# Understanding Biomineralization: Structure, Kinetics, and Applications of Ferritin

Jeffrey A. Sigman Literature Seminar November 2, 1995

The growing need for new materials having novel magnetic, electronic, and mechanical properties has raised interest in the study of biominerals. Biological systems have the ability to create materials with these types of properties as well as the ability to control their synthesis at the micro- and the macroscopic level [1]. Examples of these materials and their function include calcite and silica as exoskeletons in algae, gypsum as a gravitational device in jellyfish, and magnetite as an internal compass in bacteria [1]. Much research has been devoted to mimicking the biomineralization processes found in nature. However, the materials made using biomimetic processes still cannot surpass the materials made using non-biologically based techniques. Therefore, a detailed understanding of the structural and kinetic controls imposed on biomineral synthesis in conjunction with the use of the natural systems for materials synthesis may be needed for the production of better materials. A good candidate for such a study is the iron storage protein ferritin.

Ferritin is a highly conserved protein found in all eukaryotic cells [2]. The function of the protein is to store iron in a non-toxic biologically available form [2]. Ferritin consists of a highly symmetrical 24-subunit amino acid shell and a central core composed of a hydrous ferric oxide mineral similar to the mineral ferrihydrite [2, 3, 4,]. Two types of subunits, H (heavy) and L (light), have been identified in mammalian ferritins. The two subunits share 55% sequence homology and a common tertiary structure [3, 5, 6, 7 ].

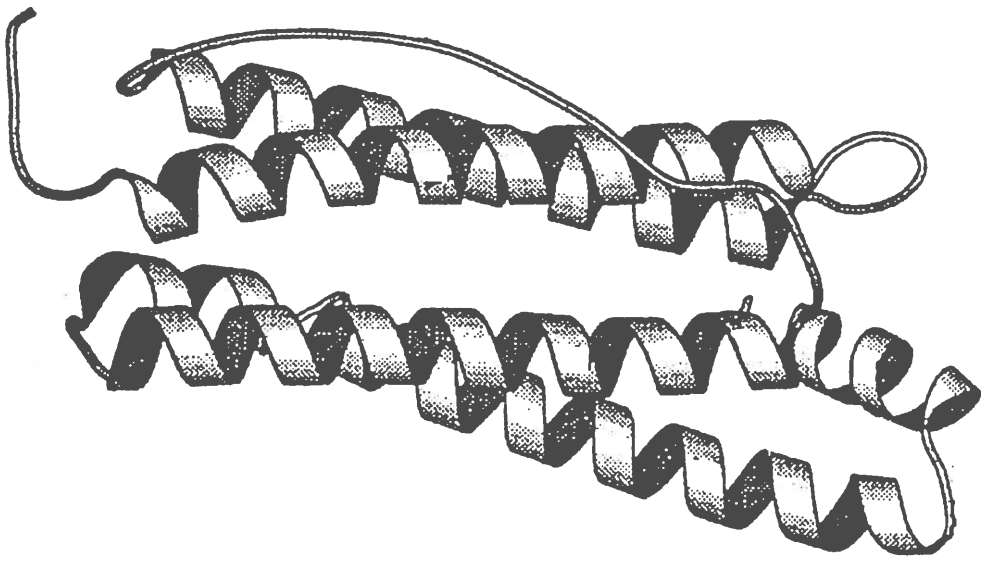

**Figure 1.** Schematic representation of the ferritin subunit [3].

Several aspects make this protein ideal for studying its biomineralization process. First, the cDNA library for several strains has been solved, making it possible to use recombinant studies and mutagenesis to aid in determining the key sequences responsible for the oxidation and deposition of iron inside the core [8, 9]. Second, crystal structures of several natural and recombinant ferritins have been solved with the aid of amino acid sequence in-

formation [3, 6, 10]. Thus, detailed information about the protein tertiary and quaternary structure is available. Furthermore, the ferritin structure is very stable, allowing it to withstand temperatures up to 80° C and incubation in concentrated buffers [11]. This stability enables the protein to be depleted of its iron core and then reconstituted *in vitro* [12, 13]. Finally, iron is spectroscopically accessible by several methods including Mössbauer, EPR, and UV-VIS, with which the kinetics of core formation can be monitored.

In recent years, three iron binding sites have been shown to lie in an intrasubunit channel of the H-subunit [6, 10]. The residues coordinating iron are highly conserved in the H-subunits and are believed to be involved in the catalytic oxidation of iron leading to core formation [6, 10, 12]. Several intermediates to core formation have been elucidated including an $Fe^{2+}$-protein complex, an $Fe^{3+}$-protein complex, $Fe^{2+}$-$Fe^{3+}$ and $Fe^{3+}$-$Fe^{3+}$ dimers, and various sizes of $Fe^{3+}$ clusters. Kinetic studies have been performed in conjunction with mutations at the proposed iron binding site [2, 13-17]. Based on these studies, a possible order of events in the formation of the above intermediates has been proposed.

The focus of this talk will be on the structure of ferritin and on the spectroscopic evidence for the known intermediates in the core formation. From this evidence, the sequence of events that lead up to the deposition of iron inside the protein cavity will be summarized. Finally, some examples of work done on depositing other minerals into ferritin will be presented [18-21].

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