

# Transition-Metal Storage, Transport, and Biomineralization

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## I. GENERAL PRINCIPLES

### A. Biological Significance of Iron, Zinc, Copper, Molybdenum, Cobalt, Chromium, Vanadium, and Nickel

Living organisms store and transport transition metals both to provide appropriate concentrations of them for use in metalloproteins or cofactors and to protect themselves against the toxic effects of metal excesses; metalloproteins and metal cofactors are found in plants, animals, and microorganisms. The normal concentration range for each metal in biological systems is narrow, with both deficiencies and excesses causing pathological changes. In multicellular organisms, composed of a variety of specialized cell types, the storage of transition metals and the synthesis of the transporter molecules are not carried out by all types of cells, but rather by specific cells that specialize in these tasks. The form of the metals is always ionic, but the oxidation state can vary, depending on biological needs. Transition metals for which biological storage and transport are significant are, in order of decreasing abundance in living organisms: iron, zinc, copper, molybdenum, cobalt, chromium, vanadium, and nickel. Although zinc is not strictly a transition metal, it shares many bioinorganic properties with transition metals and is considered with them in this chapter. Knowledge of iron storage and transport is more complete than for any other metal in the group.

The transition metals and zinc are among the least abundant metal ions in the sea water from which contemporary organisms are thought to have evolved (Table 1.1).<sup>1-5</sup> For many of the metals, the concentration in human blood plasma

Table 1.1  
Concentrations of transition metals  
and zinc in sea water and human  
plasma.<sup>a</sup>

Element	Sea water (M) × 10 <sup>8</sup>	Human plasma (M) × 10 <sup>8</sup>
Fe	0.005–2	2230
Zn	8.0	1720
Cu	1.0	1650
Mo	10.0	1000
Co	0.7	0.0025
Cr	0.4	5.5
V	4.0	17.7
Mn	0.7	10.9
Ni	0.5	4.4

<sup>a</sup> Data from References 1–5 and 12.

greatly exceeds that in sea water. Such data indicate the importance of mechanisms for accumulation, storage, and transport of transition metals and zinc in living organisms.

The metals are generally found either bound directly to proteins or in cofactors such as porphyrins or cobalamins, or in clusters that are in turn bound by the protein; the ligands are usually O, N, S, or C. Proteins with which transition metals and zinc are most commonly associated catalyze the intramolecular or intermolecular rearrangement of electrons. Although the redox properties of the metals are important in many of the reactions, in others the metal appears to contribute to the structure of the active state, e.g., zinc in the Cu-Zn dismutases and some of the iron in the photosynthetic reaction center. Sometimes equivalent reactions are catalyzed by proteins with different metal centers; the metal binding sites and proteins have evolved separately for each type of metal center.

Iron is the most common transition metal in biology.<sup>6,7</sup> Its use has created a dependence that has survived the appearance of dioxygen in the atmosphere ca. 2.5 billion years ago, and the concomitant conversion of ferrous ion to ferric ion and insoluble rust (Figure 1.1 *See color plate section, page C-1.*). All plants, animals, and bacteria use iron, except for a lactobacillus that appears to maintain high concentrations of manganese instead of iron. The processes and reactions in which iron participates are crucial to the survival of terrestrial organisms, and include ribonucleotide reduction (DNA synthesis), energy production (respiration), energy conversion (photosynthesis), nitrogen reduction, oxygen transport (respiration, muscle contraction), and oxygenation (e.g., steroid synthesis, solubilization and detoxification of aromatic compounds). Among the transition metals used in living organisms, iron is the most abundant in the environment. Whether this fact alone explains the biological predominance of iron or whether specific features of iron chemistry contribute is not clear.

Many of the other transition metals participate in reactions equivalent to those involving iron, and can sometimes substitute for iron, albeit less effectively, in natural Fe-proteins. Additional biological reactions are unique to non-ferrous transition metals.

Zinc is relatively abundant in biological materials.<sup>8,9</sup> The major location of zinc in the body is metallothionein, which also binds copper, chromium, mercury, and other metals. Among the other well-characterized zinc proteins are the Cu-Zn superoxide dismutases (other forms have Fe or Mn), carbonic anhydrase (an abundant protein in red blood cells responsible for maintaining the pH of the blood), alcohol dehydrogenase, and a variety of hydrolases involved in the metabolism of sugars, proteins, and nucleic acids. Zinc is a common element in nucleic-acid polymerases and transcription factors, where its role is considered to be structural rather than catalytic. Interestingly, zinc enhances the stereoselectivity of the polymerization of nucleotides under reaction conditions designed to simulate the environment for prebiotic reactions. Recently a group of nucleic-acid binding proteins, with a repeated sequence containing the amino acids cysteine and histidine, were shown to bind as many as eleven zinc atoms necessary for protein function (transcribing DNA to RNA).<sup>10</sup> Zinc plays a structural role, forming the peptide into multiple domains or "zinc fingers" by means of coordination to cysteine and histidine (Figure 1.2A *See color plate section, page C-1.*). A survey of the sequences of many nucleic-acid binding proteins shows that many of them have the common motif required to form zinc fingers. Other zinc-finger proteins called steroid receptors bind both steroids such as progesterone and the progesterone gene DNA (Chapter 8). Much of the zinc in animals and plants has no known function, but it may be maintaining the structures of proteins that activate and deactivate genes.<sup>11</sup>

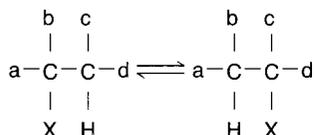
Copper and iron proteins participate in many of the same biological reactions:

- (1) reversible binding of dioxygen, e.g., hemocyanin (Cu), hemerythrin (Fe), and hemoglobin (Fe);
- (2) activation of dioxygen, e.g., dopamine hydroxylase (Cu) (important in the synthesis of the hormone epinephrine), tyrosinases (Cu), and catechol dioxygenases (Fe);
- (3) electron transfer, e.g., plastocyanins (Cu), ferredoxins, and *c*-type cytochromes (Fe);
- (4) dismutation of superoxide by Cu or Fe as the redox-active metal (superoxide dismutases).

The two metal ions also function in concert in proteins such as cytochrome oxidase, which catalyzes the transfer of four electrons to dioxygen to form water during respiration. Whether any types of biological reactions are unique to copper proteins is not clear. However, use of stored iron is reduced by copper deficiency, which suggests that iron metabolism may depend on copper proteins,

such as the serum protein ceruloplasmin, which can function as a ferroxidase, and the cellular protein ascorbic acid oxidase, which also is a ferrireductase.

Cobalt is found in vitamin B<sub>12</sub>, its only apparent biological site.<sup>12</sup> The vitamin is a cyano complex, but a methyl or methylene group replaces CN in native enzymes. Vitamin-B<sub>12</sub> deficiency causes the severe disease of pernicious anemia in humans, which indicates the critical role of cobalt. The most common type of reaction in which cobalamin enzymes participate results in the reciprocal exchange of hydrogen atoms if they are on adjacent carbon atoms, yet not with hydrogen in solvent water:



(An important exception is the ribonucleotide reductase from some bacteria and lower plants, which converts ribonucleotides to the DNA precursors, deoxyribonucleotides, a reaction in which a sugar —OH is replaced by —H. Note that ribonucleotide reductases catalyzing the same reaction in higher organisms and viruses are proteins with an oxo-bridged dimeric iron center.) The cobalt in vitamin B<sub>12</sub> is coordinated to five N atoms, four contributed by a tetrapyrrole (corrin); the sixth ligand is C, provided either by C5 of deoxyadenosine in enzymes such as methylmalonyl-CoA mutase (fatty acid metabolism) or by a methyl group in the enzyme that synthesizes the amino acid methionine in bacteria.

Nickel is a component of a hydrolase (urease), of hydrogenase, of CO dehydrogenase, and of S-methyl CoM reductase, which catalyzes the terminal step in methane production by methanogenic bacteria. All the Ni-proteins known to date are from plants or bacteria.<sup>13,14</sup> However, about 50 years elapsed between the crystallization of jack-bean urease in 1925 and the identification of the nickel component in the plant protein. Thus it is premature to exclude the possibility of Ni-proteins in animals. Despite the small number of characterized Ni-proteins, it is clear that many different environments exist, from apparently direct coordination to protein ligands (urease) to the tetrapyrrole F430 in methylreductase and the multiple metal sites of Ni and Fe-S in a hydrogenase from the bacterium *Desulfovibrio gigas*. Specific environments for nickel are also indicated for nucleic acids (or nucleic acid-binding proteins), since nickel activates the gene for hydrogenase.<sup>15</sup>

Manganese plays a critical role in oxygen evolution catalyzed by the proteins of the photosynthetic reaction center. The superoxide dismutase of bacteria and mitochondria, as well as pyruvate carboxylase in mammals, are also manganese proteins.<sup>16,17</sup> How the multiple manganese atoms of the photosynthetic reaction center participate in the removal of four electrons and protons from water is the subject of intense investigation by spectroscopists, synthetic inorganic chemists, and molecular biologists.<sup>17</sup>

Vanadium and chromium have several features in common, from a bioinorganic viewpoint.<sup>18a</sup> First, both metals are present in only small amounts in most organisms. Second, the biological roles of each remain largely unknown.<sup>18</sup> Finally, each has served as a probe to characterize the sites of other metals, such as iron and zinc. Vanadium is required for normal health, and could act *in vivo* either as a metal cation or as a phosphate analogue, depending on the oxidation state, V(IV) or V(V), respectively. Vanadium in a sea squirt (tunicate), a primitive vertebrate (Figure 1.2B), is concentrated in blood cells, apparently as the major cellular transition metal, but whether it participates in the transport of dioxygen (as iron and copper do) is not known. In proteins, vanadium is a cofactor in an algal bromoperoxidase and in certain prokaryotic nitrogenases. Chromium imbalance affects sugar metabolism and has been associated with the glucose tolerance factor in animals. But little is known about the structure of the factor or of any other specific chromium complexes from plants, animals, or bacteria.

Molybdenum proteins catalyze the reduction of nitrogen and nitrate, as well as the oxidation of aldehydes, purines, and sulfite.<sup>19</sup> Few Mo-proteins are known compared to those involving other transition metals. Nitrogenases, which also contain iron, have been the focus of intense investigations by bioinorganic chemists and biologists; the iron is found in a cluster with molybdenum (the iron-molybdenum cofactor, or FeMoCo) and in an iron-sulfur center (Chapter 7). Interestingly, certain bacteria (*Azotobacter*) have alternative nitrogenases, which are produced when molybdenum is deficient and which contain vanadium and iron or only iron. All other known Mo-proteins are also Fe-proteins with iron centers, such as tetrapyrroles (heme and chlorins), Fe-sulfur clusters, and, apparently, non-heme/non-sulfur iron. Some Mo-proteins contain additional cofactors such as the flavins, e.g., in xanthine oxidase and aldehyde oxidase. The number of redox centers in some Mo-proteins exceeds the number of electrons transferred; reasons for this are unknown currently.

## B. Chemical Properties Relative to Storage and Transport

### 1. Iron

Iron is the most abundant transition element in the Earth's crust and, in general, in all life forms. An outline of the distribution of iron in the Earth's crust<sup>20,21</sup> is shown in Table 1.2. As can be seen, approximately one-third of the Earth's mass is estimated to be iron. Of course, only the Earth's crust is relevant for life forms, but even there it is the most abundant transition element. Its concentration is relatively high in most crustal rocks (lowest in limestone, which is more or less pure calcium carbonate). In the oceans, which constitute 70 percent of the Earth's surface, the concentration of iron is low but increases with depth, since this iron exists as suspended particulate matter rather than as a soluble species. Iron is a limiting factor in plankton growth, and the rich

Table 1.2  
Iron: Its terrestrial distribution.<sup>a</sup>

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One third of Earth's mass, most abundant element by weight

Distribution in crustal rocks (weight %):

igneous	5.6
shale	4.7
sandstone	1.0
limestone	0.4

Ocean (70% of Earth's surface):

0.003–0.1 ppb, increasing with depth; limiting factor in plankton growth

Rivers:

0.07–7 ppm

$K_{sp}$  for  $\text{Fe}(\text{OH})_3$  is approximately  $10^{-39}$ , hence at pH 7  $[\text{Fe}^{3+}] 10^{-18}$  M

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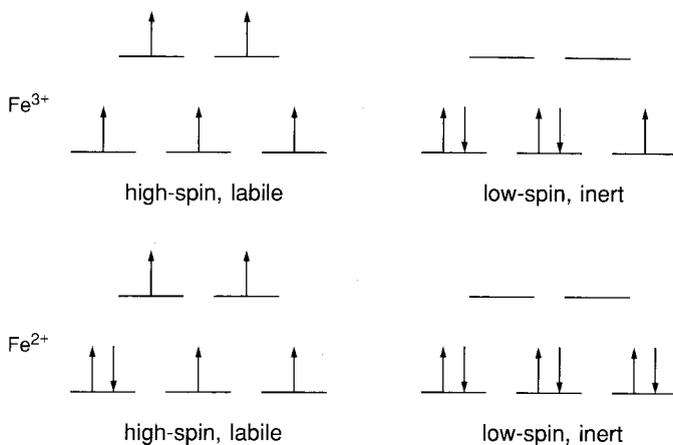
<sup>a</sup> Data from References 1a and 20.

fisheries associated with strong up-welling of ocean depths result at least in part from the biological growth allowed by these iron supplies. Properties that dominate the transport behavior of most transition metal ions are: (1) redox chemistry, (2) hydrolysis, and (3) the solubility of the metal ions in various complexes, particularly the hydroxides.

