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THE FORMATION, STRUCTURE AND DISSOLUTION OF THE FERRITIN IRON CORE STUDIED BY X-RAY ABSORPTION SPECTROSCOPY


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ABSTRACT

Ferritin is the solution to the problem of rust in biological material. Polynuclear iron complexes of up to 4500 Fe(III) atoms reversibly form a hydrous ferric oxide core inside a hollow spherical protein coat (apoferitin) that permits the controlled release of iron as needed by the organism. To study intermediates in formation of the iron core (A) and factors which may alter core structure (B), we used EXAFS. To study the factors which may influence the kinetics of iron core reduction and dissolution (C), we used dispersive x-ray absorption spectroscopy (DXAS). The results show (A) that the protein coat appears to control initiation and nucleation of the iron core; (B) that model iron core structures can be influenced by sulfate which appears to nucleate domains of hematite (Fe₂O₃) that coexist in the soluble complex with FeO·OH; and (C) that the reduction of core Fe is influenced by buffer ions, suggesting that the availability of ferritin iron in vivo may be modulated by cytoplasmatic changes in small ions.

INTRODUCTION

Iron is required in plants and animals for a variety of functions such as electron transfer, oxygen transport and activation, nitrogen reduction, and deoxyribonucleotide synthesis (for DNA). The interaction of iron and oxygen could produce rust in biological systems just as the interaction produces corrosion of iron-containing materials. However, in the case of living systems, the oxidation and hydrolysis of iron proceeds inside a hollow protein called apoferitin. Ferritin, the complex of hydrous ferric oxide and apoferitin, stores up to 4500 Fe(III) atoms in a soluble, bioavailable form. Although a great deal is known about the structure of the protein, and something is known about the iron core, little is known about the steps in the formation of the iron core and about the release of iron from the core. The steps that can be defined are illustrated below:

- Apoferritin → Fe(II)apoferitin
- Fe(III)apoferitin + O₂ → Fe(III)apoferitin
- Reduction of Fe(II)apoferitin by thiolglycolic acid
- Ferritin + 480 Fe(III) as an inner core of FeO·OH
We have investigated some of the intermediate steps in the formation and dissolution of the ferritin iron core using XAS. We selected two steady states to study by EXAFS: (A) Fe(III)apoferitin, an early intermediate in the formation of the core, and (B) iron cores formed with different biopolymers (apoferitin, polysaccharide, and polysaccharide sulfate). In addition, we chose a kinetically changing state to study by dispersive EXAFS: (C) the reduction of core Fe(III) to Fe(II) by thioglycolic acid with various buffer ions. The results provide information about (A) the role of the protein in core formation, (B) the effect of the environment on core structure, and (C) the effect of the environment on core dissolution. Horse spleen ferritin was used throughout because of its abundance and relative simplicity of structure.

RESULTS AND DISCUSSION

(A) Fe(III)apoferitin, a Nucleation Complex. When Fe(II) was added to apoferitin at 10 atoms/molecule (0.4/subunit) all of the iron appeared to be bound to the protein at distinct binding sites (1). The oxidation of the bound Fe(II) produced Fe(III), some of which was also bound by the protein, and was accompanied by the vacancy of some of the Fe(II) sites (1). This suggested that oxidation was coupled with migration of some of the iron atoms from solitary sites on the protein to clustered sites. To confirm the idea that clusters of Fe(III) formed, and to determine whether or not all Fe(III) atoms were bound to the protein, x-ray absorption spectra were obtained and analyzed. The average Fe absorber interacted with three detectable types of neighbors: a low Z atom at ca. 2.0 Å, a low Z atom at ca. 2.5 Å, and a high Z atom at ca. 3.0 Å. The data fit well with models for a protein carboxylate ligand (0 at ca. 2.0 Å and C at ca. 2.5 Å) and Fe (ca. 3.0 Å) bridged by O (ca. 2.0 Å). The iron shell is incomplete compared to full ferritin cores. Fe(III)apoferitin thus appears to be a small cluster of Fe(II) atoms (2-3) in which each Fe(III) atom also is linked to the protein via carboxylate-like ligands; the polynuclear nature of the Fe(III) in the complex was confirmed by Mössbauer spectroscopy (with B. Huynh). The formation of the Fe(III)apoferitin complex shows that the protein provides the site for nucleation, probably orienting iron core growth toward the hollow center of the apoferitin coat.

(B) Iron Cores. In order to determine if all ferritin cores would have the same structure, we used EXAFS to study soluble cores formed under three different conditions: with apoferitin (protein -COOH ligands), with dextran (polysaccharide with -OH ligands), and chondroitin sulfate (polysaccharide with -COOH, -OH, and -SO4 ligands). Only the iron cores formed in the presence of chondroitin sulfate were distinctive, with an increased order (large negative Debye Waller factor compared to ferritin). The results suggest that sulfate was the important ligand for the ordered domains, since the other ligands were present in dextran or apoferitin as well. Mössbauer analysis (with L. H. Bowen) of the chondroitin sulfate complex indicated that the ordered domains were actually soluble hematite (Fe2O3). Thus the ligands present in model experiments can determine the structure of the ferritin core. During the course of these experiments with models for ferritin cores, evidence for natural variations in ferritin cores, in this case associated with PO4, were also obtained (2).

(C) Dispersive EXAFS Analysis of Fe Reduction in Ferritin. The kinetics of reduction and release of iron from the core of ferritin has, in the past, only been studied by indirect analyses, e.g. formation of the complex of Fe(II) and bipyridyl. It is thus difficult to separate reduction from ligand binding. Because dispersive EXAFS allows spectra to be collected in a very short time (ca. 30 sec), it is possible to follow directly the reduction of iron in the ferritin core by monitoring the shift in the absorption edge from the position for Fe(III) to that of Fe(II) (Fig. 1). Preliminary data using thioglycolic acid as the electron donor showed that buffer ions [Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) vs. Tris (Tris(hydroxymethyl)aminomethane)] altered the rate and the pathway of reduction. Such data suggest that, in vivo, small changes in the cytoplasmic environment could modulate the availability of stored iron.
Figure 1. Dispersive XAS results for the Fe K edge of the iron core in horse spleen ferritin during reduction and dissolution by thioglycolic acid (TGA) in Hepes-Na buffer at pH 7.0. Spectra were collected at 32-sec intervals beginning 1 min after the addition of the reductant (TGA). The spectra displayed cover a 10-min interval; the reaction appears complete after 1.7 min. Abscissa: PIXEL number.

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