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Iron Binding Proteins without Cofactors or Sulfur Clusters

ADVANCES IN INORGANIC BIOCHEMISTRY

Gunther L. Eichhorn and Luigi G. Marzilli, *Series Editors*

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PREFACE

When iron proteins are discussed in terms of protein structure or metal binding, the major categories are heme proteins and iron-sulfur proteins. Other iron proteins, for which the metal environment is often formed primarily by the protein, are sometimes included in such discussions but more often are analyzed in terms of function; it is not uncommon to consider the iron protein interface only secondarily, and often analogies to other iron proteins are obscured. However, as discussed below, some common structural features do exist among the iron centers of the proteins. Further consideration of the metal site should aid in separating those properties necessary for iron-binding from those required for function and, incidentally, should provide a more positive description of the metal-protein interaction. Although the number of investigations of these iron-proteins has been increasing rapidly in the last few years, the reports are scattered in a wide variety of publications, each type directed to a different audience. It occurred to us that a series of reviews describing both the structural and functional features of such iron-proteins would stimulate further investigation of the similarities and differences among the proteins. Some of the proteins, e.g. ferritin, are widespread and found in plants, animals and protista while another, ribonucleotide reductase, is central to life by virtue of its role in DNA synthesis. Still another, hemerythrin, is found in only a few invertebrates but has, over the years, provided many lessons for students of iron-dioxygen interactions.

The functions of iron-proteins considered here vary widely and include deoxyribose synthesis (ribonucleotide reductase), oxygen transport (hemerythrin), oxygen activation (catechol dioxygenases), phosphoester hydrolysis (uteroferrin and the purple acid phosphatases), phosphorus storage and transport (phosvitin), iron transport (transferrin, phosvitin) and iron storage (ferritin and phosvitin). The siderophores, low molecular weight iron carriers in microorganisms, are also included in the volume because the iron-ligand interactions and mechan-

isms of metabolic regulation can serve as models for the proteins. Each chapter reviews structure, function and where possible, the regulation of synthesis. Regulation of iron proteins is particularly critical because of the extreme biological hazards of very slight excesses of iron; thus the regulation of synthesis of compounds involved in iron metabolism has some unusual features.

A property shared by all the proteins as well as the siderophores described here is the absorption of visible light. The iron proteins, without heme or sulfur clusters are a colorful group: purple, pink, red, rust and green, with extinction coefficients generally in the millimolar range. However, in the case of ferritin, the rust color is associated with the iron core; the absorption spectrum of iron bound to the protein at the nucleation sites has yet to be determined. Each protein has been analyzed by a variety of techniques including X-ray diffraction, EXAFS analysis, Mössbauer, resonance Raman, EPR and circular dichroism spectroscopy and magnetic susceptibility in addition to optical spectra. Although each technique has provided fascinating insights, in no case have the combined results yet led to a complete description of the iron environment in different functional states.

Among the properties common to several of the proteins are dimeric iron centers, aromatic hydroxyl and/or carboxylate iron ligands and interactions with phosphate. Oxygen-bridged dimeric iron centers are found in hemerythrins, ribonucleotide reductases, uteroferrin and bovine spleen purple acid phosphatase; an oxygen-bridged iron dimer has also been proposed to occur during initiation of iron polymer formation in ferritin. The existence of relatively stable, mixed valence iron pairs has recently been observed in hemerythrin, uteroferrin, and bovine spleen purple acid phosphatase and provides a new dimension in the study of iron-protein interactions. Aromatic hydroxyl iron ligands are found in the iron-tyrosinate protein subset (the catechol dioxygenases, uteroferrin, bovine spleen purple acid phosphatases, and transferrin) as well as the catechol siderophores. In addition, ribonucleotide reductase, from bacteria and mammals, contains a tyrosine radical required for function and dependent upon iron for stability. Carboxylate ligands have been detected at the iron binding sites of hemerythrin and apoferritin while carbonate is an obligatory anion at the iron centers in transferrin. Phosphate or phosphate derivatives influence the properties of the iron-protein complex in uteroferrin and transferrin, manifested as alterations in the CD spectrum or stability, respectively. In the case of phosvitin, phosphate links the iron to serine side chains, resulting in iron phosphate clusters with a Fe:P = 1:2. Finally, phosphate changes the size of the iron polymer in ferritin, appearing in disordered regions of the iron core and/

or at the protein-iron interface.