As an example of the effects of solubility, consider the enormous variation in the concentration of iron in rivers, depending on whether the water is from a clear mountain stream running over rock or a muddy river carrying large amounts of sediment. However, the amount of dissolved iron in the form of free ferric ion or its hydrolysis products, whatever the source of water, is extremely low. As can be seen from the solubility of hydrated Fe(III) ( $K_s \sim 10^{-18}$  M) (Table 1.2), the concentration of free ferric ion is extraordinarily low at neutral pH; so significant concentrations of soluble iron species can be attained only by strong complex formation.

One example of the versatility of iron as a function of its environment is how the ligand field can strongly alter the structural and ligand exchange properties of the metal ion (Figure 1.3). The ligand field can also alter the redox properties. For high-spin ferric ion, as found in the aquo complex or in many other complexes (including the class of microbial iron-transport agents called siderophores, to be discussed later), the coordination geometry is octahedral or pseudo-octahedral. In the relatively weak ligand field (high-spin ground state), the complex is highly labile. In a strong ligand field, such as an axially ligated porphyrin complex of ferric ion, or the simple example of the ferrocyanide anion, the low-spin complex is exchange-inert. Similarly, the high-spin octahedral ferrous complexes are exchange-labile, but the corresponding axially ligated porphyrin complexes, or the ferrocyanide complexes, are spin-paired (diamagnetic) and ligand exchange-inert. Large, bulky ligands or constrained ligands, such as those provided by metalloprotein and enzyme sites, can cause a tetrahedral environment, in which both ferrous ion and ferric ion form high-spin complexes.

## octahedral



## tetrahedral

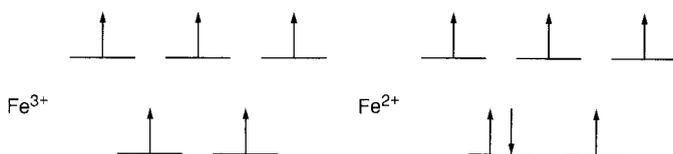


Figure 1.3  
Versatility of Fe coordination complexes.

The distribution of specific iron complexes in living organisms depends strongly on function. For example, although there are many different iron complexes in the average human, the relative amounts of each type differ more than 650-fold (Table 1.3). The total amount of iron in humans is quite large, averaging more than three and up to five grams for a healthy adult. Most of the iron is present as hemoglobin, the plasma oxygen-transport protein, where the func-

Table 1.3  
Average human Fe distribution.

Protein	Function	Oxidation state of Fe	Amount of Fe (g)	Percent of total
Hemoglobin	Plasma O <sub>2</sub> transport	2	2.6	65
Myoglobin	Muscle O <sub>2</sub> storage	2	0.13	6
Transferrin	Plasma Fe transport	3	0.007	0.2
Ferritin	Cell Fe storage	3	0.52	13
Hemosiderin	Cell Fe storage	3	0.48	12
Catalase	H <sub>2</sub> O <sub>2</sub> metabolism	2	0.004	0.1
Cytochrome <i>c</i>	Electron transport	$\frac{2}{3}$	0.004	0.1
Other	Oxidases, other enzymes, etc.		0.14	3.6

tion of the iron is to deliver oxygen for respiration. A much smaller amount of iron is present in myoglobin, a muscle oxygen-storage protein. For transport, the most important of these iron-containing proteins is transferrin, the plasma iron-transport protein that transfers iron from storage sites in the body to locations where cells synthesizing iron proteins reside; the major consumers of iron in vertebrates are the red blood cells. However, at any given time relatively little of the iron in the body is present in transferrin, in much the same way that at any given time in a large city only a small fraction of the population will be found in buses or taxis. Other examples of iron-containing proteins and their functions are included in Table 1.3 for comparison.

An example of different iron-coordination environments, which alter the chemical properties of iron, is the difference in the redox potentials of hydrated  $\text{Fe}^{3+}$  and the electron-transport protein cytochrome *c* (Table 1.4). The coordina-

Table 1.4  
Fe redox potentials.

Complex	Coord. no., type	$\text{Fe}^{3+}/\text{Fe}^{2+}$ $E^\circ$ (mV)
$\text{Fe}(\text{OH}_2)_6^{3+}$	6, aquo complex	770
Cytochrome <i>a</i> <sub>3</sub>	6, heme	390
HIPIP	4, $\text{Fe}_4\text{S}_4(\text{SR})_4^-$	350
Cytochrome <i>c</i>	6, heme	250
Rubredoxin	4, $\text{Fe}(\text{SR})_4$	-60
Ferredoxins	4, $\text{Fe}_4\text{S}_4(\text{SR})_4^{2-}$	-400

tion environment of iron in cytochrome *c* is illustrated in Figure 1.4. For example, the standard reduction potential for ferric ion in acid solution is 0.77 volts; so here ferric ion is quite a good oxidant. In contrast, cytochrome *c* has a redox potential of 0.25 volts. A wide range of redox potentials for iron is achieved in biology by subtle differences in protein structure, as listed in Table 1.4. Notice the large difference in the potential of cytochrome *c* and rubredoxin (Figure 1.5), 0.25 volts vs. -0.06 volts, respectively. In polynuclear ferredoxins, in which each iron is tetrahedrally coordinated by sulfur, reduction potentials are near -0.4 volts. Thus, the entire range of redox potentials, as illustrated in Table 1.4, is more than one volt.

## 2. Chemical properties of zinc, copper, vanadium, chromium, molybdenum, and cobalt

The chemical properties of the other essential transition elements simplify their transport properties. For zinc there is only the +2 oxidation state, and the hydrolysis of this ion is not a limiting feature of its solubility or transport. Zinc is an essential element for both animals and plants.<sup>8,9,20,21</sup> In general, metal ion uptake into the roots of plants is an extremely complex phenomenon. A cross-sectional diagram of a root is shown in Figure 1.6. It is said that both diffusion

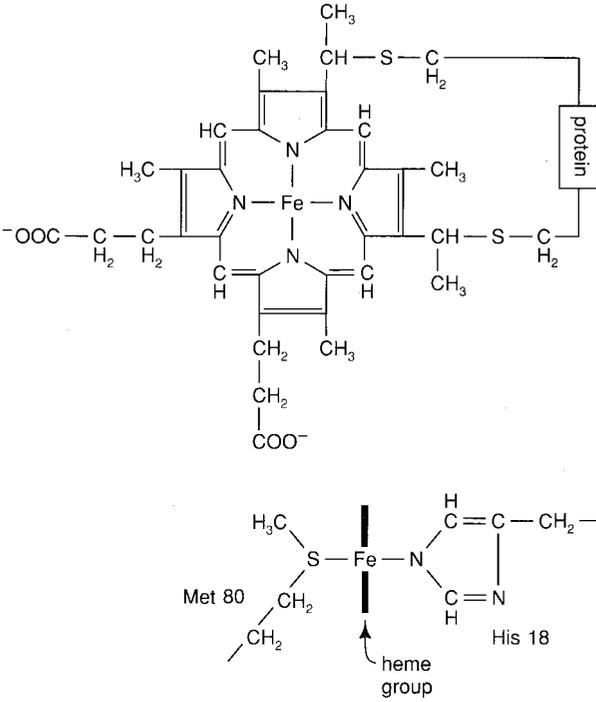


Figure 1.4  
Heme group and iron coordination in cytochrome *c*.

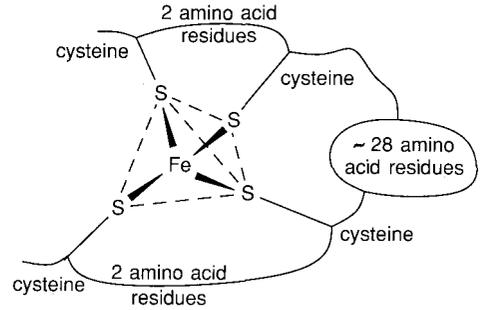


Figure 1.5  
 $Fe^{3+/2+}$  coordination in rubredoxin.

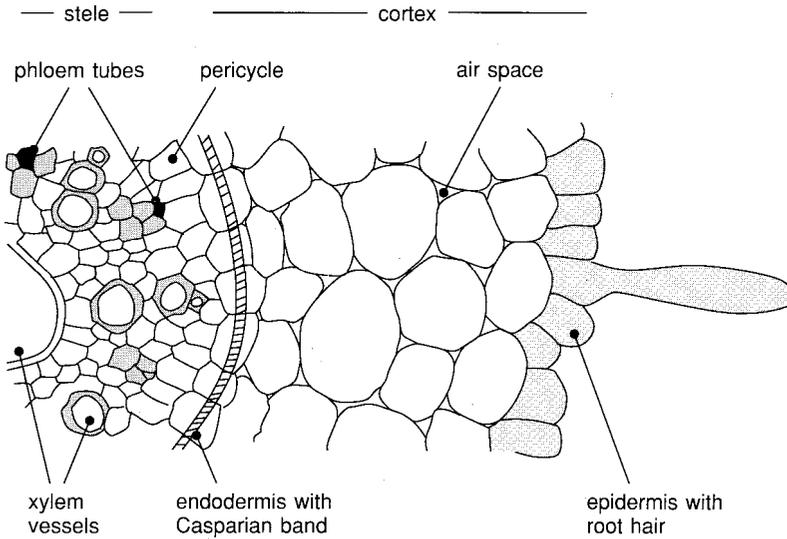


Figure 1.6  
Transverse section of a typical root.<sup>20</sup> The complex features of the root hair surface that regulate reductase and other activities in metal uptake are only beginning to be understood.

and mass flow of the soil solution are of significance in the movement of metal ions to roots. Chelation and surface adsorption, which are pH dependent, also affect the availability of nutrient metal ions. Acid soil conditions in general retard uptake of essential divalent metal ions but increase the availability (sometimes with toxic results) of manganese, iron, and aluminum, all of which are normally of very limited availability because of hydrolysis of the trivalent ions.

Vanadium is often taken up as vanadate, in a pathway parallel to phosphate.<sup>18</sup> However, its oxidation state within organisms seems to be highly variable. Unusually high concentrations of vanadium occur in certain ascidians (the specific transport behavior of which will be dealt with later). The workers who first characterized the vanadium-containing compound of the tunicate, *Ascidia nigra*, coined the name tunchrome.<sup>22</sup> The characterization of the compound as a dicatecholate has been reported.<sup>23</sup>

Quite a different chemical environment is found in the vanadium-containing material isolated from the mushroom *Amanita muscaria*. Bayer and Kneifel, who named and first described amavadine,<sup>24</sup> also suggested the structure shown in Figure 1.7.<sup>25</sup> Recently the preparation, proof of ligand structure, and (by implication) proof of the complex structure shown in Figure 1.7 have been established.<sup>26</sup> Although the exact role of the vanadium complex in the mushroom

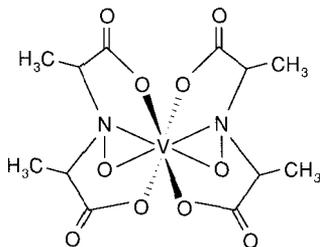


Figure 1.7  
A structure proposed for amavadine.<sup>27</sup>

remains unclear, the fact that it is a vanadyl complex is now certain, although it may take a different oxidation state *in vivo*.

