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Review

Ferritins for chemistry and for life

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ABSTRACT

Ferritins, highly symmetrical protein nanocages, are reactors for Fe^{2+} and dioxygen or hydrogen peroxide that are found in all kingdoms of life and in many different cells of multicellular organisms. They synthesize iron concentrates required for cells to make cofactors of iron proteins (heme, FeS, mono and diiron). The caged ferritin biominerals, $Fe_2O_3{\cdot}H_2O$ are also antioxidants, acting as sinks for iron and oxidants scavenged from damaged proteins; genetic regulation of ferritin biosynthesis is sensitive to both iron and oxidants. Here, the emphasis is ferritin oxidoreductase chemistry, ferritin ion channels for Fe^{2+} transit into and out of the protein cage and $Fe^{3+}O$ mineral nucleation, and uses of ferritin cages in nanocatalysis and nanomaterial synthesis. The ferritin nanocage as reactors for Fe^{2+} and oxygen, likely critical in the transition from anaerobic to aerobic life on earth, play central, contemporary roles that balance iron and oxygen chemistry in biology and have emerging roles in nanotechnology.

1. Introduction

Ferritins are natural reactors for iron and oxygen chemistry that control the biosynthesis and dissolution of caged hydrated ferric oxides [1,2]. Caged ferritin minerals can have diameters as large as 8 nm, with thousands of iron and oxygen atoms. The minerals have various amounts of phosphate (P) that reflect local phosphate concentrations; phosphate is low in animal ferritin iron minerals (Fe:P = 8:1) and high in plants or microorganisms (Fe:P = 1:1) where the ferritin minerals are largely amorphous [1]. Control of ferritin mineral order in animals, where a range of crystallinity occurs [3], depends on the number of protein-based mineral nuclei. The combinatorial structure of hybrid cages assembled from two types of ferritin gene products, H (catalytically active) and L (catalytically inactive) protein subunits, controls the number of protein-based mineral nuclei [1]. Plant ferritins are localized in plastids; the microbial origin of plastids likely relates to the high phosphate

Abbreviation: DFP, diferric peroxo.

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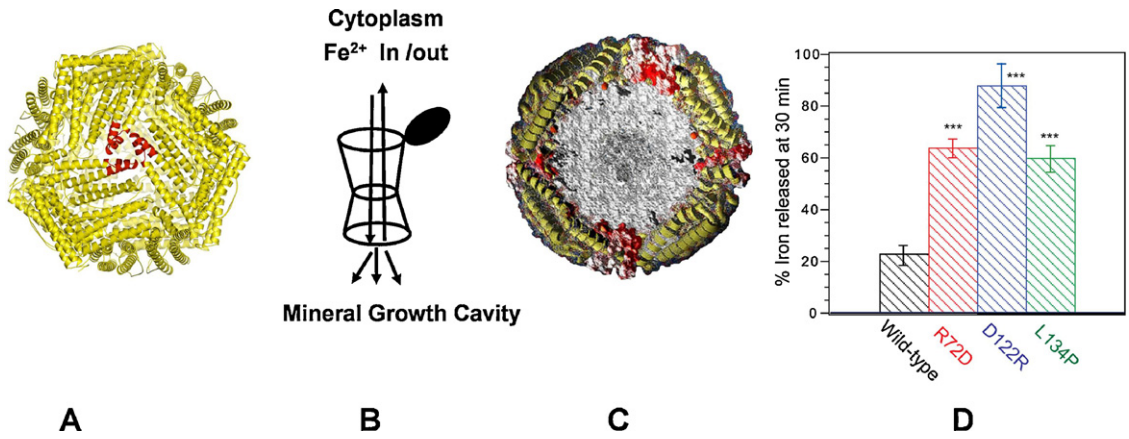

Fig. 1. Eukaryotic ferritin. (A) The protein cage. (B) Outline of one of the eight Fe^{2+} entry and exit ion channels showing the N-terminal gate (black) and mid channel constriction [28,32]. Downward arrows show the direction of Fe^{2+} transit and distributions to multiple oxidoreductase sites; the upward show Fe^{2+} leaving the cage after mineral dissolution by external reductant. (C) A cross-section of the ferritin protein cage showing a full $Fe_2O_3{\cdot}H_2O$ mineral; in nature the average filling of the cage is 1000–2000 Fe atoms but the range form a given tissue can be from <1 iron to 4000 Fe/cage. Helices are shown in yellow; the channels form from helical segments of three polypeptide subunits around the three fold axes of the cage. (D) Fe^{2+} exit increases in ferritin variants with amino acid substitutions at conserved Fe^{2+} ion channel residues. The figure is constructed from the author's work in [1,28,32].

content of plant ferritin minerals. Nevertheless, the plant ferritin protein cage sequence, encoded in the nucleus, is more closely related to animals (55% sequence identity). Ferritins are required to concentrate iron to match requirements for synthesis of proteins with iron cofactors (Fe–porphyrin, Fe–S, and Fe); they also are antioxidants and retrieve iron released from proteins, damaged by oxidation, for future cellular use.

