Arthur S. Brill

Transition Metals in Biochemistry

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With 49 Figures

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Preface

Transition metal ions in biological systems are of interest in biology, biochemistry, chemistry, medicine, and physics. Scientists with rather different viewpoints, employing many methods, have contributed to this area. A concise review of the current state of the field will, to some extent, reflect the special knowledge of the person writing it – in this case application of physical methods to the investigation of metal coordination. X-ray diffraction is one of the most important of these methods, but a useful treatment of X-ray structure analysis would be comparable in size with and beyond the scope of the monograph. Many results of X-ray diffraction studies are, of course, presented. Electron paramagnetic resonance spectroscopy has played a major part in the rapid advance in knowledge of the electronic structures of transition metal ions in biological systems. More generally, measurements involving light, microwaves, and magnetic fields are capable of producing much new information, and the required instrumentation is available at most research institutions. Therefore light absorption and paramagnetic resonance are treated in depth. The principles described in the latter discussions are broadly applicable, for example to the promising techniques of X-ray spectroscopy (utilizing synchrotron radiation) and lanthanide-perturbed, very high-resolution nuclear magnetic resonance spectroscopy.

Those aspects of theory are presented which can be directly employed in the quantification and interpretation of experimental data; sufficient background, formulas, and examples are given so that the reader is equipped to understand similar analyses in the literature and to begin to proceed on his own. Only material generally accepted as factual is given in the text; references are provided so that the reader is able to find and evaluate for himself recent data and current speculation. The author believes that the monograph can aid in providing a basis for closer communication among scientists of different backgrounds with a common interest in the biochemistry of transition metal ions.

I thank many colleagues for help in the forms of photographs of figures, references, criticism, and encouragement.

Charlottesville, Virginia
April, 1977

ARTHUR S. BRILL

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Chapter 1

The Role of Transition Metal Ions in Biological Oxidation and Related Processes

Proteins perform many functions in support of life. Some serve as structural materials; others transport gases in the bloodstream between lungs and tissues; still others are involved in blood coagulation; a few are hormones. Large in number and diverse in structure are the enzymes, catalysts for the multitude of chemical reactions of degradation, synthesis, and transformation continuously going on in all biological systems. Many enzymes, particularly those involved in oxidation-reduction reactions, contain metal ions of the first and second "transition series" as "prosthetic groups". We begin by describing what is meant by the terms "transition series" and "prosthetic groups".

1. Transition Metal Ions

The extra-nuclear description of an atom or ion starts with its ground state configuration which specifies the number of electrons in each spatial orbital. Thus the electron configuration of argon is

$$(1s)^2(2s)^2(2p)^6(3s)^2(3p)^6$$

where, for example, $2p^6$ indicates that there is a pair of electrons in each of the three spatial orbitals characterized by principal quantum number 2 and orbital angular momentum quantum number 1 (see Chap. 6-1). The 2p "shell" can, by the Pauli principle, hold just six electrons and is seen to be filled in this example. Indeed, all s, p, and d shells can contain at most two, six, and ten electrons respectively.

The transition groups or series in the periodic table are those in which an inner shell is being filled with electrons while an outer shell of slightly lower energy remains populated. In the first or iron group of transition elements, it is the 3d shell which receives additional electrons after the 4s shell has initially received two. The latter two electrons come into 4s with potassium and calcium, and the first transition element is scandium with the following electron configuration:

$$(1s)^2(2s)^2(2p)^6(3s)^2(3p)^6(3d)^1(4s)^2.$$

The ground configurations of ions of the iron group are given in Table 1-1. While zinc is not considered a transition element, we include the zinc ion in Table 1-1 so that it can be related electronically to members of the 3d group. Table 1-1 is discussed

Table 1-1. Ground states of ions of the iron group

Ions	Ground configuration[a]	Free ion ground state
Ti(III)	$3d^1$	^2D
V(III)	$3d^2$	^3F
Cr(III),V(II)	$3d^3$	^4F
Cr(II)	$3d^4$	^5D
Fe(III),Mn(II)	$3d^5$	^6S
Co(III),Fe(II)	$3d^6$	^5D
Co(II)	$3d^7$	^4F
Ni(II)	$3d^8$	^3F
Cu(II)	$3d^9$	^2D
Zn(II),Cu(I)	$3d^{10}$	^1S