The role of chromium in biology remains even more mysterious. In human beings the isolation of "glucose tolerance factor" and the discovery that it contains chromium goes back some time. This has been well reviewed by Mertz, who has played a major role in discovering what is known about this elusive and apparently quite labile compound.<sup>27</sup> It is well established that chromium is taken up as chromic ion, predominantly via foodstuffs, such as unrefined sugar, which presumably contain complexes of chromium, perhaps involving sugar hydroxyl groups. Although generally little chromium is taken up when it is administered as inorganic salts, such as chromic chloride, glucose tolerance in many adults and elderly people has been reported to be improved after supplementation with 150–250 mg of chromium per day in the form of chromic chloride. Similar results have been found in malnourished children in some studies in Third World countries. Studies using radioactively labeled chromium have shown that, although inorganic salts of chromium are relatively unavailable to mam-

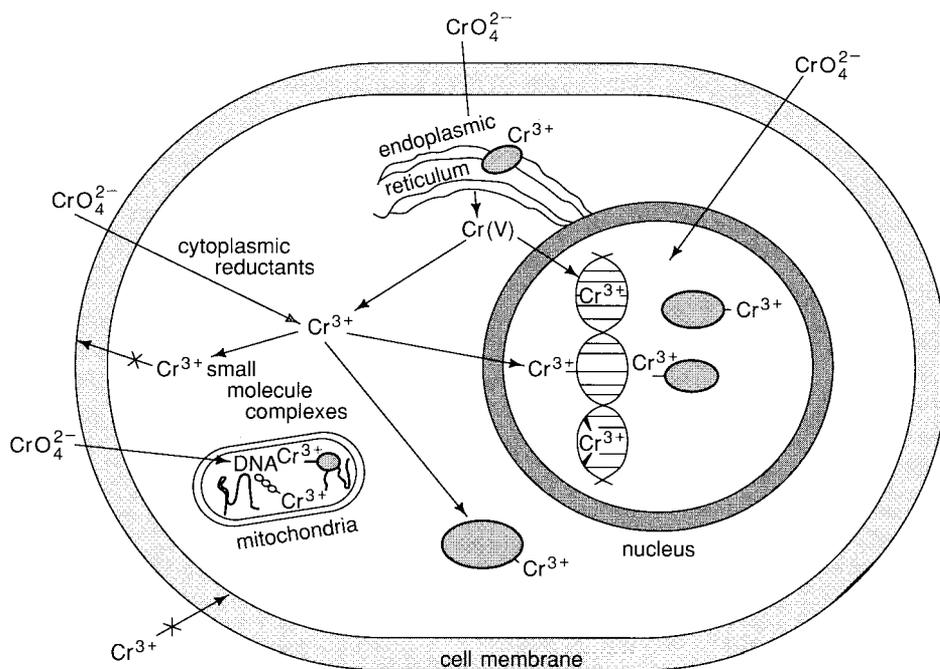


Figure 1.8

The uptake-reduction model for chromate carcinogenicity. Possible sites for reduction of chromate include the cytoplasm, endoplasmic reticulum, mitochondria, and the nucleus.<sup>27</sup>

mals, brewer's yeast can convert the chromium into a usable form; so brewer's yeast is today the principal source in the isolation of glucose tolerance factor and has been used as a diet supplement.

Although chromium is essential in milligram amounts for human beings as the trivalent ion, as chromate it is quite toxic and a recognized carcinogen.<sup>30</sup> The uptake-reduction model for chromate carcinogenicity as suggested by Connett and Wetterhahn is shown in Figure 1.8. Chromate is mutagenic in bacterial and mammalian cell systems, and it has been hypothesized that the difference between chromium in the +6 and +3 oxidation states is explained by the "uptake-reduction" model. Chromium(III), like the ferric ion discussed above, is readily hydrolyzed at neutral pH and extremely insoluble. Unlike  $\text{Fe}^{3+}$ , it undergoes extremely slow ligand exchange. For both reasons, transport of chromium(III) into cells can be expected to be extremely slow unless it is present as specific complexes; for example, chromium(III) transport into bacterial cells has been reported to be rapid when iron is replaced by chromium in the siderophore iron-uptake mediators. However, chromate readily crosses cell membranes and enters cells, much as sulfate does. Because of its high oxidizing power, chromate can undergo reduction inside organelles to give chromium(III), which binds to small molecules, protein, and DNA, damaging these cellular components.

In marked contrast to its congener, molybdenum is very different from chromium in both its role in biology and its transport behavior, again because of fundamental differences in oxidation and coordination chemistry properties. In contrast to chromium, the higher oxidation states of molybdenum dominate its chemistry, and molybdate is a relatively poor oxidant. Molybdenum is an essential element in many enzymes, including xanthine oxidase, aldehyde reductase, and nitrate reductase.<sup>19</sup> The range of oxidation states and coordination geometries of molybdenum makes its bioinorganic chemistry particularly interesting and challenging.

The chemistry of iron storage and transport is dominated by high concentrations, redox chemistry (and production of toxic-acting oxygen species), hydrolysis ( $pK_a$  is about 3, far below physiological pH), and insolubility. High-affinity chelators or proteins are required for transport of iron and high-capacity sequestering protein for storage. By comparison to iron, storage and transport of the other metals are simple. Zinc, copper, vanadium, chromium, manganese, and molybdenum appear to be transported as simple salts or loosely bound protein complexes. In vanadium or molybdenum, the stable anion, vanadate or molybdate, appears to dominate transport. Little is known about biological storage of any metal except iron, which is stored in ferritin. However, zinc and copper are bound to metallothionein in a form that may participate in storage.

## II. BIOLOGICAL SYSTEMS OF METAL STORAGE, TRANSPORT, AND MINERALIZATION

### A. Storage

#### 1. The storage of iron

Three properties of iron can account for its extensive use in terrestrial biological reactions:

- (a) facile redox reactions of iron ions;
- (b) an extensive repertoire of redox potentials available by ligand substitution or modification (Table 1.4);
- (c) abundance and availability (Table 1.1) under conditions apparently extant when terrestrial life began (see Section I.B.).

Ferrous ion appears to have been the environmentally stable form during prebiotic times. The combination of the reactivity of ferrous ion and the relatively large amounts of iron used by cells may have necessitated the storage of ferrous ion; recent results suggest that ferrous ion may be stabilized inside ferritin long enough to be used in some types of cells. As primitive organisms began to proliferate, the successful photosynthetic cells, which trapped solar energy by reducing  $\text{CO}_2$  to make carbohydrates  $(\text{CH}_2\text{O})_n$  and produce  $\text{O}_2$ , exhausted from the environment the reductants from  $\text{H}_2$  or  $\text{H}_2\text{S}$  or  $\text{NH}_3$ . The abil-

ity of primitive organisms to switch to the use of  $\text{H}_2\text{O}$  as a reductant, with the concomitant production of dioxygen, probably produced the worst case of environmental pollution in terrestrial history. As a result, the composition of the atmosphere, the course of biological evolution, and the oxidation state of environmental iron all changed profoundly. Paleogeologists and meteorologists estimate that there was a lag of about 200–300 million years between the first dioxygen production and the appearance of significant dioxygen concentrations in the atmosphere, because the dioxygen produced at first was consumed by the oxidation of ferrous ions in the oceans. The transition in the atmosphere, which occurred about 2.5 billion years ago, caused the bioavailability of iron to plummet and the need for iron storage to increase. Comparison of the solubility of  $\text{Fe}^{3+}$  at physiological conditions (about  $10^{-18}$  M) to the iron content of cells (equivalent to  $10^{-5}$  to  $10^{-8}$  M) emphasizes the difficulty of acquiring sufficient iron.

Iron is stored mainly in the ferritins, a family\* of proteins composed of a protein coat and an iron core of hydrous ferric oxide [ $\text{Fe}_2\text{O}_3(\text{H}_2\text{O})_n$ ] with various amounts of phosphate.<sup>6,7</sup> As many as 4,500 iron atoms can be reversibly stored inside the protein coat in a complex that is soluble; iron concentrations equivalent to 0.25 M [about  $10^{16}$ -fold more concentrated than  $\text{Fe(III)}$  ions] can be easily achieved *in vitro* (Figure 1.1). Ferritin is found in animals, plants, and even in bacteria; the role of the stored iron varies, and includes intracellular use for Fe-proteins or mineralization, long-term iron storage for other cells, and detoxification of excess iron. Iron regulates the synthesis of ferritin, with large amounts of ferritin associated with iron excess, small or undetectable amounts associated with iron deficiency. [Interestingly, the template (mRNA) for ferritin synthesis is itself stored in cells and is recruited by intracellular iron or a derivative for efficient translation into protein.<sup>31</sup> Iron does not appear to interact directly with ferritin mRNA nor with a ferritin mRNA-specific regulatory (binding) protein; however, the specific, mRNA regulatory (binding) protein has sequence homology to aconitase, and formation of an iron-sulfate cluster prevents RNA binding.] Because iron itself determines in part the amount of ferritin in an organism, the environmental concentration of iron needs to be considered before one can conclude that an organism or cell does not have ferritin.

Ferritin is thought to be the precursor of several forms of iron in living organisms, including hemosiderin, a form of storage iron found mainly in animals. The iron in hemosiderin is in a form very similar to that in ferritin, but the complex with protein is insoluble, and is usually located within an intracellular membrane (lysosomes). Magnetite ( $\text{Fe}_3\text{O}_4$ ) is another form of biological iron derived, apparently, from the iron in ferritin. Magnetite plays a role in the behavior of magnetic bacteria, bees, and homing pigeons (see Section II.C).

The structure of ferritin is the most complete paradigm for bioinorganic chemistry because of three features: the protein coat, the iron-protein interface, and the iron core.<sup>6,7</sup>

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\* A family of proteins is a group of related but distinct proteins produced in a single organism and usually encoded by multiple, related genes.

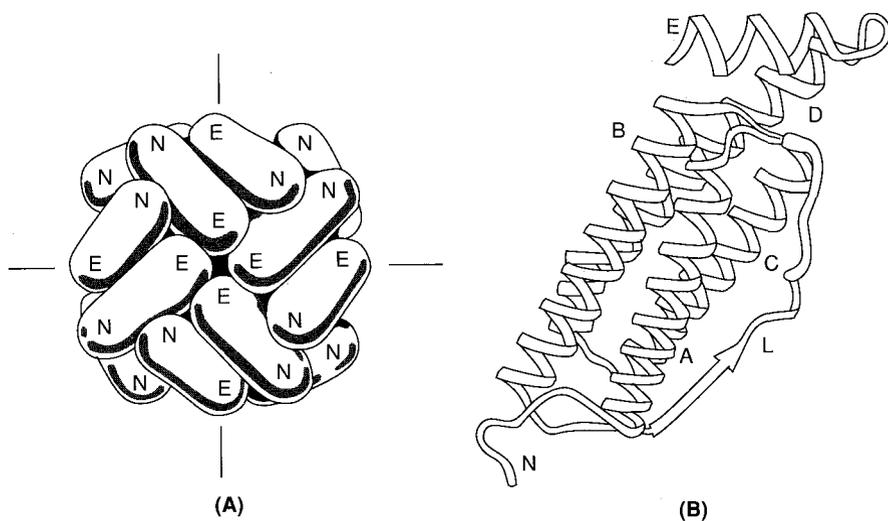


Figure 1.9

(A) The protein coat of horse spleen apoferritin deduced from x-ray diffraction of crystals of the protein.<sup>32</sup> The outer surface of the protein coat shows the arrangement of the 24 ellipsoidal polypeptide subunits. N refers to the N-terminus of each polypeptide and E to the E-helix (see B). Note the channels that form at the four-fold axes where the E-helices interact, and at the three-fold axes near the N-termini of the subunits. (B) A ribbon model of a subunit showing the packing of the four main alpha-helices (A, B, C, and D), the connecting L-loop and the E-helix.