To our knowledge, no collection of review articles on iron-proteins without heme or sulfur clusters has previously appeared. It remains to be seen whether interest in the proteins will be enhanced by the book and whether features of structure common to all the proteins will become apparent. Even now it is clear that as a group these proteins have novel structural features.

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April, 1983

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FERRITIN: STRUCTURE, FUNCTION, AND REGULATION

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I. INTRODUCTION

The necessity for ferritin in living cells is illustrated by examining the behavior of iron salts in solution in the presence of air at physiological pH (Figure 1); in the figure the solution containing apoferritin (6.7 μM) contains

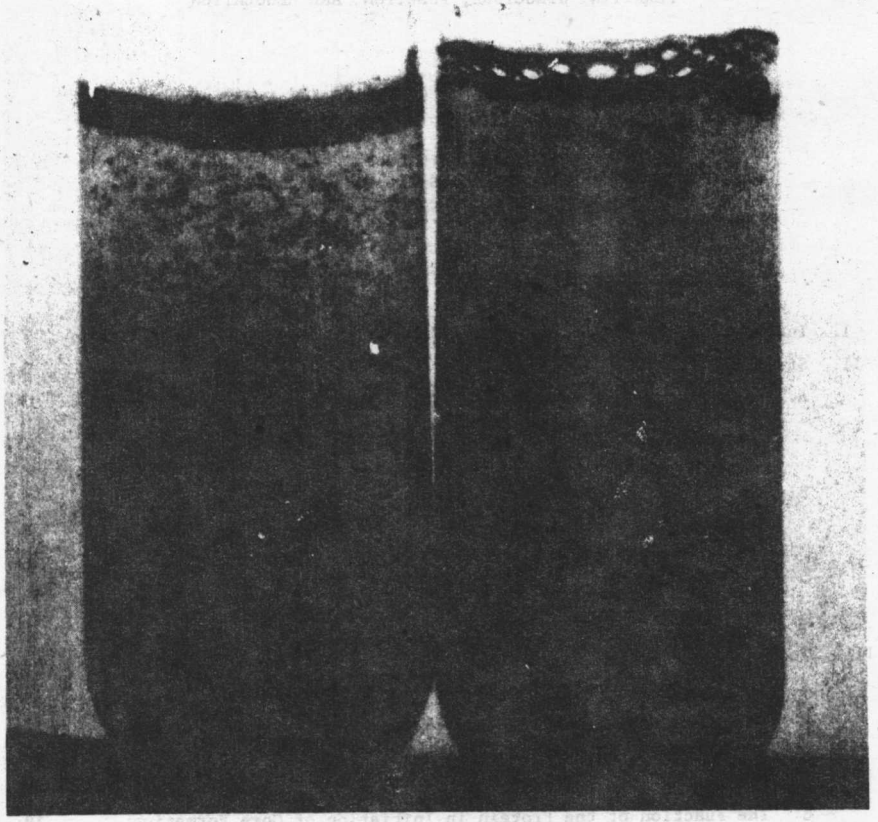

Fig. 1. Solutions of iron in the presence and absence of apoferritin. A freshly prepared solution of $FeSO_4$ was added to 0.02 M imidazole, pH 7.0, with or without horse spleen apoferritin (6.7 μM); the final concentration of iron was 2mM. Note the absence of precipitate in the tube on the right containing apoferritin, although the iron concentration exceeds that of the hydrated iron ion by 10^{14} fold.