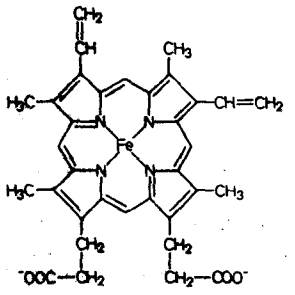
[a]Not explicitly shown is the closed shell (argon) inner configuration, e.g., Ti(III): $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^1$

further in Chapter 6-3. The iron series has among its members several elements of widespread biological occurrence and of great importance in oxygen transport and utilization, and, more generally, in biological oxidation-reduction catalysis, biosynthesis, and in other metabolic processes. The next transition series, the palladium group, is characterized by the filling of the 4d shell. In this series only molybdenum plays an appreciable role in living systems. The remaining transition groups (the lanthanides or "rare earths", a 4f series; the platinum group, a 5d series; and the actinide series, 5f) are not known to be of biological importance.

2. Prosthetic Groups

As an adjective, the word "protein" describes molecules, or those parts of molecules, which are composed entirely of α-amino acid residues, covalently united head-to-tail by peptide bonds to form long, unbranched polymers. These polymers yield only amino acids upon hydrolysis. The reader is assumed to be familiar with the structural chemistry of such polypeptide chains. As a noun, the word protein is commonly used for two kinds of molecule. "Simple" proteins are as just described. In "conjugated" proteins, there are, in addition, firmly bonded non-amino acid com-

Fig. 1-1. Heme



ponents which can be organic or inorganic (or both) in nature. These components are called "prosthetic groups".

The contribution of a prosthetic group to the bulk of a conjugated protein varies greatly. Consider, for example, azurins and myoglobins, both of which have a molecular weight in the neighborhood of 16,000 daltons. The former proteins contain a single copper ion as prosthetic group, the metal contributing about 0.4% of the weight. The myoglobin molecules have iron protoporphyrin IX or "heme" (Fig. 1-1) as prosthetic group, this complicated compound constituting about 4% of the weight. In cytochrome c_3, with three or four heme groups per molecule of weight 12,000 daltons, the prosthetic mass is 17 to 22% of the total.

The terms cofactor, coenzyme, and prosthetic group are not synonymous. If an enzyme requires a nonamino acid derived component for its activity, the component is called a "cofactor". If the cofactor is not simply a metal ion but is a complex organic or organometallic group, it is called a "coenzyme". The "holoenzyme" is the entire, active, enzyme molecule consisting of the cofactor plus the protein moiety. The protein moiety, which by itself is not active, is called an "apoenzyme". The attachment of cofactors to apoenzymes can vary from strong and permanent (covalent) to weak and dissociable. Prosthetic groups are, by definition, stably bound components of conjugated proteins. Thus, some cofactors are prosthetic groups (e.g. the heme group of cytochrome c), some are not (e.g. nicotinamide adenine dinucleotide), and conversely (e.g. hemoglobin is not an enzyme, and the heme prosthetic groups in it are not cofactors).

3. Equilibrium Considerations in Reactions of Transition Metals

a) Site Stability: Equivalent Ligands, Chelation, and Other Factors

In the binding of metal ions to proteins there is competition between these ions and protons for the ionizable residues which serve as ligands. The dissociation of protons from proteins is governed by

$$\log \frac{x_i}{1 - x_i} = pH - pK_i + 0.868\ w\ Z$$

where x_i is the extent of proton dissociation, pK_i is the intrinsic pK for group i, and 0.868 w Z is an electrostatic correction factor which depends upon the net charge Z (Tanford, 1961). If the charge on the macromolecule under acid conditions (ionizable groups all protonated) is Z_a, the charge at an arbitrary pH is

$$Z = Z_a + m\bar{v} - R$$

where m is the charge on the metal ion, $\bar{v}$ is the average number of metal ions bound (per molecule), R is the number of protons dissociated, and it is assumed that no other charged species are bound in significant amount. Clearly Z is highly pH dependent. In pH regions where there is neither protein conformational change nor association of protein units, w is approximately constant and the electrostatic correction factor is proportional to the net charge Z. Whether or not this is the case, the following relation describes the association of the metal ion with residue j:

