31-14(c). The products would then vacate the active site, leaving it ready to bind another molecule of substrate and repeat the cycle.

Copper Metalloenzymes

More than 20 of these have been isolated, but in no case is structure or function well understood. The copper enzymes are mostly oxidases, that is, enzymes that catalyze oxidations. Examples are (1) *Ascorbic acid oxidase* (MW = 140,000; 8 Cu), which is widely distributed in plants and microorganisms. It catalyzes oxidation of ascorbic acid (vitamin C) to dehydroascorbic acid. (2) *Cytochrome oxidase*, the terminal electron acceptor in the oxidative pathway of cell mitochondria. This enzyme also contains heme. (3) Various *tyrosinases*, which catalyze the formation of pigments (melanins) in a host of plants and animals.

In many lower animals, such as crabs and snails, the oxygen-carrying molecule is a copper-containing protein *hemocyanin*, which despite the name, contains no heme group. The hemocyanins represent the third system in Nature (besides hemoglobins and hemerythrins) for oxygen carrying from the point of intake to those tissues where O_2 is required. Like hemoglobin, hemocyanins have many subunits in the complete molecule and, therefore, exhibit cooperativity in O_2 binding. The active sites consist of two copper atoms ($\sim 3.8 \text{ \AA}$ apart) that jointly bind one O_2 molecule. The way they do this apparently involves the conversion of the colorless $Cu^I \cdots Cu^I$ deoxy center to a peroxide-bridged $Cu^{II}-O-O-Cu^{II}$, which is bright blue.

31-10 Nitrogen Fixation

Elemental nitrogen (N_2) is relatively unreactive. In order to "fix" nitrogen, that is, make nitrogen react with other substances to produce nitrogen compounds, it is generally necessary to use energy-rich conditions. High temperatures or electrical discharges can supply the necessary activation energy. However, primitive bacteria and some blue-green algae can fix nitrogen under mild conditions, that is, ambient temperature and pressure. Metalloenzymes play a key role in this process.

Bacterial Nitrogenase Systems

Our more detailed information about nitrogen fixation comes mainly from studies of free-living soil bacteria. These can be cultured in the laboratory and essential components can be isolated and purified. Biological nitrogen fixation is

a reductive process. An important fact, which was established by using $^{15}\text{N}_2$, is that the first recognizable product is always NH_3 . Apparently, all intermediates remain bound to the enzyme system.

It has been known since 1930 that molybdenum is essential for bacterial nitrogen fixation, since this function can be turned off and on by removing and then restoring molybdenum to the environment. Magnesium and iron are also essential components.

In 1960, the first active cell-free extracts were prepared, and since then, *nitrogenases*, as the enzymes are called, have been obtained in fairly pure condition from several bacteria. In each case the nitrogenase can be separated into two proteins, one with molecular weight of about 260,000 (the Fe-protein) and the other around 240,000 (the MoFe protein). Neither of these proteins is separately active, but on mixing them activity is obtained immediately. The Fe-protein consists of two identical subunits that clasp a ferredoxin unit (Fe_4S_4) between them by forming Fe—S bonds to two cysteine residues in each subunit. It is believed that the Fe-protein plays its role by coupling electron transfer and hydrolysis of ATP, but that the actual conversion of N_2 to NH_3 is carried out at the active site of the larger protein, the MoFe-protein, so-called because it contains both molybdenum and iron.

Until very recently, there has been no direct indication of how the iron and molybdenum atoms are arranged in the MoFe protein, nor did we have any completely reliable knowledge of exactly how many of each type of metal atom is present. However, in late 1992 an X-ray crystallographic study revealed a metal cluster arrangement, as shown in Fig. 31-15.

This structure is still somewhat inaccurate and one of the bridging groups (Y) has not yet been conclusively identified. Overall, the structure has had an enormous impact. Previously, it had been correctly assumed that some sort of mixed iron-molybdenum-sulfur species was present, but it was also assumed that the molybdenum atom was the seat of reactivity, that is, the atom to which N_2 would first become attached and then reduced. In view of the apparent coordinative saturation of the Mo atom and the possibility that the middle part of the cluster, where the two halves are joined by the $\mu\text{-S}$, $\mu\text{-S}$, and $\mu\text{-Y}$ linkages, might be capable of accepting the N_2 molecule and retaining the various intermediates, the mechanism of action might be quite different from what was previously imagined.

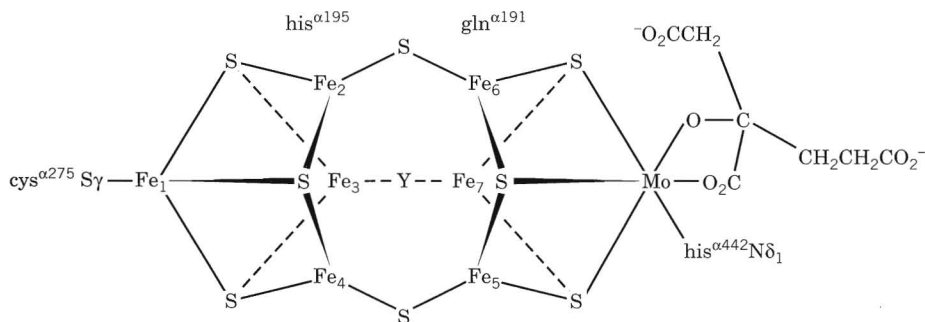


Fig. 31-15 The Fe₇Mo-sulfur cluster system, and its immediate surroundings, found in the MoFe-protein of nitrogenase.

