Biochimica et Biophysica Acta 1800 (2010) 719–731

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

The iron redox and hydrolysis chemistry of the ferritins

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ARTICLE INFO

Article history:
Received 6 January 2010
Received in revised form 23 March 2010
Accepted 26 March 2010
Available online 9 April 2010

Keywords:
Iron oxidation
Mineral core formation
Iron detoxification
Ferritin channel
Ferroxidase center
Di-iron peroxo complex
Di-iron oxo complex
Heteropolymer
H- and L-subunit

ABSTRACT

Background: Ferritins are ubiquitous and well-characterized iron storage and detoxification proteins. In bacteria and plants, ferritins are homopolymers composed of H-type subunits, while in vertebrates, they typically consist of 24 similar subunits of two types, H and L. The H-subunit is responsible for the rapid oxidation of Fe(II) to Fe(III) at a dinuclear center, whereas the L-subunit appears to help iron clearance from the ferroxidase center of the H-subunit and support iron nucleation and mineralization.
Scope of review: Despite their overall similar structures, ferritins from different origins markedly differ in their iron binding, oxidation, detoxification, and mineralization properties. This chapter provides a brief overview of the structure and function of ferritin, reviews our current knowledge of the process of iron uptake and mineral core formation, and highlights the similarities and differences of the iron oxidation and hydrolysis chemistry in a number of ferritins including those from archaea, bacteria, amphibians, and animals.
General Significance: Prokaryotic ferritins and ferritin-like proteins (Dps) appear to preferentially use H_2O_2 over O_2 as the iron oxidant during ferritin core formation. While the product of iron oxidation at the ferroxidase centers of these and other ferritins is labile and is retained inside the protein cavity, the iron complex in the di-iron cofactor proteins is stable and remains at the catalytic site. Differences in the identity and affinity of the ferroxidase center ligands to iron have been suggested to influence the distinct reaction pathways in ferritins and the di-iron cofactor enzymes.
Major conclusions: The ferritin 3-fold channels are shown to be flexible structures that allow the entry and exit of different ions and molecules through the protein shell. The H- and L-subunits are shown to have complementary roles in iron oxidation and mineralization, and hydrogen peroxide appears to be a by-product of oxygen reduction at the FC of most ferritins. The di-iron(III) complex at the FC of some ferritins acts as a stable cofactor during iron oxidation rather than a catalytic center where Fe(II) is oxidized at the FC followed by its translocation to the protein cavity.

1. Introduction

Iron is the most abundant transition metal on Earth and an essential element for many forms of life. It is found in the active sites of many enzymes and oxygen carrier proteins and is an important component of many cellular processes including respiration, electron transfer reactions, energy metabolism, DNA synthesis, and gene regulation. At physiological conditions, free iron exists primarily in one of two oxidation states, the relatively soluble ferrous (Fe^{2+}) state and the very insoluble ferric (Fe^{3+}) state with the solubility of the two ions being ~ 10^{-1} and 10^{-18} M, respectively. The physiological ferric ion concentration is far too low to support the growth of aerobic microorganisms that have developed various strategies to overcome this limitation.

Moreover, the large variability in redox potential contributes significantly to the role of iron as an essential biological metal. The biological use of iron is thus limited by its low solubility and its propensity to participate in harmful free radical reactions. Consequently, living organisms were compelled to adopt efficient iron transport and storage mechanisms to obtain and safely balance the deleterious and beneficial effects of iron. For example, in bacteria and fungi, iron is mainly acquired by small organic molecules called "siderophores" that have a very high affinity for Fe(III) [1–3]. In plants, at least two strategies to acquire iron from the soil have evolved based on either reducing (i.e., ferric–chelate reductase) or chelating (i.e., siderophores) iron [4]. In animals, iron is mainly acquired from food with good to limited bioavailability depending on the source (i.e., iron is readily absorbed from red meat but has limited bioavailability in plants because of the presence of phosphates, phytates, and polyphenols that inhibit absorption by formation of insoluble complexes) and is transported and stored by a number of key proteins via a complex but fairly well-understood mechanism [5].