$$\log \frac{\bar{v}_j}{(1-\bar{v}_j)(F-\bar{v})} = \log x_j + \log P + \log K - 0.868\ mwZ$$

where the protein concentration is P, F is the ratio of the total number of metal ions to the total number of protein molecules, and K is the intrinsic association constant between metal ions and the ligand j. The binding of each metal ion eliminates the attached ligands from the equilibrium given in the first equation, a situation explicitly included in the next relation which gives the hydrogen ion dissociation function (titration curve) R:

$$R = \sum_{\text{all } i} \bar{v}_i + \sum_{\text{all } i} (1-\bar{v}_i) x_i .$$

In the four equations above, the unknowns (each a function of pH) are the x_i, the $\bar{v}_i$, $\bar{v}$, w, Z, and R, of which only R and sometimes $\bar{v}$ can be readily measured. In practice the application of these equations is considerably simplified by the large range of the pK_i (many of the ionizing groups do not have significantly overlapping regions of titration) and by the frequent occurrence of two or more identical ligands in the coordination sphere. If we denote the site consisting of ℓ identical ligands of type j by ℓ_j, the second equation above becomes

$$\log \frac{\bar{v}_{\ell j}}{(1-\bar{v}_{\ell j})(F-\bar{v})} = \ell \log x_j + \log P + \ell \log K - .868\ mwZ$$

since the fraction of these sites free of protons is given by $(1-\bar{v}_{\ell j})(x_j)^{\ell}$.

Consider now the stability of a metal-protein complex against dropping pH under the conditions F = 1 (total amount of metal present corresponds to that in the fully formed complex) and $\bar{v}_{\ell j} = \bar{v}$ (ligands involved are only of type j). The preceding equation becomes

$$\log \frac{\bar{v}}{(1-\bar{v})^2} = \ell \log x_j + \log P + \ell \log K - .868\ mwZ$$

which we rewrite in the form

$$(x_j)^{\ell} = \frac{\bar{v}}{(1-\bar{v})^2} \frac{1}{(K')^{\ell} P}$$

where $\ell \log K' = \ell \log K - .868\ mwZ$ defines K'. The value of x_j (the dissociation of free groups j) corresponding to 50% complex formation ($\bar{v} = 1/2$) is then

$$x_j = \frac{1}{K'} \left(\frac{2}{P}\right)^{\frac{1}{\ell}}.$$

The effect of ℓ upon stability can be seen by taking typical values of P (10^{-4} M) and K' (5×10^2). Then for ℓ = 1,2,3,4 one finds x_j = 40, 0.28, 0.054, 0.023, where $(x_j)_{max} = 1$. For these P and K' values the ℓ = 1 complex cannot ever be fully formed, the ℓ = 2 complex forms in the pH region where group j titrates in the absence of the metal, and the ℓ = 3 and 4 complexes are forming at pHs well below the latter titration region. Furthermore, in the region where the change in metal bound with pH is greatest, the buffering capacity dR/dpH is proportional to ℓ^2 (Brill and Venable, 1967). It follows that the slope of the metal association function increases as ℓ^2. In short, as ℓ increases, the apparent pK of the ionizing groups to which the metal ions are bound decreases and the slope of the metal association function increases, the two factors together extending the stability of the complexes to significantly lower pH.

It should be noted that, if a metal ion is on an n-fold symmetry axis, there will be stabilization of the site since it is then necessary for ℓ to be greater than or equal to n.

In the binding of independent small molecule ligands, the entropy decrease accompanying the formation of a link is responsible for a significant positive contribution to the free energy change. When two or more ligand atoms of a molecule are fixed in the proper position to bind to a metal ion, the entropy change is reduced and the association constant increases, a well-known phenomenon called *chelation*. Clearly the quadridentate nitrogen structure of the inner ring of porphyrin is strongly chelating. While, in the absence of a metal ion, the ligands of binding sites on proteins move relative to each other, they are constrained compared with small molecules in solution. Thus binding sites on proteins have at least some chelation character, and stability is gained through this.