STUDY GUIDE

Scope and Purpose

We have sketched some important inorganic aspects of the chemistry of life. This has been an area of great recent interest among researchers, and new understandings develop so frequently, that the reader should expect to consult recent journal articles for more up-to-date information. Continued study in the references provided under "Supplementary Reading" is highly encouraged.

This chapter's major message is that the chemistry of life involves more than 20 elements besides those traditionally treated in organic chemistry. Though these other elements tend to have limited roles, life processes require them just as surely as they require proteins, carbohydrates, and lipids.

Study Questions

A. Review

1. Name four transition metals and two nontransition metals that play important roles in biological processes.
2. Draw the structure of porphine and explain how the structures of heme and chlorophyll are related to it.
3. What role does the magnesium ion play in the functioning of chlorophyll?
4. What constitutes a heme protein? Name three of them.
5. What are the functions of hemoglobin and myoglobin? What are the principal similarities in their structures?
6. What changes occur in the heme groups of hemoglobin on going from deoxy- to oxy-hemoglobin?
7. What is the structure of the redox center of HiPIP and of the 4-Fe and 8-Fe ferredoxins?
8. What functions do ferrichromes and ferrioxamines have? What are their chief chemical features?
9. State the main components of cobalamin. How do B_{12} , B_{12r} , and B_{12s} differ?
10. What role does the zinc ion play in the action of carboxypeptidase?
11. What is the principal function of nitrogenase?
12. List the ways in which the cobaloximes resemble cobalamin.

C. Questions from the Literature of Inorganic Chemistry

1. Consider the paper by J. Halpern, "Mechanisms of Coenzyme B_{12} -Dependent Rearrangements," *Science*, **1985**, 227, 869.
 - (a) What is the significance of the observation that reactions involving the coenzyme B_{12} give scrambling of the methylene hydrogens from the 5'-deoxyadenosine of the coenzyme with the hydrogen atom involved in the migration [e.g., Eq. (1)] at the substrate?
 - (b) Through what various spin states does the cobalt atom of the coenzyme B_{12} progress during the operation of the mechanism shown in Fig. 2 of this article? What is the difference in the number of d electrons on B_{12} and B_{12r} ?
 - (c) What factors are said to influence the critical cobalt-carbon bond dissociation energies?

- (d) What features do the “DH” and the “saloph” cobalt complex model systems have in common with the coenzyme B_{12} ?
 - (e) What analogy does the author draw between the reversible cobalt–carbon bond dissociation of coenzyme B_{12} and the reversible binding of dioxygen as in Eqs. 23 and 24?
2. Consider the extensive work by J. P. Collman and students, represented by the following paper, and the references therein: J. P. Collman, J. I. Brauman, B. I. Iverson, J. L. Sessler, R. M. Morris, and Q. H. Gibson, *J. Am. Chem. Soc.*, **1983**, *105*, 3052.
 - (a) What are the main similarities and differences, structurally, between the “picket fence” and “pocket” porphyrins that are described in this article?
 - (b) How is solvation thought to reduce affinities for O_2 of the unprotected iron(II) porphyrins?
 - (c) What advantages in O_2 binding do the “picket fence” and “pocket” porphyrins have over those iron(II) porphyrins that are “unprotected” from solvation effects?
 - (d) How do the O_2 and CO affinities of the “picket fence” porphyrins compare with those of the “pocket” porphyrins?
 - (e) What geometries for $M-O_2$ and $M-CO$ groups seem to make sense in explaining the observations in (d)?
 3. Consider the work by J. Chatt on nitrogen fixation analogs: J. Chatt, A. J. Pearman, and R. L. Richards, *J. Chem. Soc. Dalton Trans.*, **1977**, 1852.
 - (a) The N_2 complexes reported here are protonated to give ammonia. How is this reaction of interest to the molybdenum nitrogenase systems?
 - (b) In other studies mentioned in the introduction to this paper, other complexes were protonated to give not ammonia, but intermediate reduction products. Enumerate the findings concerning the formation of diazenido, diazine, and hydrazido ligands.
 - (c) What is the difference between protonation of the N_2 ligand in complexes containing two bidentate dppe ligands and protonation of N_2 ligand in complexes containing four monodentate $P(CH_3)_2C_6H_5$ ligands? What bonding arguments do the authors present to account for these differences?
 - (d) At what stage do the authors propose a splitting of the $N-N$ bond? When is this likely to occur in the overall stepwise process that is proposed?
 - (e) How is the oxidation state of the metal at the end of reaction sequence (5) different from the oxidation state that is likely in the enzymic system? How do the authors propose that the enzyme avoids this high an oxidation state?

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