Abbreviations: Dps, DNA binding proteins from starved cell; HuHF and HuLF, human H-chain and L-chain ferritins; MtF, human mitochondrial ferritin; HoSF, horse spleen ferritin; BfMF, bullfrog M-chain ferritin; EcBFR and EcFtnA, heme- and nonheme-containing *Escherichia coli* ferritins; AvBF and DdBF, *Azotobacter vinelandii* and *Desulfovibrio desulfuricans* bacterioferritins; PfFtn and AfFtn, *Pyrococcus furiosus* and *Archaeoglobus fulgidus* archaeal ferritins; ITC, isothermal titration calorimetry; EXAFS, extended X-ray absorption fine structure; EPR, electron paramagnetic resonance spectroscopy; FC, ferroxidase center

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doi:10.1016/j.bbagen.2010.03.021

The wide range of the Fe^{2+}/Fe^{3+} redox potential (from approximately −500 to +600 mV depending on the iron ligands and environment) and the facile ability of iron to gain and lose electrons allows it to participate in a wide variety of oxidation–reduction reactions [6] but at the same time underlies its toxicity. The one-electron reduction of dioxygen by Fe^{2+} results in the formation of superoxide radical $O_2^{\bullet -}$, which can accept another electron and two protons to produce hydrogen peroxide, H_2O_2. Superoxide and hydrogen peroxide are the by-products of incomplete O_2 reduction, and their balance is regulated by the enzyme superoxide dismutase:

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

On the other hand, ferrous ions can react with hydrogen peroxide to generate the very reactive and damaging hydroxyl radical species ($^{\bullet}OH$) via Fenton reaction while superoxide radical ($O_2^{\bullet -}$) can reduce ferric iron to ferrous ions [7]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH (\text{Fenton})$$

$$O_2^{\bullet -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

The sum of these two reactions produces hydroxyl anion, hydroxyl radical and O_2 and is known as the Haber–Weiss reaction that only manifests in the presence of catalytic amounts of a redox metal such as iron:

$$H_2O_2 + O_2{\bullet}^- \xrightarrow{Fe} OH^- + {}^{\bullet}OH + O_2 (\text{Haber–Weiss})$$

One of the means to protect cells from the potentially toxic effects of free iron and radical chemistry is ferritin, a ubiquitous iron storage and detoxification protein [1,8–11]. Ferritins are a family of natural, highly conserved supramolecular nanostructures designed to sequester thousands of iron atoms in a mineralized and biologically available form for later use in heme and nonheme iron proteins and in biochemical reactions. The increased sensitivity to oxidative stress and the lethal effect on embryonic growth following deletion of the ferritin genes emphasize the importance of these proteins in early life development [12–16]. Another important property of ferritin and ferritin-like protein (also called Dps, DNA binding proteins from starved cell) is the protection of DNA from oxidative damage and organism nutritional deprivation and stress [17–21]. In this chapter, we will discuss our current knowledge of the process of iron uptake and accumulation in ferritin and review the chemistry of iron oxidation and deposition in a number of archaeal, bacterial, amphibian, and animal ferritins from a mechanistic and stoichiometric standpoint. Plant ferritins and the mechanism of iron release and the movement of iron and other ions through the ferritin shell are the subjects of separate chapters in this special issue and will not be discussed here.

2. Ferritin structural overview

2.1. Ferritins in mammals

Ferritins are composed of a protein shell surrounding a cavity where up to 4500 iron atoms can be accommodated as a macroinorganic iron complex. They have a unique molecular architecture typically composed of 24 structurally similar or identical subunits. Their main function is to detoxify and store cellular iron by coupling iron and oxygen (or hydrogen peroxide) to form a stable but biologically available ferric oxide mineral at nonreactive sites inside the ferritin cavity.