Apart from the effects just discussed, the association constant of a complex is influenced by the natures both of the ligands and the metal ion. All the factors involved cannot realistically be combined into a simple mathematical formula, but useful generalizations can be made in such terms as polarizability, "hard" and "soft" acids and bases, and binding order (e.g. the Irving-Williams series for divalent metal ions (Angelici, 1973; Buckingham, 1973)).

b) Electron Transfer and Redox Potentials

The Nernst equation gives the free energy change, expressed as EMF (electromotive force or voltage), for the n electron reduction of an electron acceptor (oxidant):

$$\text{electron acceptor} + ne^- \longleftrightarrow \text{electron donor}$$

$$E = E^\circ + \frac{2.303RT}{nF} \log \frac{\text{acceptor activity}}{\text{donor activity}} .$$

$E^\circ = E^{\circ\prime}$ is the "standard (redox) potential" corresponding to 1 M concentrations of reductant and oxidant in solution at pH 7.0, 25°C, under 1.0 atm pressure, as measured against a standard (-0.42 volt) hydrogen (gas, 1.0 atm) - hydrogen ion half-cell at the same pH and temperature. At this temperature

$$E(\text{volts}) = E^{\circ\prime} + \frac{0.059}{n} \log \frac{\gamma_a[\text{acceptor}]}{\gamma_d[\text{donor}]}$$

where γ_i, the activity coefficients, are unity under the standard conditions. Closely related to $E^{\circ\prime}$ are the "midpoint" or "half-reduction" potentials, E_m, obtained when the acceptor and donor are at the same concentration. To the extent that the measured EMF and hence the γ_i, are independent of this concentration, $E_m = E^{\circ\prime}$. The more positive these potentials are, the greater the oxidizing potential and, as in an ordinary battery, the greater the affinity of the positive electrode for electrons.

The transfer of electrons is often accompanied by the release or uptake of protons, (as would be associated with a change in the number of groups coordinated to the metal), e.g.

$$\text{electron acceptor} + ne^- \longleftrightarrow \text{electron donor} + mH^+,$$

a process with which is associated a pH dependent EMF of -0.059 m/n volt per pH unit at 25°. Thus potentiometric titration provides a three-dimensional surface on which $E^{\circ\prime}$ (or E_m) is a function of both the concentration of an oxidizing agent and the pH. From such data one can obtain the number n of electrons transferred and the number m of protons involved in the reaction.

In electron transfer reactions involving transition metal valence couples (e.g. cuprous, cupric), n will almost always be 1.0 since the usual case is a unit change in metal valence without any change in ligand oxidation state. No general statement can be made about m and pH dependence. In protein complexes it is not uncommon for m to be different in different regions of pH. Standard (or midpoint) potentials for copper and iron in representative proteins are given in Table 1-2. The couples are either cuprous, cupric or ferrous, ferric so that in each case n = 1.0. Where known, the pH dependence is given. Williams (1976) reviews factors involved in the potentials of one-electron reactions.

Table 1-2. Redox potentials for unit valence changes in metal components of some proteins

Protein	$E^{0'}$ (or E_m) (mV)	$dE^{0'}/dpH$ (mV/pH unit)	
		pH < 7	pH > 7
Azurin (Ps.)	+330 (pH 6.4)		
Plastocyanin	+380	-60	0
Peroxidases	-190 (average)	see Ricard et al. (1972)	
Myoglobin	+ 50		
Cytochromes b	~ 0 (average)	~ 0	-60
Cytochromes c	+250 (average)	~ 0	~ 0
Cytochrome a	+210	~20	~20
Cytochrome a_3	+390	0	60
Ferredoxins	-400 (average)		
Rubredoxins	- 60 average)		
High potential iron-sulfur protein	+350		

References: Dus et al. (1967); Dutton et al. (1970); Hughes (1975); Malkin and Malmström (1970); Palmer (1975); Ricard et al. (1972); Wilson and Dutton (1970); Wilson and Leigh (1972); Wilson et al. (1972a,b)

Transition metals in several proteins (e.g. the cupric ion in azurin and the ferric ion in cytochrome c) are "spontaneously" reduced at alkaline pH (> 10). In the presence of an oxidant such as ferricyanide, cycling between valence states will proceed. Quantitative measurements of the consumption of the oxidant reveal levels of reductant far in excess of the protein concentration (Brill et al., 1968). Not yet known are the identity of the reductant and the nature of the process.