Ferritin protein cages self-assemble into highly symmetrical cages, from polypeptides, after spontaneous folding of each polypeptide into bundles of 4α helices. Variations are observed in the N-terminal extensions of microbial and eukaryotic ferritins, the location of the active sites, the ferrous oxidants, and the number of ion channels. A central, mineral growth cavity accounts for 30% of the volume of ferritin protein cages (Fig. 1). Animal ferritins contrast with all other ferritins (Archaeal, bacterial, and plant) in forming cages from tissue-specific combinations of different H and L subunits; plant ferritin cages are hybrids of different H subunits. Microbes assemble ferritin cages that have identical subunits; the different ferritin gene products (protein subunits) are synthesized at different times during the culture cycle or in response to different environmental conditions [4].

The multiple, natural, iron–protein interactions occurring within ferritin protein cages throughout the entire length of each subunit: di-Fe^{2+} sites within each polypeptide where oxidant (O_2 or H_2O_2) produces di-Fe mineral precursors, Fe^{2+} entry/exit channels formed by three subunits at the 3-fold cage symmetry axes, that feed multiple di-iron catalytic sites, and, specific to animal ferritins, nucleation channels that can enhance mineral order and exit at the 4-fold symmetry axes of the cage [1,5]; the rates may differ widely as in millisecond dispersal from single channels to multiple catalytic sites or slow (hours) helix–helix changes as ferric oxo nuclei move through nucleation channels. While localized metal–protein interactions occur throughout individual ferritin polypeptides, cooperative effects on metal ion distribution occur throughout the protein cage [6–8]. Here, we focus in Section 2 on oxidoreductase chemistry, in Section 3 on Fe^{2+} transit into and out of the protein cage, $Fe^{3+}O$ mineral nucleation and in Section 4 on uses of ferritin cages in nanocatalysis and nanomaterial synthesis. The ancient and widespread occurrence of ferritins Fe^{2+} and O biological reactors suggests a role in the transition from anaerobic to aerobic life on earth, as well as the critical contemporary roles of balancing iron and oxygen chemistry in health and disease and in emerging roles for nano technology.

2. Oxidoreductase chemistry

The ferritins oxidize Fe^{2+} and reduce either dioxygen or hydrogen peroxide to synthesize the $Fe_2O_3{\cdot}H_2O$, protein-caged biomineral iron concentrates. Active site ligands among the ferritins are diverse and mechanisms vary. The most extensively studied group in the ferritin family is the maxi-ferritin of eukaryotes. In the 24-subunit cages, subunits with active sites use Fe^{2+} and dioxygen as substrates; all subunits are catalytically active except in animals where L catalytically inactive subunits, co-assemble with the H active subunits to alter mineral nucleation and crystal order; see Section 3.2 and [1]. In microorganisms, there are several types of ferritins; (i) bacterial and archaea maxi-ferritins, which are similar to eukaryotic ferritins in terms of the 3D structure [9–11], albeit with low sequence similarity to eukaryotic ferritins. (ii) Bacterioferritins (BFRs), which contain heme and are widespread in bacteria; BFR have not been detected in archaea. The 24 subunits of the BFR protein cage contain one *b*-type heme between dimer pairs [12–14]. (iii) Mini-ferritins, called Dps (DNA binding proteins from starved cells) proteins, self-assembled from 12-subunits [15]. Dps proteins like many other ferritins protect cells from oxidant damage by removing iron and oxygen, which in mini-ferritins is predominantly hydrogen peroxide rather than dioxygen, from the cytoplasm and converting into iron biomineral. Ferritin proteins are encoded in genes sensitive to cellular oxidant status. The shared properties of all ferritins are hollow, nanoproteins, of multiple α-helices, containing iron channels and sites for catalytic reactions between two ferrous and oxygen atoms to synthesize caged iron biominerals.

Eukaryotic ferritins catalyze the oxidoreduction reaction for Fe^{2+} and O_2 at the multiple diiron binding sites embedded in the center of a 4-α-helix bundle. Although ferritins utilize Fe^{2+} as a substrate, the ferritin diiron site shares the structural and functional characteristics with the diiron cofactor site in diiron enzymes. Such diiron cofactor enzymes include methane monooxygenase, ribonucleotide reductase, and Δ^9-fatty acid desaturase [16–21]. The first detectable reaction intermediate for the oxidoreduction reaction is a diferric peroxo (DFP) species, which is also detected in the diiron containing enzymes. DFP formation in ferritin has been extensively characterized by kinetics [1,2] and multiple spectroscopies (UV/vis, resonance Raman, Mössbauer, and EXAFS) [22–24]. DFP decays to Fe^{3+} oxo or hydroxo dimers or multimers with concomitant production of H_2O_2; peroxide production is stoichiometric with DFP