*Protein Coat* Twenty-four peptide chains (with about 175 amino acids each), folded into ellipsoids, pack to form the protein coat,\* which is a hollow sphere about 100 Å in diameter; the organic surface is about 10 Å thick (Figure 1.9). Channels which occur in the protein coat at the trimer interfaces may be involved in the movement of iron in and out of the protein.<sup>62,63,65</sup> Since the protein coat is stable with or without iron, the center of the hollow sphere may be filled with solvent, with  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ , or, more commonly, with both small aggregates of iron and solvent. Very similar amino-acid sequences are found in ferritin from animals and plants. Sorting out which amino acids are needed to form the shape of the protein coat and the ligands for iron core formation requires the continued dedication of bioinorganic chemists; identification of tyrosine as an Fe(III)-ligand adds a new perspective.<sup>64</sup>

*Iron-Protein Interface* Formation of the iron core appears to be initiated at an Fe-protein interface where Fe(II)-O-Fe(III) dimers and small clusters of Fe(III) atoms have been detected attached to the protein and bridged to each other by oxo/hydroxo bridges. Evidence for multiple nucleation sites has been obtained

\* Some ferritin subunits, notably in ferritin from bacteria, bind heme in a ratio of less than one heme per two subunits. A possible role of such heme in the oxidation and reduction of iron in the core is being investigated.

from electron microscopy of individual ferritin molecules (multiple core crystallites were observed) and by measuring the stoichiometry of binding of metal ions, which compete with binding of monoatomic iron, e.g., VO(IV) and Tb(III) (about eight sites per molecule). EXAFS (*Extended X-ray Absorption Fine Structure*) and Mössbauer spectroscopies suggest coordination of Fe to the protein by carboxyl groups from glutamic (Glu) and aspartic (Asp) acids. Although groups of Glu or Asp are conserved in all animal and plant ferritins, the ones that bind iron are not known. Tyrosine is an Fe(III)-ligand conserved in rapid mineralizing ferritins identified by Uv-vis and resonance Raman spectroscopy.<sup>64</sup>

*Iron Core* Only a small fraction of the iron atoms in ferritin bind directly to the protein. The core contains the bulk of the iron in a polynuclear aggregate with properties similar to ferrihydrite, a mineral found in nature and formed experimentally by heating neutral aqueous solutions of Fe(III)(NO<sub>3</sub>)<sub>3</sub>. X-ray diffraction data from ferritin cores are best fit by a model with hexagonal close-packed layers of oxygen that are interrupted by irregularly incomplete layers of octahedrally coordinated Fe(III) atoms. The octahedral coordination is confirmed by Mössbauer spectroscopy and by EXAFS, which also shows that the average Fe(III) atom is surrounded by six oxygen atoms at a distance of 1.95 Å and six iron atoms at distances of 3.0 to 3.3 Å.

Until recently, all ferritin cores were thought to be microcrystalline and to be the same. However, x-ray absorption spectroscopy, Mössbauer spectroscopy, and high-resolution electron microscopy of ferritin from different sources have revealed variations in the degree of structural and magnetic ordering and/or the level of hydration. Structural differences in the iron core have been associated with variations in the anions present, e.g., phosphate<sup>29</sup> or sulfate, and with the electrochemical properties of iron. Anion concentrations in turn could reflect both the solvent composition and the properties of the protein coat. To understand iron storage, we need to define in more detail the relationship of the ferritin protein coat and the environment to the redox properties of iron in the ferritin core.

Experimental studies of ferritin formation show that Fe(II) and dioxygen are needed, at least in the early stages of core formation. Oxidation to Fe(III) and hydrolysis produce one electron and an average of 2.5 protons for iron atoms incorporated into the ferritin iron core. Thus, formation of a full iron core of 4,500 iron atoms would produce a total of 4,500 electrons and 11,250 protons. After core formation by such a mechanism inside the protein coat, the pH would drop to 0.4 if all the protons were retained. It is known that protons are released and electrons are transferred to dioxygen. However, the relative rates of proton release, oxo-bridge formation, and electron transfer have not been studied in detail. Moreover, recent data indicate migration of iron atoms during the early stages of core formation and the possible persistence of Fe<sup>2+</sup> for periods of time up to 24 hours. When large numbers of Fe(II) atoms are added, the protein coat appears to stabilize the encapsulated Fe(II).<sup>34a,b</sup> Formation of the iron core

of ferritin has analogies to surface corrosion, in which electrochemical gradients are known to occur. Whether such gradients occur during ferritin formation and how different protein coats might influence proton release or alter the structure of the core are subjects only beginning to be examined.

## 2. The storage of zinc, copper, vanadium, chromium, molybdenum, cobalt, nickel, and manganese

Ions of nonferrous transition metals require a much less complex biological storage system, because the solubilities are much higher ( $\geq 10^{-8}$  M) than those for  $\text{Fe}^{3+}$ . As a result, the storage of nonferrous transition metals is less obvious, and information is more limited. In addition, investigations are more difficult than for iron, because the amounts in biological systems are so small. Essentially nothing is known yet about the storage of vanadium, chromium, molybdenum, cobalt, nickel, and manganese, with the possible exception of accumulations of vanadium in the blood cells of tunicates.

Zinc and copper, which are used in the highest concentrations of any of the non-ferrous transition metals, are specifically bound by the protein metallothionein<sup>35,36</sup> (see Figure 1.10). Like the ferritins, the metallothioneins are a family of proteins, widespread in nature and regulated by the metals they bind. In contrast to ferritin, the amounts of metal stored in metallothioneins are smaller (up to twelve atoms per molecule), the amount of protein in cells is less, and the template (mRNA) is not stored. Because the cellular concentrations of the metallothioneins are relatively low and the amount of metal needed is relatively small, it has been difficult to study the biological fate of copper and zinc in living organisms, and to discover the natural role of metallothioneins. However, the regulation of metallothionein synthesis by metals, hormones, and growth factors attests to the biological importance of the proteins. The unusual metal environments of metallothioneins have attracted the attention of bioinorganic chemists.

Metallothioneins, especially in higher animals, are small proteins<sup>35,36</sup> rich in cysteine (20 per molecule) and devoid of the aromatic amino acids phenylalanine and tyrosine. The cysteine residues are distributed throughout the peptide chain. However, in the native form of the protein (Figure 1.10), the peptide chains fold to produce two clusters of  $-\text{SH}$ , which bind either three or four atoms of zinc, cadmium, cobalt, mercury, lead, or nickel. Copper binding is distinct from zinc, with 12 sites per molecule.

In summary, iron is stored in iron cores of a complicated protein. Ferritin, composed of a hollow protein coat, iron-protein interface, and an inorganic core, overcomes the problems of redox and hydrolysis by directing the formation of the quasi-stable mineral hydrous ferric oxide inside the protein coat. The outer surface of the protein is generally hydrophilic, making the complex highly soluble; equivalent concentrations of iron are  $\leq 0.25$  M. By contrast to iron, storage of zinc, copper, chromium, manganese, vanadium, and molybdenum is relatively simple, because solubility is high and abundance is lower. Little is known

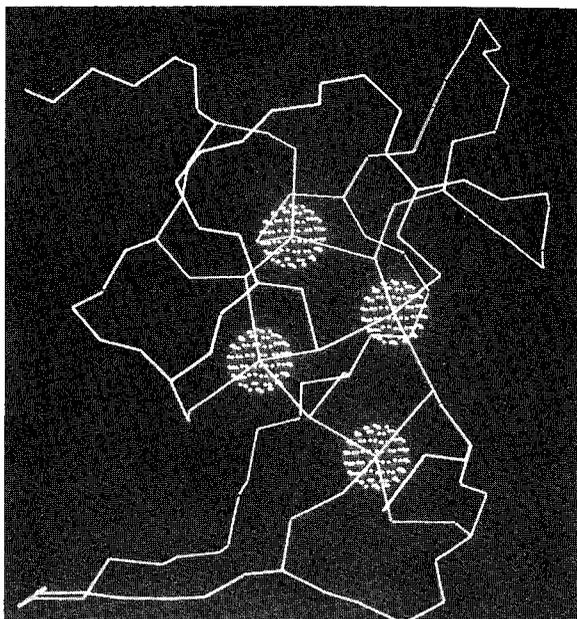


Figure 1.10

The three-dimensional structure of the  $\alpha$  domain from rat  $cd_7$  metallothionein-2, determined by NMR in solution (Reference 36a), based on data in Reference 36b. The four metal atoms, bonded to the sulfur of cysteine side chains, are indicated as spherical collections of small dots. A recent description of the structure of the  $cd_5Zn_2$  protein, determined from x-ray diffraction of crystals, agrees with the structure determined by NMR (Reference 36c).

about the molecules that store these metals, with the possible exception of metallothionein, which binds small clusters of zinc or copper.

## B. Transport

### 1. Iron

The storage of iron in humans and other mammals has been dealt with in the previous section. Only a small fraction of the body's inventory of iron is in transit at any moment. The transport of iron from storage sites in cellular ferritin or hemosiderin occurs via the serum-transport protein transferrin. The transferrins are a class of proteins that are bilobal, with each lobe reversibly (and essentially independently) binding ferric ion.<sup>37-39</sup> This complexation of the metal cation occurs via prior complexation of a synergistic anion that *in vivo* is bicarbonate (or carbonate). Serum transferrin is a monomeric glycoprotein of molecular weight 80 kDa. The crystal structure of the related protein, lactoferrin,<sup>39</sup> has been reported, and recently the structure of a mammalian transferrin<sup>40</sup> has been deduced.

Ferritin is apparently a very ancient protein and is found in higher animals, plants, and even microbes; in plants and animals a common ferritin progenitor

is indicated by sequence conservation.<sup>41</sup> In contrast, transferrin has been in existence only relatively recently, since it is only found in the phylum Chordata. Although the two iron-binding sites of transferrin are sufficiently different to be distinguishable by kinetic and a few other studies, their coordination environments have been known for some time to be quite similar. This was first discovered by various spectroscopies, and most recently was confirmed by crystal-structure analysis, which shows that the environment involves two phenolate oxygens from tyrosine, two oxygens from the synergistic, bidentate bicarbonate anion, nitrogen from histidine, and (a surprise at the time of crystal-structure analysis) an oxygen from a carboxylate group of an aspartate.<sup>39</sup>

The transferrins are all glycoproteins, and human serum transferrin contains about 6 percent carbohydrate. These carbohydrate groups are linked to the protein, and apparently strongly affect the recognition and conformation of the native protein.

Although transferrins have a high molecular weight and bind only two iron atoms, transferrin is relatively efficient, because it is used in many cycles of iron transport in its interaction with the tissues to which it delivers iron. Transferrin releases iron *in vivo* by binding to the cell surface and forming a vesicle inside the cell (endosome) containing a piece of the membrane with transferrin and iron still complexed. The release of the iron from transferrin occurs in the relatively low pH of the endosome, and apoprotein is returned to the outside of the cell for delivery of another pair of iron atoms. This process in active reticulocytes (immature red blood cells active in iron uptake) can turn over roughly a million atoms of iron per cell per minute.<sup>38</sup> A schematic structure of the protein, deduced from crystal-structure analysis, is shown in Figure 1.11. Transferrin is an ellipsoidal protein with two subdomains or lobes, each of which binds iron. The two halves of each subunit are more or less identical, and are connected by a relatively small hinge. In human lactoferrin, the coordination site of the iron is the same as the closely related serotransferrin site. A major question that remains about the mechanism of iron binding and release is how the protein structure changes in the intracellular compartment of low pH to release the iron when it forms a specific complex with cell receptors (transferrin binding proteins) and whether the receptor protein is active or passive in the process. Recent studies suggest that the cell binding site for transferrin (a membrane, glycoprotein called the transferrin receptor) itself influences the stability of the iron-transferrin complex. The path of iron from the endosome to Fe-proteins has not been established; and the form of transported intracellular iron is not known.