4. Molecular Functions of Proteins Containing Transition Metal Ion Prosthetic Groups

Part of the material discussed in this section can be found in various sections of comprehensive textbooks of biochemistry (e.g. Cantarow and Schepartz, 1967; Lehninger, 1975). Details of topics can be filled in and differences of viewpoint and emphasis explored by consulting more specialized expositions such as those appearing in Antonini and Brunori (1971), Boyer (1975, 1976), Buchanan and Arnon (1970), Burns and Hardy (1975), Chance et al. (1966), Chance et al. (1971), Dunford and Stillman (1976), Florkin and Stotz (1966), Ghiretti (1968), Gray (1971), Hayaishi (1969), King et al. (1973), Lemberg and Barrett (1973), Peisach et al. (1966), Weissbluth (1974). Frieden (1974) discusses rela-

tions among the molecular functions of iron and copper proteins and finds that these considerations suggest an evolutionary sequence in respiratory biochemistry.

a) Oxygen Carriage

Substances which are to carry or store molecular oxygen in living systems must have the capacity to take up the gas and to release it under suitable conditions. This function is elegantly met by the well-known protein hemoglobin, a tetramer of molecular weight 65,000 daltons, each subunit of which contains a ferrous protoporphyrin IX prosthetic group. The subunits interact ("cooperate") in such a way that the equilibrium saturation is an S-shaped function of oxygen pressure. Under physiological conditions, this dissociation behavior increases the efficiency of gas loading and unloading by hemoglobin as compared with myoglobin (a monomer with the same prosthetic group) which exhibits the customary hyperbolic dissociation curve. The nature of cooperativity in hemoglobin has been and remains a topic of considerable interest.

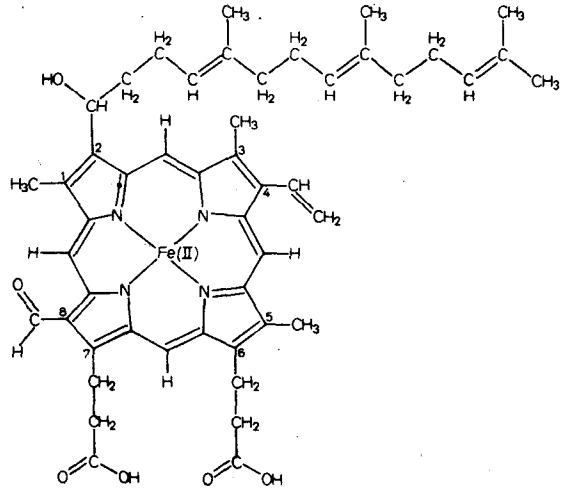
The iron-containing proteins chlorocruorin and hemerythrin also transport oxygen. The prosthetic group of chlorocruorin is a heme, but the porphyrin moiety is not the same as in hemoglobin and myoglobin. Hemerythrin is not a heme protein but resembles hemoglobin in having oxy, deoxy, and ferric (met) forms. The hemocyanins are a large class of oxygen-transporting proteins which contain copper (not in a porphyrin complex) and no heme. Vanadium complexes in combination with protein were believed to serve as the carrier of oxygen in sea squirts, but recent data do not support this view.

b) Oxygen Utilization

Depending upon the size and development of the aerobic organism, molecular oxygen will move to the cells requiring it either in solution or else largely carried by one of the proteins just discussed. In small, simple cells (prokaryotes), just within the wall there is a membrane to which oxygen molecules diffuse and where the reduction of these molecules is catalyzed by several kinds of enzyme, with practical consequences (cellular respiration). In complex cells (eukaryotes), the oxygen molecules diffuse inside to the membranous organelles (mitochondria, microbodies) which contain the enzymes of cellular respiration.