Extensive and important helix–helix interactions occur between ferritin subunits and loops resulting in unusually high protein stability (i.e., up to 80 °C and to 6 M guanidine at neutral pH). Despite this web of inter- and intrasubunits interactions, the protein maintains a flexible and dynamic structure that controls the flow of iron, oxidants, reductants, chelators, and small ligands and molecules in and out of its shell [22]. Unlike bacterial, fish, and amphibian ferritins, mammalian ferritins (with the exception of mitochondrial and serum ferritin) consist of two functionally and genetically distinct subunit types, H (heavy, ~21,000 kDa) and L (light, ~19,000 kDa) subunits. These two subunits coassemble in various ratios with a tissue-specific distribution to form a shell-like structure with 4/3/2 octahedral symmetry [1,8–11]. The H-subunit has a dinuclear iron center consisting of A and B binding sites (Fig. 1) where the fast conversion of Fe(II) to Fe(III) by dioxygen (or hydrogen peroxide) occurs, whereas the L-subunit is thought to contribute to the nucleation of the iron core and thus stores iron at a lower rate compared to the H-subunit [1,8–11,23]. The distribution of the H/L subunit ratio is tissue specific where up to 70% H subunits can be found in tissues exhibiting high ferroxidation activity (i.e., heart and brain) and up to 90% L subunits are found in tissues having mainly a storage function and much less iron oxidation activities (i.e., spleen and liver) [8,10]. More specifically, isoferritins from the following tissues are composed of these H to L ratios: human placenta (~20% H, 80% L), human spleen (~10% H, 90% L), human liver (~50% H, 50% L), human heart (~90% H, 10% L), human serum (~0% H, 100% L), horse spleen (~8% H, 92% L), and rat liver (~35% H, 65% L) [8,24]. Exclusively homopolymer ferritins (i.e., H-type in bacterial ferritins, mammalian mitochondrial ferritins, and M-type in fish and amphibian ferritins) seem to be able to carry out both reactions of iron oxidation and iron mineralization [25–27].

2.2. Ferritins and ferritin-like proteins in bacteria

In bacteria, there are at least three types of iron storage proteins: the archetypal ferritins, the heme-containing bacterioferritins, and the Dps proteins [6]. The archetypal ferritins are similar to those found in eukaryotes and are composed of the typical but identical 24 homopolymer H-type subunits. The bacterioferritins are found only in eubacteria and consist of 24 identical H-subunits and up to 12 protoporphyrin IX heme groups of unknown function [28,29]. In *Escherichia coli*, these latter bind covalently between 2-fold symmetry-related subunits and are ligated by methionines Met-52 and Met-52'.

The Dps proteins are present only in prokaryotes and are smaller compared to ferritin (i.e., ~250 kDa vs. ~500 kDa). They consist of only 12 similar subunits organized in 3/2 octahedral symmetry and can accumulate just ~500 iron atoms per protein shell [30]. Interestingly, all three protein types can exist in the same bacterium but bind and oxidize iron quite differently. In the 24-mer proteins, the ferroxidation reaction takes place at conserved amino acid residues located within the H-subunit, whereas iron oxidation in Dps occurs at sites located at the 2-fold interface between the two subunits [30]. Each ferritin subunit is folded into four α-helix bundles (helices A–D) with helices B and C connected by a loop L that traverses the length of the bundle and a short fifth helix E (Fig. 1) that ends at the C-terminal [1,8–11]. Plant and animal ferritins share a high amino acid sequence homology and a very similar 3D structure with, however, two major differences that are related to localization and expression. Firstly, animal ferritins are localized in the cytoplasm of cells whereas plant ferritins are found in the plastids, a family of specific plant organelles responsible for the proper functioning of the plant (refer to the chapters by Zhao and by Briat et al. in this special BBA issue). Secondly, animal ferritins are mainly expressed and regulated at the translational level, whereas plant ferritins are regulated at the transcriptional level [4].

3. Ferritin channels

3.1. Vertebrate channels

In most species, the assembled 24 subunits in ferritin are tightly packed together leaving eight narrow hydrophilic channels around