1.2: Biological Systems of Metal Storage

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 CO_2 to make carbohydrates $(CH_2O)_n$ and produce O_2, exhausted from the environment the reductants from H_2 or H_2S or NH_3. The ability of primitive organisms to switch to the use of H_2O 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 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 $[Fe_2O_3(H_2O)_n]$ with various amounts of phosphate.[6,7] 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 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.[31] 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 (Fe_3O_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.[6,7]

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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.

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.[62,63,65] Since the protein coat is stable with or without iron, the center of the hollow sphere may be filled with solvent, with $Fe_2O_3 \cdot H_2O$, 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.[64]

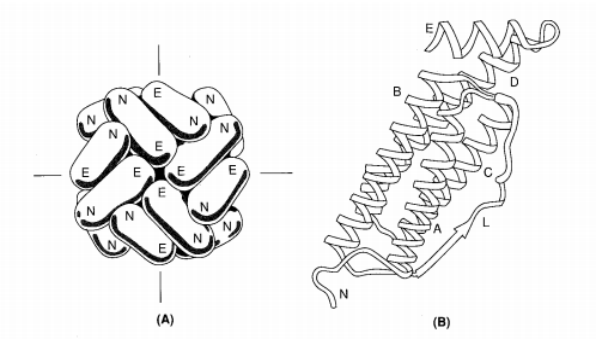

Figure 1.9 - (A) The protein coat of horse spleen apoferritin deduced from x-ray diffraction of crystals of the protein.[32] The outer surface of the protein coat shows the arrangement of the 24 ellipsoidal polypeptide subunits. N refers to the N-terminus of each polypeptide and E to the E-helix (see B). Note the channels that form at the four-fold axes where the E-helices interact, and at the threefold 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.

* 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.

Iron-Protein Interface

Formation of the iron core appears to be initiated at an Fe-protein interface where Fe(II)-O-Fe(Ill) dimers and small clusters of Fe(Ill) 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 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 (*E*xtended *X*-ray *A*bsorption *F*ine *S*tructure) 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.[64]

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 $Fe(III)(NO_3)_3$. 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[29] 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^{2+} 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(III).[34a,b] 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.

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 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[35,36] (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[35,36] 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 -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 ≤ 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 about the molecules that store these metals, with the possible exception of metallothionein, which binds small clusters of zinc or copper.

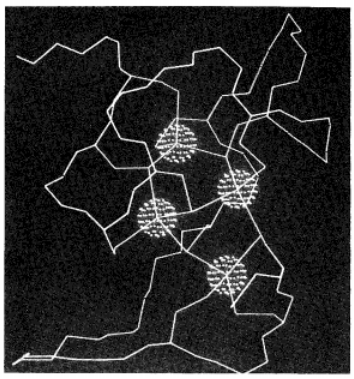

Figure 1.10 - The three-dimensional structure of the a domain from rat cd_7 metallothionein-2, determined by NMR in solution (Reference 36a), based on data in Reference 36b. The four metal atoms, bonded to the sulfur of cysteine side chains, are indicated as spherical collections of small dots. A recent 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).