Cytochrome c oxidase is found in all aerobic cells. At concentrations of cyanide which effectively inhibit cytochrome oxidase, the oxygen uptake of these cells is severely reduced and death results. This most important enzyme reacts directly with molecular oxygen, transferring to it electrons (received from cytochrome c) and directing the negative free energy made available in this process to the phosphorylation of adenosine diphosphate. Cytochrome oxidase is known to contain two iron atoms,

Fig. 1-2. Heme a. (After Caughey et al., 1975)



bound in heme a[1] (Fig. 1-2) and two copper atoms. The two hemes are spectroscopically distinguishable in the response of the enzyme to chemical changes and have been designated a and a_3 for some time, where a_3 is the autoxidizable site (Atmungsferment of Warburg). In view of the dependence of the chemical reactivity and spectrally revealed electronic structure of each of these heme sites upon the state of the other, a and a_3 must be regarded as components of one enzyme. Because the reaction with oxygen and succeeding processes are exceedingly fast at room temperature, it has been difficult to resolve functional intermediates in the sequence of electron transfers. However, after flash photolysis of the carbon monoxide-a_3 complex in the presence of dissolved oxygen at -100°C, it is possible to observe recombination of a_3 with the oxygen followed by a succession of intramolecular oxidations (Chance et al., 1975). These experiments indicate that copper is oxidized before "cytochrome a", and hence that the copper sites as well as a_3 and a are components of a single enzyme. Cytochrome oxidase consists of several units, estimates ranging from two to six, and has a total molecular weight of about 200,000 daltons.

The overall reaction of the respiratory chain can be written

$$2NADH + 2H^+ + 6ADP + 6P_i + O_2 \rightarrow 2NAD^+ + 8H_2O + 6ATP$$

where NADH and NAD^+ are the reduced and oxidized forms of nicotinamide adenine dinucleotide respectively (Fig. 1-3). ADP and ATP are adenosine di- and triphosphate, respectively, and P_i is inorganic orthophosphate. Both atoms of oxygen are reduced to water and only electrons are transferred from the NADH. Apart from the (important) occurrence of phosphorylations, all of the enzymes of the respiratory chain could be called electron transferases. Thus cytochrome oxidase engages in electron transport

[1]Other heme(s) may be present in certain bacterial cytochrome oxidases.

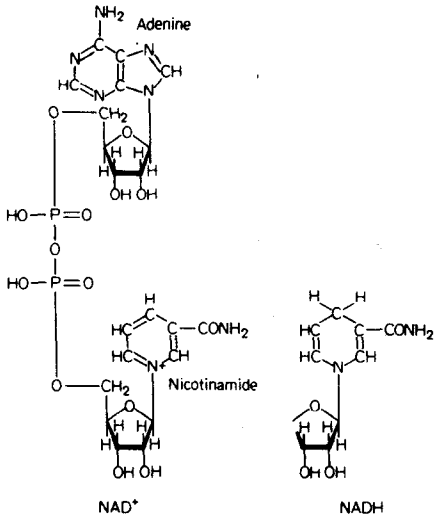


Fig. 1-3. Nicotinamide adenine dinucleotide: oxidized form: *NAD*$^+$; reduced form: *NADH*

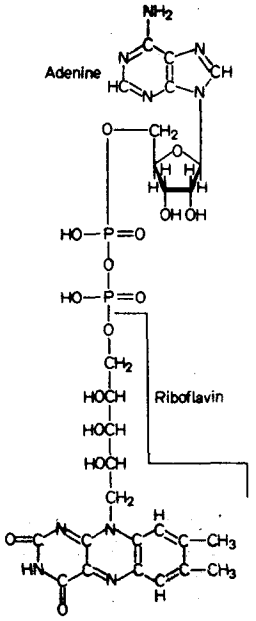
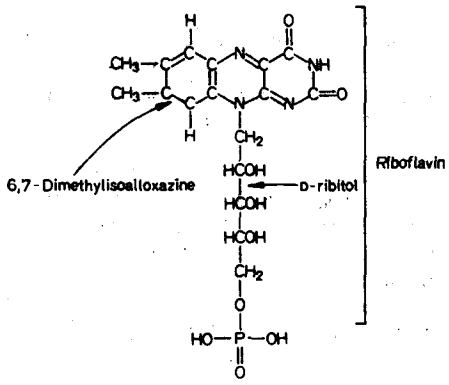