Another major type of biological iron transport occurs at the biological opposite of the higher organisms. Although almost all microorganisms have iron as an essential element, bacteria, fungi, and other microorganisms (unlike humans and other higher organisms) cannot afford to make high-molecular-weight protein-complexing agents for this essential element when those complexing agents would be operating extracellularly and hence most of the time would be lost to the organism. As described earlier, the first life forms on the surface of the

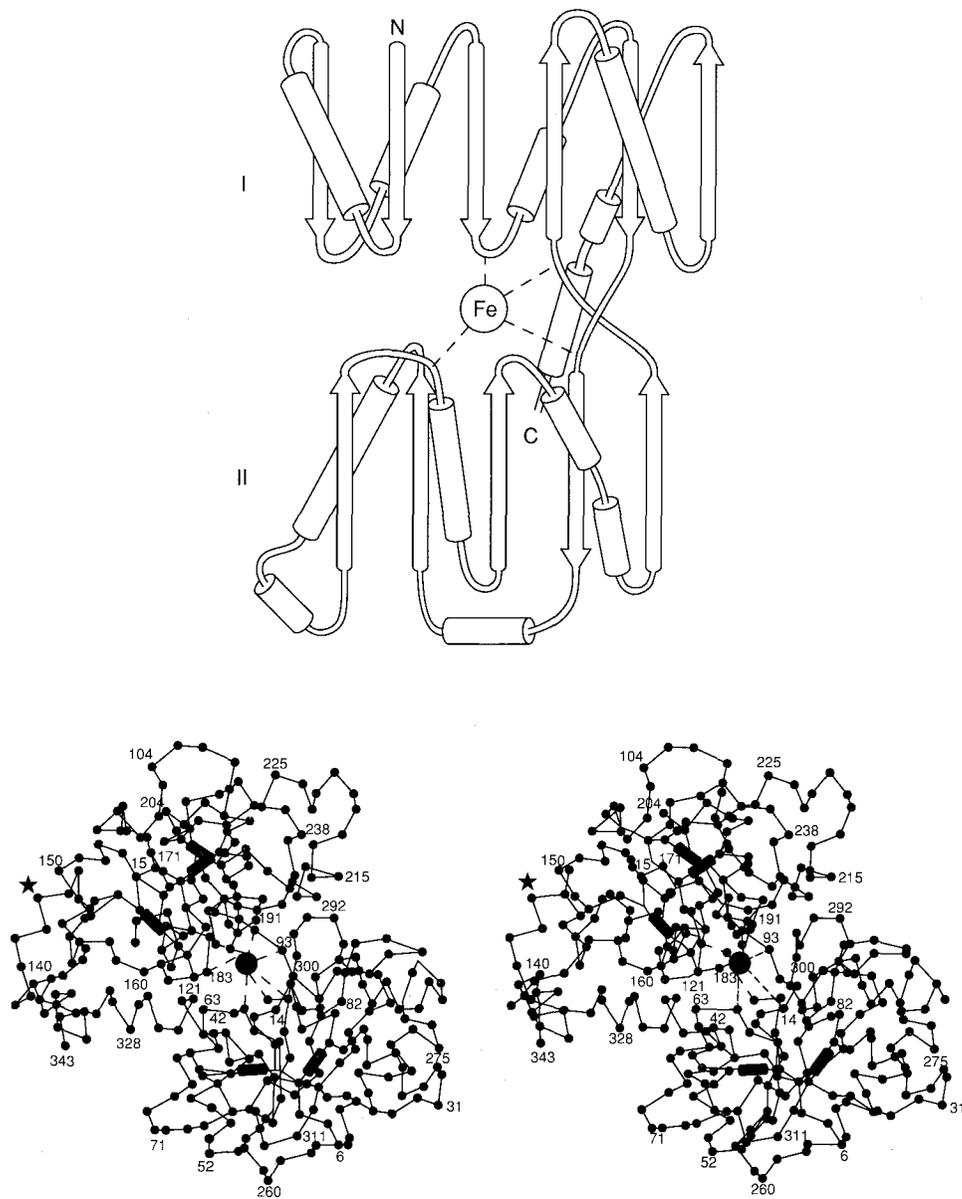


Figure 1.11

Three-dimensional structure of lactoferrin. *Top*: schematic representation of the folding pattern of each lactoferrin lobe; Domain I is based on a beta-sheet of four parallel and two antiparallel domains; Domain II is formed from four parallel and one antiparallel strand. *Bottom*: stereo C $\alpha$  diagram of the N lobe of lactoferrin; (●) iron atom between domain I (residues 6–90+) and domain II (residues 91–251); (■) disulfide bridges; (★) carbohydrate attachment site. See Reference 39.

Earth grew in a reducing atmosphere, in which the iron was substantially more available because it was present as ferrous-containing compounds. In contrast to the profoundly insoluble ferric hydroxide, ferrous hydroxide is relatively soluble at near neutral pH. It has been proposed that this availability of iron in the ferrous state was one of the factors that led to its early incorporation in so many metabolic processes of the earliest chemistry of life.<sup>6,38</sup> In an oxidizing environment, microorganisms were forced to deal with the insolubility of ferric hydroxide and hence when facing iron deficiency secrete high-affinity iron-binding compounds called siderophores (from the Greek for iron carrier). More than 200 naturally occurring siderophores have been isolated and characterized to date.<sup>42</sup>

Most siderophore-mediated iron-uptake studies in microorganisms have been performed by using cells obtained under iron-deficient aerobic growth conditions. However, uptake studies in *E. coli* grown under anaerobic conditions have also established the presence of siderophore-specific mechanisms. In both cases, uptake of the siderophore-iron complex is both a receptor- and an energy-dependent process. In some studies the dependence of siderophore uptake rates on the concentration of the iron-siderophore complex has been found to conform to kinetics characteristic of protein catalysts, i.e., Michaelis-Menten kinetics. For example, saturable processes with very low apparent dissociation constants of under one micromolar ( $1 \mu\text{M}$ ) have been observed for ferric-enterobactin transport in *E. coli* (a bacterium), as shown in Figure 1.12. Similarly, in a very

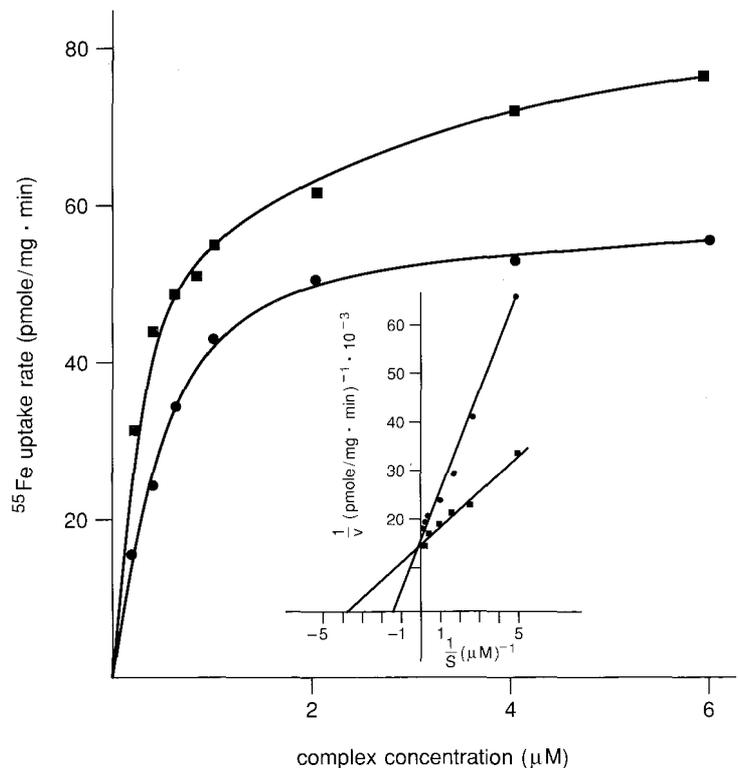


Figure 1.12  
Effect of MECAM analogues  
on iron uptake from *E. coli*.  
Iron transport by  $2 \mu\text{M}$  ferric  
enterobactin is inhibited by  
ferric MECAM.

different microorganism, the yeast *Rhodotorula pilimanae*, Michaelis-Menten kinetics were seen again with a dissociation constant of approximately  $6 \mu\text{M}$  for the ferric complex of rhodotoroulic acid; diagrams of some representative siderophores are shown in Figure 1.13. The siderophore used by the fungus *Neurospora crassa* was found to have a dissociation constant of about  $5 \mu\text{M}$  and, again, saturable uptake kinetics.

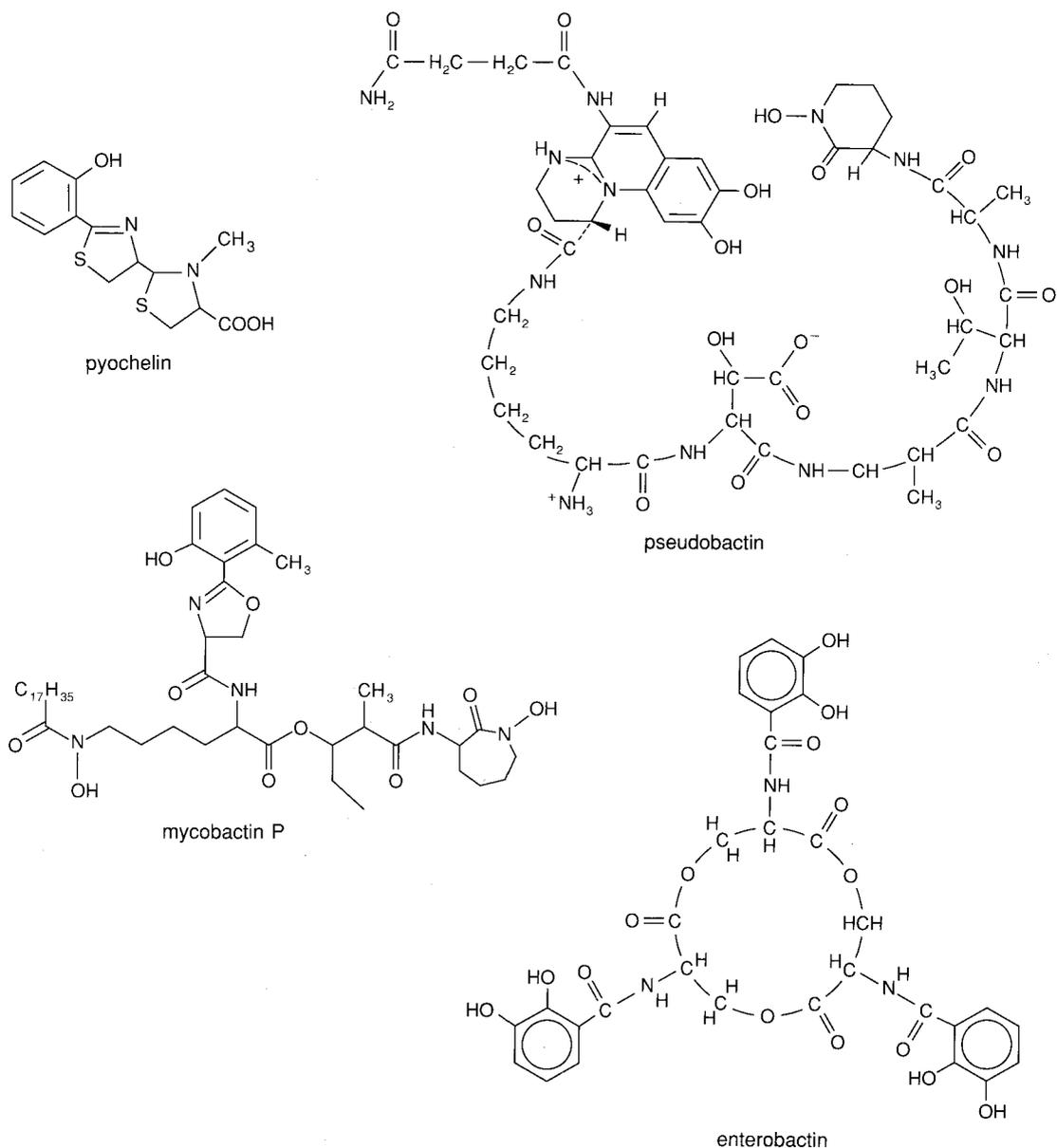


Figure 1.13  
Examples of bacterial siderophores. See Reference 42.

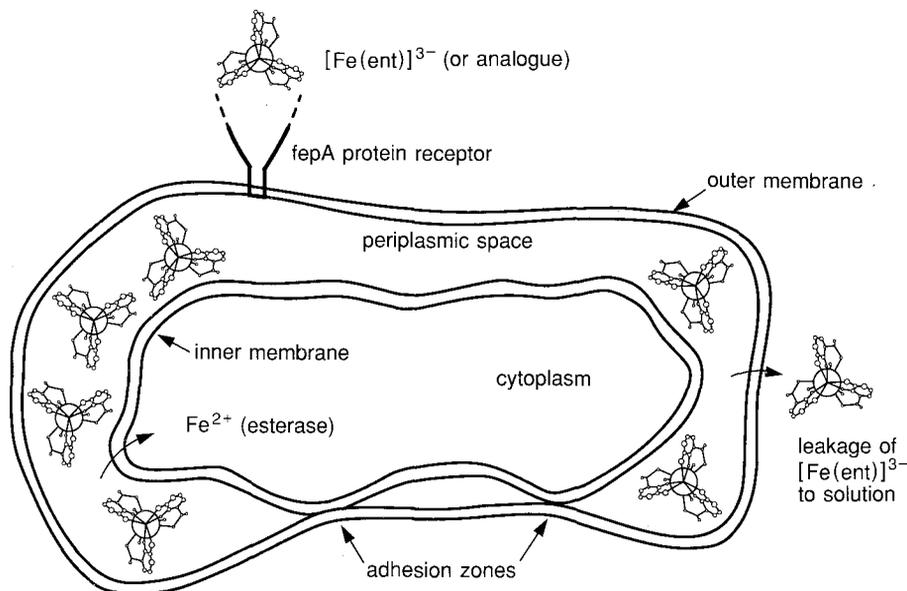


Figure 1.14  
Model for enterobactin-mediated Fe uptake in *E. coli*.