Fe^{3+} at a concentration equivalent to 10^{14} times that of the hydrated ion in the tube without apoferritin. Several unusual features of ferritin structure and function such as the extraordinarily large number of iron atoms per molecule, the large number of protein subunits, and the self-regulation of synthesis by iron are sufficiently striking that attention can be drawn away from the interaction between individual iron atoms and the protein. However, recent advances in understanding the primary and three-dimensional structure of apoferritin and metal binding by the protein allow a sharper focus upon the nature of the iron-protein interface. Many excellent reviews on ferritin have appeared (e.g., 1-3). The goal of this review is to complement them by taking advantage of more recent data to emphasize the contribution of the protein shell to the function of ferritin.

II. STRUCTURE

Ferritin consists of a multi-subunit protein shell, apoferritin, surrounding a core of hydrous ferric oxide. The core is variable in size and may consist of as many as 4500 atoms.

A. The Iron Core

1. The Three-dimensional Organization of Iron and Oxygen Atoms. The polymeric iron core of ferritin has an average composition of $(FeOOH)_8 \cdot FeO \cdot OPO_3H_2$ (4) and exhibits crystalline behavior in a variety of analyses including X-ray and electron diffraction (5-8), ultra high resolution electron microscopy (9), and Mössbauer spectroscopy (10,11). The size of the cores, estimated from Mössbauer spectra, measured between 10 and 300 K, ranges from 40 to 88 Å; 64 Å was the most common size (10).

X-ray and electron diffraction measurements of the ferritin core made independently by several investigators (5-8, e.g.) are very similar (for example, Table I). The diffraction pattern of the core is unaffected by hydration, deproteinization with 1 N NaOH (5), 5.25% NaOCl (7), or reconstitution from the apoprotein shell and $FeSO_4$ or $Fe(NH_4)_2(SO_4)_2$ in the presence of $NaHCO_3$ or histidine or KIO_3/S_2O_3 (5). Since reconstitution in the absence of phosphate, a natural component of the core, produces the same diffraction pattern as native ferritin (5), it is apparent that phosphate does not detectably affect the three-dimensional structure of the iron core. The diffraction pattern of the ferritin core is very similar to that of ferrihydrite ($5\ Fe_2O_3 \cdot 9\ H_2O$), a polymer formed from solutions of ferric nitrate after heating to 80 or 90 °C (6,7) and/or the slow addition of NH_4OH (8); ferrihydrite is devoid of phosphate.

TABLE I

INTERPLANAR SPACINGS OF THE IRON CORE IN HORSE SPLEEN FERRITIN FROM X-RAY AND ELECTRON DIFFRACTION[a]

d_0	I_0 (X-ray, (5))	I_0 (E.D.,(7))
2.54	Strong	Strong
2.47		
2.24	Strong	Strong
1.98	Medium	Medium
1.73	Weak	Weak[b]
1.51	Very weak	Weak
1.47	Strong	Strong
1.34	Very weak	-
1.23	Weak	-

[a]The data are taken from references (5) and (7). The abbreviations used are: d_0, observed d-spacing in angstroms; I_0, observed intensity, visually estimated.
[b]1.72.

Two models have been proposed to fit the diffraction data for ferritin, both of which involve hexagonal close-packed layers of oxygen with iron atoms between the layers. In one model, an arrangement of 12 iron sites/unit cell was proposed, involving both tetrahedral and octahedral coordination (5). Subsequent analyses of electronic and Mössbauer spectra (11) and EXAFS analyses (12,13) indicated that essentially all the iron atoms in the ferritin core are octahedrally coordinated. Another early model (6) correctly predicted the octahedral coordination of the iron but required regular spacing of the iron layers with vacancies in order to fit the X-ray data.