Fig. 1-4. Flavin adenine dinucleotide (FAD)

to oxygen and not in the addition of oxygen to another molecule. There are blue, copper-containing proteins, not in the respiratory chain and not otherwise producing high-energy compounds, that catalyze the transfer of electrons from substrate to molecular oxygen with dehydrogenation of substrate and formation of water. Ascorbic acid oxidase and the laccases are well-established examples. Ceruloplasmin is a blue protein containing seven copper atoms per mole (160,000 daltons), which is found in the blood plasma of most vertebrates. It has recently been established that ceruloplasmin effectively catalyzes the oxidation of the ferrous ion to ferric by molecular oxygen, and the protein is now called ferroxidase. There are a larger number of enzymes which catalyze the transfer of electrons from substrate to oxygen molecules with dehydrogenation of substrate and formation of hydrogen peroxide (or precursor superoxide radicals). This is the case in the reactions catalyzed by the copper-containing enzymes galactose oxidase, uricase, monoamine oxidase and diamine oxidase. The latter two proteins may also contain organic prosthetic factors (flavin or pyridoxal phosphate). The complex protein xanthine oxidase, which contains eight iron atoms, two molybdenum atoms, and two FAD groups (flavin adenine dinucleotide, Fig. 1-4), and has a molecular weight of 300,000 daltons, utilizes molecular oxygen to oxidize certain purines by hydrogen removal, superoxide or peroxide being formed. All of the iron atoms are believed to be present in complexes with an equal number of acid-labile (inorganic) sulfurs. Iron proteins in which the iron is coordinated to sulfur, either inorganic or from cysteine, are now generally called "iron-sulfur" proteins. Aldehyde oxidase is closely related to xanthine oxidase in composition and function. Dihydroorotic dehydrogenase,

Fig. 1-5. Flavin mononucleotide (FMN)



an iron-sulfur protein which contains also both FMN (flavin mononucleotide, Fig. 1-5) and FAD, catalyzes the dehydrogenation of dihydroorotic acid with passage of electrons to oxygen molecules. NAD^+ is a better electron acceptor than oxygen for this system.

In the reactions just discussed, no oxygen atoms are transferred to the substrate. At the other extreme are the oxygen transferases or oxygenases which insert both atoms of the oxygen molecule into the substrate. The iron-containing proteins pyrocatechase, protocatechuic oxidase, homogentisic acid oxidase, and tryptophan oxidase are in this category. In between are the hydroxylases or mixed-function or mono-oxygenases which insert one atom of molecular oxygen into a substrate molecule, the other atom reacting with a separate reducing agent. For example cytochrome P_{450}, found in nonphosphorylating electron transport chains (extramitochondrial) utilizes molecular oxygen to hydroxylate substrates. Other examples are the iron-containing protein proline hydroxylase and the copper-containing proteins tyrosinase and dopamine-β-hydroxylase.

c) Hydroperoxide Reactions

Peroxidases and catalase are heme-containing proteins which catalyze reactions between hydroperoxides (e.g. H_2O_2, hydrogen peroxide; MeOOH, methyl hydroperoxide; EtOOH, ethyl hydroperoxide) and many oxidizable compounds. The defining difference between peroxidases and catalase is in their effectiveness in catalyzing the decomposition of H_2O_2 into oxygen and water, the so-called "catalatic" reaction, catalase being 10^4 more effective. One molecule of catalase cycling through the reaction sequence can free a million molecules of oxygen from hydrogen peroxide (in buffered aqueous solution at room temperature) in one minute. Peroxidases efficiently catalyze the oxidation of hydrogen donors (other than H_2O_2) by H_2O_2. Catalase will do this efficiently only when the level of H_2O_2 is kept relatively low. Catalase from all sources consists of four subunits with a total molecular weight of about 250,000 daltons; four hemes are present. The molecular weights of the peroxidases are variable, averaging about one-fourth that of catalase. The most studied