Although the behavior just described seems relatively simple, transport mechanisms in living cells probably have several more kinetically distinct steps than those assumed for the simple enzyme-substrate reactions underlying the Michaelis-Menten mechanism. For example, as ferric enterobactin is accumulated in *E. coli*, it has to pass through the outer membrane, the periplasm, and the cytoplasm membrane, and is probably subjected to reduction of the metal in a low-pH compartment or to ligand destruction.

A sketch of a cell of *E. coli* and some aspects of its transport behavior are shown in Figure 1.14. Enterobactin-mediated iron uptake in *E. coli* is one of the best-characterized of the siderophore-mediated iron-uptake processes in microorganisms, and can be studied as a model. After this very potent iron-sequestering agent complexes iron, the ferric-enterobactin complex interacts with a specific receptor in the outer cell membrane (Figure 1.14), and the complex is taken into the cell by active transport. The ferric complexes of some synthetic analogs of enterobactin can act as growth agents in supplying iron to *E. coli*. Such a feature could be used to discover which parts of the molecule are involved in the sites of structural recognition of the ferric-enterobactin complex. Earlier results suggested that the metal-binding part of the molecule is recognized by the receptor, whereas the ligand platform (the triserine lactone ring; see Figure 1.13) is not specifically recognized.

To find out which domains of enterobactin are required for iron uptake and recognition, rhodium complexes were prepared with various domains of enterobactin (Figure 1.15) as ligands to use as competitors for ferric enterobactin.<sup>44</sup> The goal was to find out if the amide groups (labeled Domain II in Figure 1.15),

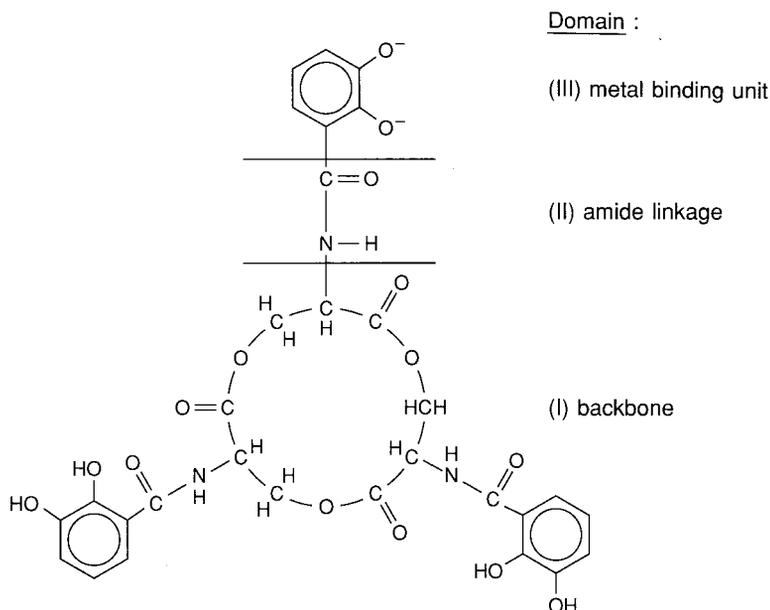
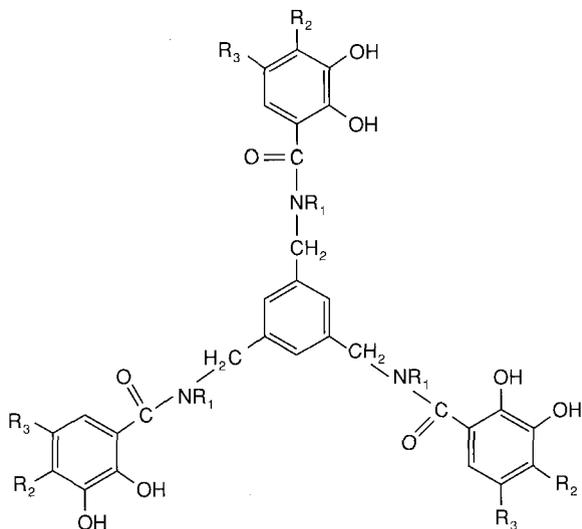


Figure 1.15  
Definition of recognition domains in enterobactin.

which linked the metal-binding catechol groups (Domain III, Figure 1.15) to the central ligand backbone (Domain I, Figure 1.15), are necessary for recognition by the receptor protein. In addition, synthetic ligands were prepared that differed from enterobactin by small changes at or near the catechol ring. Finally, various labile trivalent metal cations, analogous to iron, were studied to see how varying the central metal ion would affect the ability of metal enterobactin complexes to inhibit competitively the uptake of ferric enterobactin by the organism. For example, if rhodium MECAM (Figure 1.16) is recognized by the receptor for ferric enterobactin on living microbial cells, a large excess of rhodium MECAM will block the uptake of radioactive iron added as ferric enterobactin. In fact, the rhodium complex completely inhibited ferric-enterobactin uptake, proving that Domain I is not required for recognition of ferric enterobactin.

However, if only Domain III is important in recognition, it would be expected that the simple tris(catecholato)-rhodium(III) complex would be an equally good inhibitor. In fact, even at concentrations in which the rhodium-catechol complex was in very large excess, no inhibition of iron uptake was observed, suggesting that Domain II is important in the recognition process.

The role of Domain II in the recognition process was probed by using a rhodium dimethyl amide of 2,3-dihydroxybenzene (DMB) as a catechol ligand, with one more carbonyl ligand than in the tris(catecholato)-rhodium(III) complex. Remarkably, this molecule shows substantially the same inhibition of enterobactin-mediated iron uptake in *E. coli* as does rhodium MECAM itself. Thus, in addition to the iron-catechol portion of the molecule, the carbonyl groups



MECAM ( $R_1 = R_2 = R_3 = H$ )

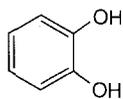
MECAM-Me ( $R_1 = R_3 = H, R_2 = CH_3$ )

MECAMS ( $R_1 = R_2 = H, R_3 = SO_3^-$ )

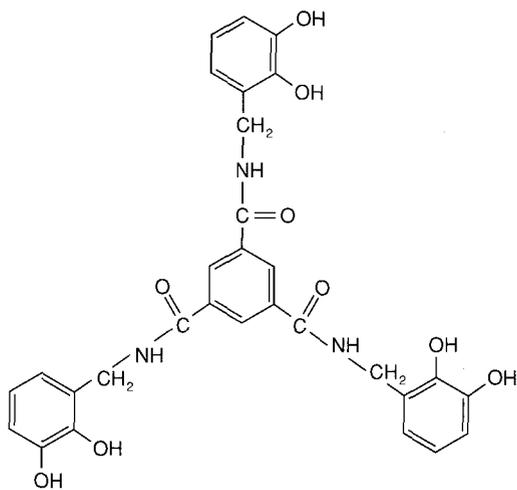
Me<sub>3</sub>MECAM ( $R_2 = R_3 = H, R_1 = CH_3$ )



DMB



catechol



TRIMCAM

Figure 1.16  
MECAM and related enterobactin analogues.

(Domain II) adjacent to the catechol-binding subunits of enterobactin and synthetic analogs are required for recognition by the ferric-enterobactin receptor. In contrast, when a methyl group was attached to the "top" of the rhodium ME-CAM complex, essentially no recognition occurred.

In summary, although the structure of the outer-membrane protein receptor of *E. coli* is not yet known, the composite of the results just described gives a sketch of what the ferric-enterobactin binding site must look like: a relatively rigid pocket for receiving the ferric-catecholate portion of the complex, and proton donor groups around this pocket positioned to hydrogen bond to the carbonyl oxygens of the ferric amide groups. The mechanisms of iron release from enterobactin, though followed phenomenologically, are still not known in detail.

## 2. Zinc, copper, vanadium, chromium, molybdenum, and cobalt

As described in an earlier section, transport problems posed by the six elements listed in the heading are somewhat simpler (with the exception of chromium) than those for iron. One very interesting recent development has been the characterization of sequestering agents produced by plants which complex a number of metal ions, not just ferric ions. A key compound, now well-characterized, is mugenic acid (Figure 1.17).<sup>45</sup> The structural and chemical similari-

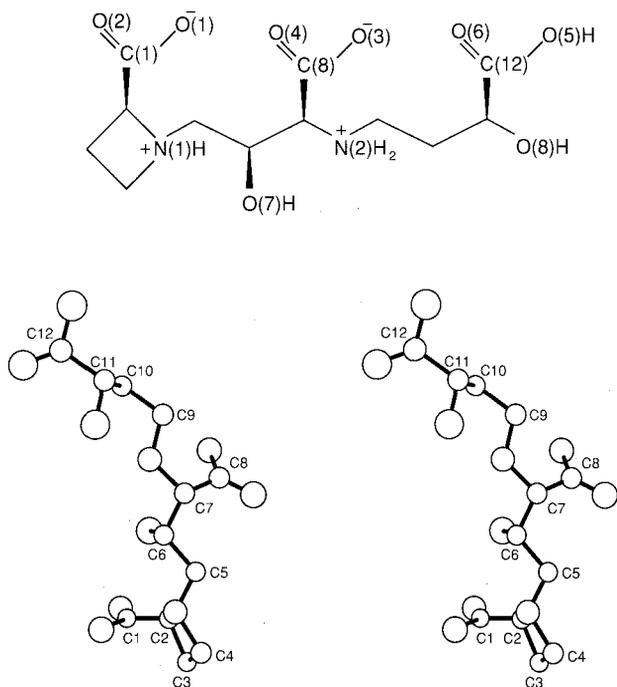


Figure 1.17  
Structure and a stereo view of mugenic acid. See Reference 42.

ties of mugenic acid to ethylenediaminetetraacetic acid (EDTA) have been noted. Like EDTA, mugenic acid forms an extremely strong complex with ferric ion, but also forms quite strong complexes with copper, zinc, and other transition-metal ions. The structure of the cobalt complex (almost certainly essentially identical with that of the iron complex) is shown in Figure 1.18. Like the siderophores produced by microorganisms, the coordination environment accommodated by mugenic acid is essentially octahedral. Although the coordination properties of this ligand are well laid out, and it has been shown that divalent metal cations, such as copper, competitively inhibit iron uptake by this ligand, the detailed process of metal-ion delivery by mugenic acid and related compounds has not been elucidated.