Examination of individual ferritin cores by ultra high resolution electron microscopy confirmed the ordering of iron atoms indicated by X-ray and electron diffraction studies of populations of molecules (9). Ferritin was observed with single large crystals or multiple small crystals. Essentially every iron core, regardless of the crystal size, exhibited lattice defects with discontinuities and/or variable scattering densities, suggesting incomplete occupancy of the iron layers. However, the spacing of such incomplete layers was irregular, in contrast to the model based on the X-ray data (6) and occurred at intervals of 2, 3, or 6 oxygen layers (9). The frequency of lattice imperfections, such as site vacancies, indicates that 20-33% of the iron atoms in a completed ferritin core should be in a different environment from the rest (9). If, however, the environment of each iron atom were the same, using the average Fe-O bond lengths

measured with EXAFS analysis and a density of 3.5 gm/cc, a core structure can be proposed (12) with hexagonal symmetry in which iron atoms are coordinated to oxygen in a flattened octahedral arrangement; the plane with iron atoms lies between two planes of approximately close-packed oxygen. The sets of O-Fe-O layers are only weakly bound to each other to form the three-dimensional structure. In such a model, each plane of iron atoms terminates with a phosphate group at the core-protein interface.

The variety of models proposed to explain the existing data on the structure of the ferritin core reflects, in part, the problems of reconciling core composition and density with a regular array of all the iron atoms. While the ferritin core may be entirely crystalline, albeit imperfectly crystalline, it may also be that highly disordered or amorphous regions of the iron polymer exist within the crystallites in analogy to crystals of organic polymers (14).

2. The Distribution of Phosphate. The phosphate in the ferritin core may be both in disordered regions and at the surface, since reconstitution of ferritin in the absence of phosphate has no detectable effect on the X-ray or electron diffraction pattern (5); the similarity between the three-dimensional structure of ferrihydrite and the phosphorus-containing ferritin core reflects the location of phosphorus in disordered regions of the core. Treating ferritin with 0.1 M ammonium magnesium citrate precipitates only 60% of the phosphorus even after 5 days (4), indicating that some of the phosphate is inaccessible to the reagent and may be tightly bound inside the core.

Several observations suggest that phosphate may represent chain termination sites at the surface and/or in the core interior. For example, if phosphate is present during reconstitution of ferritin from apoferritin and $FeSO_4$ (Fe/PO_4 = 5:1), the core size is reduced, measured both chemically (15) and by Mössbauer spectroscopy (10); the most common crystallite diameter deduced from Mössbauer spectroscopy is 48 Å, compared to 64 Å when phosphate is absent. Studies of phosphate release from such reconstituted cores also suggest both surface and interior positions for the phosphates (15). In addition, when native ferritin molecules are fractionated according to iron content, the phosphate/iron ratio is higher in ferritin molecules with a low iron content than in those with a high iron content (15 and references therein). Since iron polymers grow by branching, and since there are multiple sites in ferritin for initiation of polymerization (e.g., 16), it is possible to envision chain termini both with exterior and with interior positions caused by branched growth around a termination point or at the junction of several polymer chains, placing the phosphate at chain termini in the core interior and/or on the surface. An alternative

view of the location of the phosphate is that it is adventitiously bound on the core surface and at faults in the interior of the crystal (15). Whatever the location of the phosphate, the environment of iron near phosphate is different from other iron atoms in terms of three-dimensional order and possibly because of coordination to the phosphate. Such differences in environment can account, in part at least, for the heterogeneity of the rate of iron released from ferritin observed in vitro (17,18).

