Structural and mechanistic insights into iron processing and biomineralization by vertebrate ferritins

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Ferritins are ubiquitous multimeric protein systems showing a nanocage structure able to include thousands of iron atoms as oxoferric biomineral. In mammals, these twentyfour-mer protein shells are generally heteropolymers composed by two different types of subunits classified, according to their relative molecular weight, as heavy H and light L (183 and 175 amino acids, respectively, in human chains). The relative ratios between the two types in heteropolymers is tissue-dependent: ferritins in iron storage organs (e.g. liver and spleen) are richer in L-subunits, while those with fast iron metabolism (e.g. brain and heart) are richer in the H type. The H-subunit contains a ferroxidase center characterized by the so-called Fe1 and Fe2 sites and able to rapidly oxidize Fe^{2+} to Fe^{3+}. Besides the high structural conservation of the catalytic center, the mechanism by which the ferroxidase reaction occurs is not fully understood and different models have been proposed. We have developed a soaking/flash freezing method to allow aerobic and anaerobic addition of iron(II) to frog and human ferritin crystals (1,2,3) Multi-wavelength anomalous diffraction data have been exploited to unambiguously detect iron atoms. Through this method we have observed for the first time the iron binding sites in X-ray crystal structures of vertebrate ferritins and how they become populated with time (1,2,3). Interestingly, accessories transient metal sites have also been identified in the proximity of the ferroxidase site and demonstrated to play a key role in the reaction turnover (3,4). On the other hand, L-subunits lack the ferroxidase site, and hence iron incorporation in nanocages rich in L-chains is much slower. Nevertheless, homopolymeric L-ferritins are able to biomineralize iron. The proposed mechanism involves the presence of a putative nucleation site on the inner cage surface (5). Through a time-dependent series of X-ray crystal structures of iron-loaded homopolymeric human L-ferritin we have observed the progressive formation of a triiron cluster on the inner cage surface of each subunit (6). After 60 minutes exposure, a fully formed (μ^3-oxo)tris[(μ^2-peroxo)(μ^2-glutamato-κO:κO')](glutamato-κO)(diaquo)triiron(III) anionic cluster was clearly visible in a structure determined at 1.98 Å resolution. The functional significance of the protein carboxylates involved in the coordination of the metallocluster for biomineralization was clearly demonstrated by the lower iron oxidation rate measured in the E60A-E61A-E64A triple variant of human L-ferritin. A similar metallocluster was also observed in the lower resolution (2.22 Å) structure of horse spleen ferritin, suggesting that it constitutes a common feature of mammalian ferritins representing the yet unobserved, nucleation site of L-type proteins. This cluster structure is unprecedent in biological systems even though it shows striking structural similarities to a synthetic hexanuclear iron cluster reported about 20 years ago and proposed as possible model of a ferritin biomineral (7). Structural data, together with stopped-flow kinetic data, provide new clues to explain the ferroxidase and biomineralization processes in vertebrate ferritins.

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