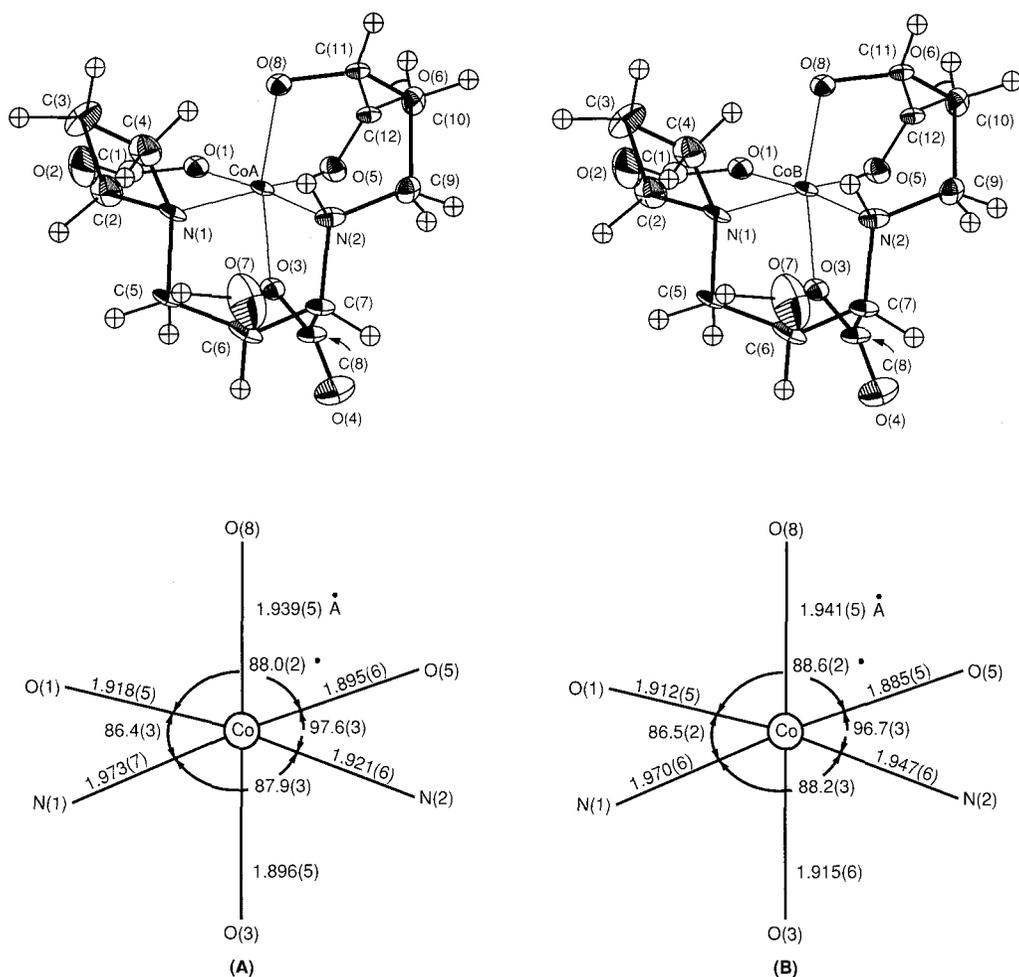


Figure 1.18

Molecular structures of the complexes (molecules A and B) and coordination about the cobalt ion in molecules A and B of the mugenic acid-Co(III) complex. Bond lengths in Å; angles in degrees. See Reference 42.

As noted in an earlier section, the biochemistry of vanadium potentially involves four oxidation states that are relatively stable in aqueous solution. These are  $V^{2+}$ ,  $V^{3+}$ ,  $VO^{2+}$ , and  $VO_2^+$  (the oxidation states 2, 3, 4, and 5, respectively). Since even without added sequestering agents,  $V^{2+}$  slowly reduces water to hydrogen gas, it presumably has no biological significance. Examples of the remaining three oxidation states of vanadium have all been reported in various living systems. One of the most extensively investigated examples of transition-metal-ion accumulation in living organisms is the concentration of vanadium in sea squirts (tunicates), which is reported to be variable; many species have vanadium levels that are not exceptionally high. Others such as *Ascidia nigra* show exceptionally high vanadium concentrations.<sup>46</sup>

In addition to showing a remarkable concentration of a relatively exotic transition-metal ion, tunicates are a good laboratory model for uptake experiments, since they are relatively simple organisms. They possess a circulation system with a one-chambered heart, and a digestive system that is essentially a pump and an inlet and outlet valve connected by a digestive tract. The organism can absorb dissolved vanadium directly from sea water as it passes through the animal. The influx of vanadate into the blood cells of *A. nigra* has been studied by means of radioisotopes. The corresponding influx of phosphate, sulfate, and chromate (and the inhibition of vanadate uptake by these structurally similar oxoanions) has been measured. In the absence of inhibitors, the influx of vanadate is relatively rapid (a half-life on the order of a minute near 0°C) and the uptake process shows saturation behavior as the vanadate concentration is increased. The uptake process (in contrast to iron delivery in microorganisms, for example, and to many other uptake processes in microorganisms or higher animals) is *not* energy-dependent. Neither inhibitors of glycolysis nor decouplers of respiration-dependent energy processes show any significant effect on the rate of vanadate influx.

Phosphate, which is also readily taken up by the cells, is an inhibitor of vanadate influx. Neither sulfate nor chromate is taken up significantly, nor do they act as significant inhibitors for the vanadate uptake. Agents that inhibit transport of anions, in contrast, were found to inhibit uptake of vanadate into the organism. These results have led to the model proposed in Figure 1.19:

- (1) vanadate enters the cell through anionic channels; this process eliminates positively charged metal ion or metal-ion complexes present in sea water;
- (2) vanadate is reduced to vanadium(III); since the product is a cation, and so cannot be transported through the anionic channels by which vanadate entered the cell, the vanadium(III) is trapped inside the cell—the net result is an accumulation of vanadium. [It has been proposed that the tunichrome could act either as a reducing agent (as the complex) or (as the ligand) to stabilize the general vanadium(III); however, this seems inconsistent with its electrochemical properties (see below).]

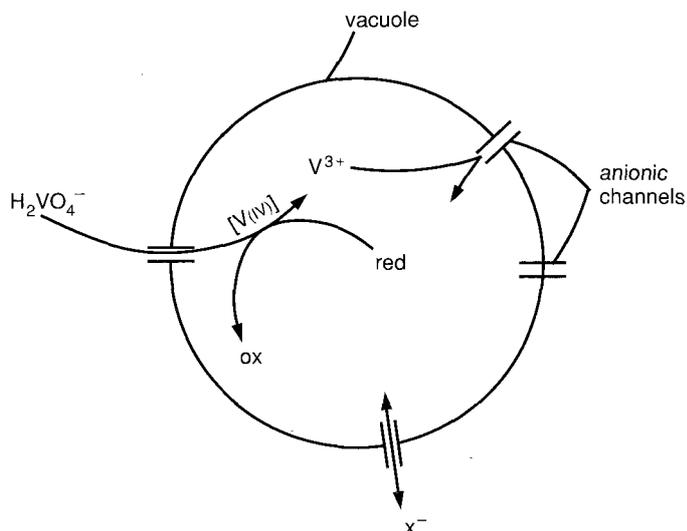


Figure 1.19

Diagram of a vanadium accumulation mechanism. Vanadium enters the vacuole within the vanadocyte as mononegative  $\text{H}_2\text{VO}_4^-$ , although it may be possible for the dinegative anion,  $\text{HVO}_4^{2-}$ , to enter this channel as well ( $\text{X}^-$  stands for any negative ion such as  $\text{Cl}^-$ ,  $\text{H}_2\text{PO}_4^-$ , etc., that may exchange across the membrane through the anionic channel). Reduction to  $\text{V}^{3+}$  takes place in two steps, via a  $\text{V(IV)}$  intermediate. The resulting cations may be trapped as tightly bound complexes, or as free ions that the anionic channel will not accept for transport. The nature of the reducing species is unknown.

Synthetic models of tunichrome b-1 (Figure 1.20) have been prepared. Tunichrome is a derivative of pyrogallol whose structure precludes the formation of an octahedral complex of vanadium as a simple 1:1 metal:ligand complex. The close analogue, described as 3,4,5-TRENPAMH<sub>9</sub>, also cannot form a simple octahedral 1:1 complex. In contrast, the synthetic ligands TRENCAM and 2,3,4-TRENPAM can form pseudo-octahedral complexes. The structure of the vanadium TRENCAM complex shows that it is indeed a simple pseudo-octahedral tris-catechol complex.<sup>47</sup> The electrochemical behavior of these complexes is similar, with vanadium(IV/III) potentials of about  $-0.5$  to  $-0.6$  volts versus NHE. These results indicate that tunichrome b-1 complexes of vanadium(IV/III) would show similar differences in their redox couples at high pH. At neutral pH, in the presence of excess pyrogallol groups, vanadium(IV) can be expected to form the intensely colored tris-catechol species. However, comparison of the EPR properties reported for vanadium-tunichrome preparations with model vanadium(IV)-complexes would indicate predominantly bis(catechol) vanadyl coordination. In any case, the vanadium(III) complexes must remain very highly reducing. It has been pointed out that the standard potential of pyrogallol is  $0.79$  V and decreases  $60$  mV per pH unit (up to about pH 9), so that at pH 7 the potential is about  $0.4$  V. The potentials of the vanadium couples for the tunichrome analogs are about  $-0.4$  V. It has been concluded, therefore, that tunichrome or similar ligands cannot reduce the vanadium(IV) complex; so the

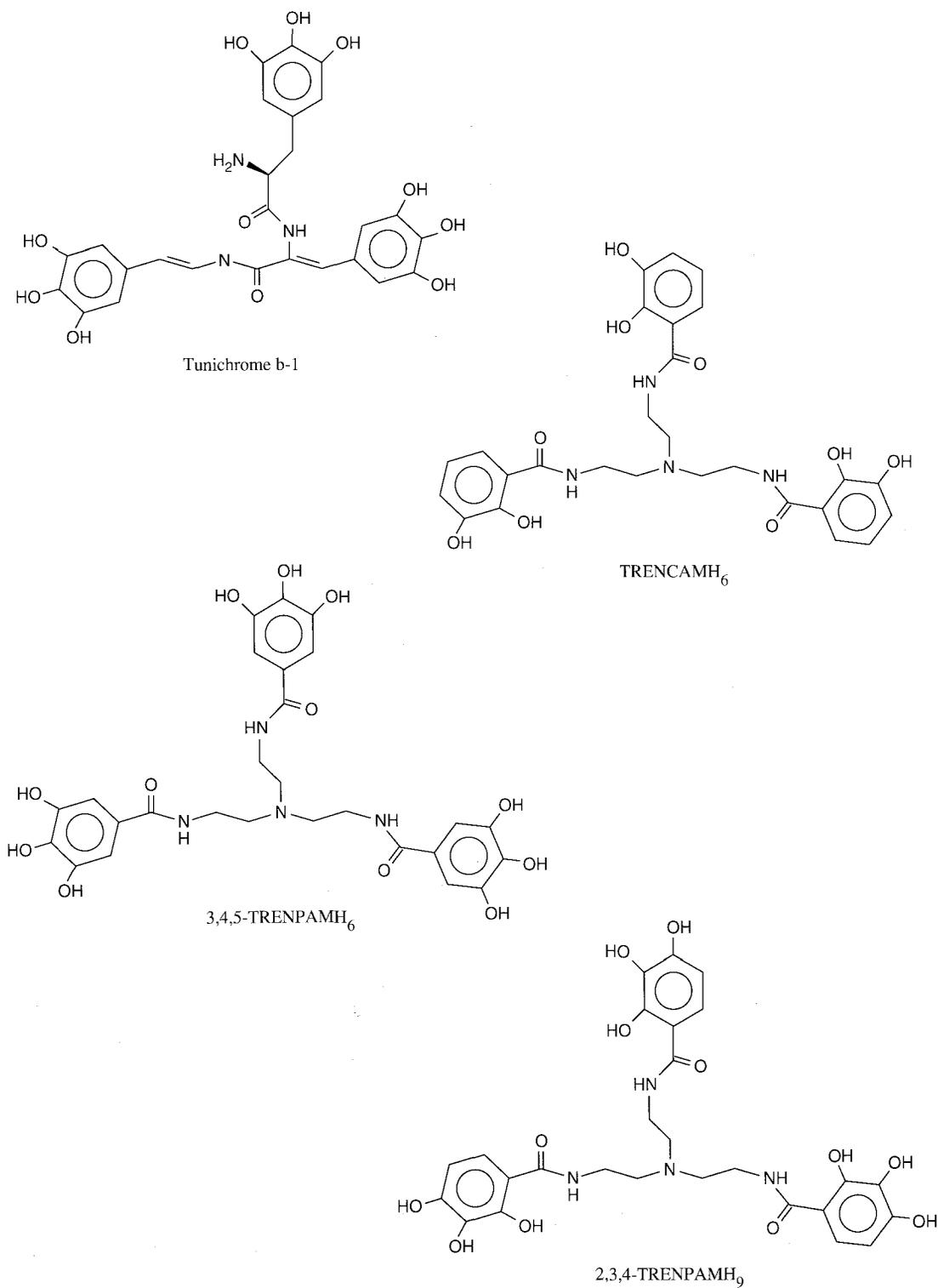


Figure 1.20  
Structures of tunichrome b-1 and synthetic analogues.<sup>43</sup>

highly reducing vanadium(III) complex of tunichrome must be generated in some other way.<sup>47</sup>

Although a detailed presentation of examples of the known transport properties of essential transition-metal ions into various biological systems could be the subject of a large book, the examples that we have given show how the underlying inorganic chemistry of the elements is used in the biological transport systems that are specific for them. The regulation of metal-ion concentrations, including their specific concentration when necessary from relatively low concentrations of surrounding solution, is probably one of the first biochemical problems that was solved in the course of the evolution of life.