B. The Protein Shell

1. General Properties. The apoferritin shell defines the physical limits of the iron core, affects the three-dimensional structure of the iron core (5), and may, via cell-specific variations, influence the storage function in vivo (19). The shell is assembled from subunits before iron core formation (20,21). Recently X-ray diffraction data on horse spleen apoferritin have been analyzed at high resolution and are described in detail in the next chapter (22). Briefly, the shell is composed of 24 elliptical subunits arranged in a hollow sphere. In eukaryotes the shell is entirely protein, but bacterioferrins, isolated from Escherichia coli (23) and Azotobacter vinelandii (24) contain heme. In addition to being found in higher and lower plants (e.g., 25), fungi (26), bacteria (e.g. 23,24), and invertebrates (e.g., 27), ferritin is found in many different organs and cell types of vertebrates, g.g., liver, spleen, heart, muscle, and blood.

2. The Primary Structure. The amino acid composition and tryptic peptide maps (19,24-35) of ferritin from a variety of sources are quite similar, suggesting close homology. The similarity of ferritin from different sources is emphasized by the immunological cross-reactivity of antisera with a wide variety of ferritins (e.g., 27,36-39).

Despite the common features among ferritin molecules from different organisms, specific differences occur among species, organs, and cells. Some of the differences appear to reflect the cellular location of ferritin molecules rather than function. For example, ferritin is most often an intracellular protein, frequently sequestered in subcellular components, e.g., lysosomes or vesicles (40,41). However, small amounts of ferritin are normally present in the serum.* The ferritin found in serum conforms to the observation that glycosylation of proteins is often associated with secretion and stability in the extracellular

*The facile detection of serum ferritin by radioimmunometric analysis (35) is clinically important for measuring iron stores. The prognostic value of analyses for serum ferritin concentration is also being evaluated, since changes in serum ferritin are associated with tissue damage and inflammation and directly or indirectly with malignancies and infection (see 42 and 43 for reviews).

COMPARISON OF FERRITIN SEQUENCES

(1) Sequence of horse spleen ferritin
(2) Sequence of the principal component of human spleen apoferritin
(3) Sequence of the minor component of human spleen apoferritin

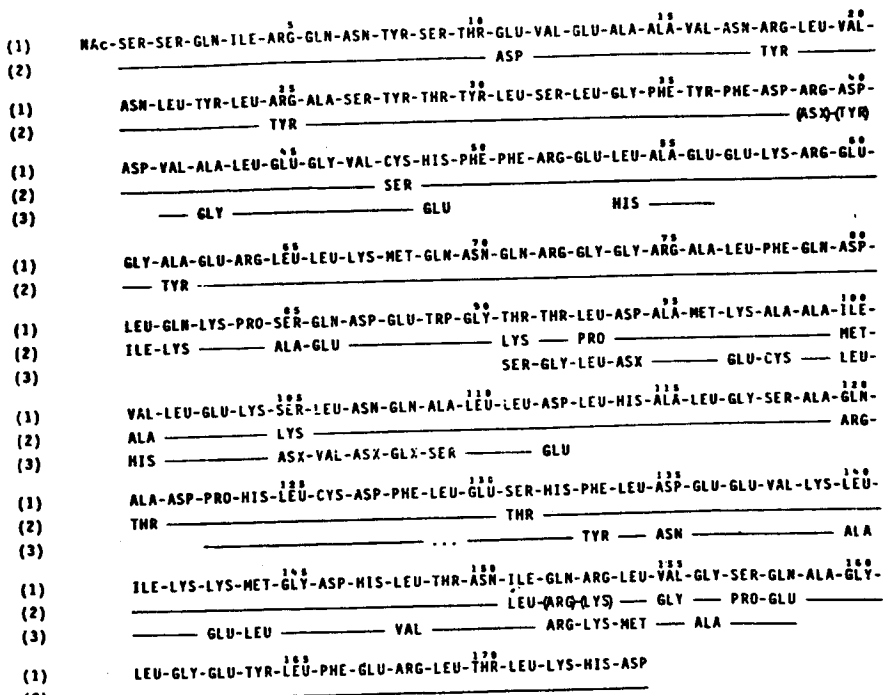

Fig. 2. The amino acid sequence of apoferritin from horse and human spleen. Reprinted from reference (46) with permission.