Iron is transported in forms in which it is tightly complexed to small chelators called siderophores (microorganisms) or to proteins called transferrins (animals) or to citrate or mugenic acid (plants). The problem of how the iron is released in a controlled fashion is largely unresolved. The process of mineral formation, called biomineralization, is a subject of active investigation. Vanadium and molybdenum are transported as stable anions. Zinc and copper appear to be transported loosely associated with peptides or proteins (plants) and possibly mugenic acid in plants. Much remains to be learned about the biological transport of nonferrous metal ions.

### C. Iron Biomineralization

Many structures formed by living organisms are minerals. Examples include apatite [ $\text{Ca}_2(\text{OH})\text{PO}_4$ ] in bone and teeth, calcite or aragonite ( $\text{CaCO}_3$ ) in the shells of marine organisms and in the otoconia (gravity device) of the mammalian ear, silica ( $\text{SiO}_2$ ) in grasses and in the shells of small invertebrates such as radiolaria, and iron oxides, such as magnetite ( $\text{Fe}_3\text{O}_4$ ) in birds and bacteria (navigational devices) and ferrihydrite  $\text{FeO}(\text{OH})$  in ferritin of mammals, plants, and bacteria. Biomineralization is the formation of such minerals by the influence of organic macromolecules, e.g., proteins, carbohydrates, and lipids, on the precipitation of amorphous phases, on the initiation of nucleation, on the growth of crystalline phases, and on the volume of the inorganic material.

Iron oxides, as one of the best-studied classes of biominerals containing transition metals, provide good examples for discussion. One of the most remarkable recent characterizations of such processes is the continual deposition of single-crystal ferric oxide in the teeth of chiton.<sup>48</sup> Teeth of chiton form on what is essentially a continually moving belt, in which new teeth are being grown and moved forward to replace mature teeth that have been abraded. However, the study of the mechanisms of biomineralization in general is relatively recent; a great deal of the information currently available, whether about iron in ferritin or about calcium in bone, is somewhat descriptive.

Three different forms of biological iron oxides appear to have distinct relationships to the proteins, lipids, or carbohydrates associated with their formation and with the degree of crystallinity.<sup>49</sup> Magnetite, on the one hand, often forms almost perfect crystals inside lipid vesicles of magneto-bacteria.<sup>50</sup> Ferrihydrite,

on the other hand, exists as large single crystals, or collections of small crystals, inside the protein coat of ferritin; however, iron oxides in some ferritins that have large amounts of phosphate are very disordered. Finally, goethite [ $\alpha$ -FeO(OH)] and lepidocrocite [ $\gamma$ -FeO(OH)] form as small single crystals in a complex matrix of carbohydrate and protein in the teeth of some shellfish (limpets and chitons); magnetite is also found in the lepidocrocite-containing teeth. The differences in the iron-oxide structures reflect differences in some or all of the following conditions during formation of the mineral: nature of co-precipitating ions, organic substrates or organic boundaries, surface defects, inhibitors, pH, and temperature. Magnetite can form in both lipid and protein/carbohydrate environments, and can sometimes be derived from amorphous or semicrystalline ferrihydrite-like material (ferritin). However, the precise relationship between the structure of the organic phase and that of the inorganic phase has yet to be discovered. When the goal of understanding how the shape and structure of biominerals is achieved, both intellectual satisfaction and practical commercial and medical information will be provided.

Synthetic iron complexes have provided models for two stages of ferritin iron storage and biomineralization:<sup>51-59</sup> (1) the early stages, when small numbers of clustered iron atoms are bound to the ferritin protein coat, and (2) the final stages, where the bulk iron is a mineral with relatively few contacts to the protein coat. In addition, models have begun to be examined for the microenvironment inside the protein coat.<sup>54</sup>

Among the models for the early or nucleation stage of iron-core formation are the binuclear Fe(III) complexes with  $[\text{Fe}_2\text{O}(\text{O}_2\text{CR}_2)]^{2+}$  cores;<sup>55,56</sup> the three other Fe(III) ligands are N. The  $\mu$ -oxo complexes, which are particularly accurate models for the binuclear iron centers in hemerythrin, purple acid phosphatases, and, possibly, ribonucleotide reductases, may also serve as models for ferritin, since an apparently transient Fe(II)-O-Fe(III) complex was detected during the reconstitution of ferritin from protein coats and Fe(II). The facile exchange of ( $\text{O}_2\text{CR}$ ) for ( $\text{O}_2\text{PR}$ ) in the binuclear complex is particularly significant as a model for ferritin, because the structure of ferritin cores varies with the phosphate content. An asymmetric trinuclear  $(\text{Fe}_3\text{O})^{7+}$  complex<sup>57</sup> and an  $(\text{FeO})_{11}$  complex (Figure 1.21) have been prepared; these appear to serve as models for later stages of core nucleation (or growth).<sup>59</sup>

Models for the full iron core of ferritin include ferrihydrite, which matches the ordered regions of ferritin cores that have little phosphate; however, the site vacancies in the lattice structure of ferrihydrite [ $\text{FeO}(\text{OH})$ ] appear to be more regular than in crystalline regions of ferritin cores. A polynuclear complex of iron and microbial dextran ( $\alpha$ -1,4-D-glucose)<sub>n</sub> has spectroscopic (Mössbauer, EXAFS) properties very similar to those of mammalian ferritin, presumably because the organic ligands are similar to those of the protein ( $-\text{OH}$ ,  $-\text{COOH}$ ). In contrast, a polynuclear complex of iron and mammalian chondroitin sulfate ( $\alpha$ -1,4- $[\alpha$ -1,3-D-glucuronic acid-N-acetyl-D-galactosamine-4-sulfate]<sub>n</sub>) contains two types of domains: one like mammalian ferritin [ $\text{FeO}(\text{OH})$ ] and one like hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>), which was apparently nucleated by the sulfate, emphasizing

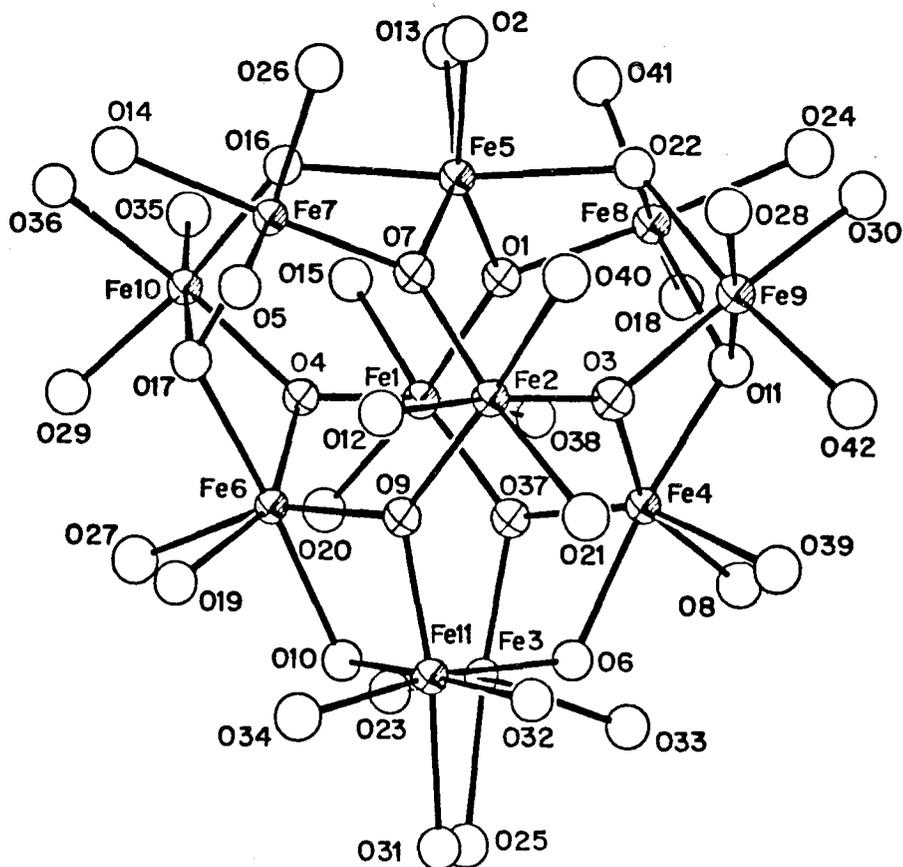


Figure 1.21

The structure of a model for a possible intermediate in the formation of the ferritin iron core. The complex consists of 11 Fe(III) atoms with internal oxo-bridges and a coat of benzoate ligands; the Fe atoms define a twisted, pentacapped trigonal prism. See Reference 53.

the importance of anions in the structure of iron cores.<sup>60</sup> Finally, a model for iron cores high in phosphate, such as those from bacteria, is Fe-ATP (4:1), in which the phosphate is distributed throughout the polynuclear iron complex, providing an average of 1 or 2 of the 6 oxygen ligands for iron.<sup>61</sup>

The microenvironment inside the protein coat of ferritin has recently been modeled by encapsulating ferrous ion inside phosphatidylcholine vesicles and studying the oxidation of iron as the pH is raised. The efficacy of such a model is indicated by the observation of relatively stable mixtures of Fe(II)/Fe(III) inside the vesicles, as have also been observed in ferritin reconstituted experimentally from protein coats and ferrous ion.<sup>43,54</sup>

Models for iron in ferritin must address both the features of traditional metal-protein interactions and the bulk properties of materials. Although such modeling may be more difficult than other types of bioinorganic modeling, the difficulties are balanced by the availability of vast amounts of information on Fe-

protein interactions, corrosion, and mineralization. Furthermore, powerful tools such as x-ray absorption, Mössbauer and solid state NMR spectroscopy, scanning electron and proton microscopy, and transmission electron microscopy reduce the number of problems encountered in modeling the ferritin ion core.

Construction of models for biomineralization is clearly an extension of modeling for the bulk phase of iron in ferritin, since the major differences between the iron core of ferritin and that of other iron-biominerals are the size of the final structure, the generally higher degree of crystallinity, and, at this time, the more poorly defined organic phases. A model for magnetite formation has been provided by studying the coulometric reduction of half the  $\text{Fe}^{3+}$  atoms in the iron core of ferritin itself. Although the conditions for producing magnetite have yet to be discovered, the unexpected observation of retention of the  $\text{Fe}^{2+}$  by the protein coat has provided lessons for understanding the iron core of ferritin. Phosphatidyl choline vesicles encapsulating  $\text{Fe}^{2+}$  appear to serve as models for both ferritin and magnetite; only further investigation will allow us to understand the unique features that convert  $\text{Fe}^{2+}$  to  $[\text{FeO}(\text{OH})]$ , on the one hand, and  $\text{Fe}_3\text{O}_4$ , on the other.

### III. SUMMARY

Transition metals (Fe, Cu, Mo, Cr, Co, Mn, V) play key roles in such biological processes as cell division (Fe, Co), respiration (Fe, Cu), nitrogen fixation (Fe, Mo, V), and photosynthesis (Mn, Fe). Zn participates in many hydrolytic reactions and in the control of gene activity by proteins with "zinc fingers." Among transition metals, Fe predominates in terrestrial abundance; since Fe is involved in a vast number of biologically important reactions, its storage and transport have been studied extensively. Two types of Fe carriers are known: specific proteins and low-molecular-weight complexes. In higher animals, the transport protein transferrin binds two Fe atoms with high affinity; in microorganisms, iron is transported into cells complexed with catecholates or hydroxamates called siderophores; and in plants, small molecules such as citrate, and possibly plant siderophores, carry Fe. Iron complexes enter cells through complicated paths involving specific membrane sites (receptor proteins). A problem yet to be solved is the form of iron transported in the cell after release from transferrin or siderophores but before incorporation into Fe-proteins.

Iron is stored in the protein ferritin. The protein coat of ferritin is a hollow sphere of 24 polypeptide chains through which  $\text{Fe}^{2+}$  passes, is oxidized, and mineralizes inside in various forms of hydrated  $\text{Fe}_2\text{O}_3$ . Control of the formation and dissolution of the mineral core by the protein and control of protein synthesis by Fe are subjects of current study.

Biomineralization occurs in the ocean (e.g., Ca in shells, Si in coral reefs) and on land in both plants (e.g., Si in grasses) and animals (e.g., Ca in bone, Fe in ferritin, Fe in magnetic particles). Specific organic surfaces or matrices of protein and/or lipid allow living organisms to produce minerals of defined shape and composition, often in thermodynamically unstable states.

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