Application of Biomineralization : Metal Adsorption and Synthesis of Inorganic Nanometer Particles

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Biomineralization means the formation of inorganic minerals in organisms. It is widespread in Nature and major biomineralization products are calcium, silicon and iron minerals. Several anions involved in biominerals are carbonate, phosphate, oxide and hydroxide etc(1). Biominerals are often formed as composite materials in association with an organic framework such as proteins and polysaccharides which influence the morphology and crystallinity of the biomineral formed.

The iron storage protein ferritin serves as a good example of biomineralization called "organic matrix-mediated" process. This process is distinguished from the other process called "biologically induced" mineralization which results in bulk extracellular and/or intracellular mineral formation. Ferritin consists of spherical protein shell, apoferritin, of 24 subunits enclosing a cavity of ca. 8 nm diameter which contains an iron mineral in the form of ferrihydrite($5Fe_2O_3 \cdot 9H_2O$). The protein shell includes hydrophilic and hydrophobic channels by which iron atoms can be accumulated within and sequestered from the molecule. The protein itself is known to play an active role in the nucleation and growth of ferrihydrite(2). Although the mineralization occurs in the cavity, it is not very strictly controlled and can be influenced by surrounding environments including metal concentration, PO_4^{3-} content, pH, redox potential and other factors.

We aim to utilize the biomineralization process in relation to metal adsorption and synthesis of inorganic nanometer particles since the supramolecular protein cage ferritin offers several advantages over other systems already developed such as surface-bound organic groups, polymers, porous glasses and zeolites. The protein is rather rigid and stable at high temperature(80-85 ℃) and pH(8.5-9) for limited periods of time without significant damage of its structure. In the native protein the iron core can be readily removed from the molecule and reconstituted *in vitro*. The protein has affinity to other metal ions and can easily be reconstituted with these metal ions(3, 4).

In this study, we prepared apoferritin from horse spleen ferritin by reductive dissolution and reconstituted the molecule with Fe^{2+}, Mn^{2+}, Al^{3+} and UO_2^{2+}. The inorganic nanometer particles formed in the core were examined using transmission electron microscopy(TEM), electron diffraction and energy dispersive X-ray analysis(EDXA).

Metal adsorption

Apoferritin(2 μM) prepared by the method described earlier was adsorbed by the addition of $Fe(NH_4)_2(SO_4)_2$, $MnCl_2 \cdot 4H_2O$, $(CH_3COO)_2UO_2 \cdot 2H_2O$ and $AlCl_3$ solutions in 25 mM TES buffer(pH 8). Each metal solution was added to give a concentration of about 2 mM(theoretically corresponding to a loading of 1000 metal ions per ferritin molecule). Following the reaction with the metal ions for 24 hrs, dialysis was performed against d-H_2O for 1 hr. Fe^{2+} solution was prepared under anaerobic condition provided by N_2 gas. UO_2^{2+} was added in a dark condition.

Transmission electron microscopy

Protein samples were examined under a JEOL 2000FXII(200

KeV) and a ZEISS EM912 Omega(120 KeV) TEMs. Adsorption of Mn^{2+} by the empty protein cages(apoferritin) at pH 8 is observed as spherical electron dense particles(Fig. 1a) and the EDXA spectrum(Fig. 1c) confirms that the particles consist of Fe and Mn. Stained protein molecules show the mineral formed within the protein cavity(data not shown). Although the detailed structure of the core mineral needs to be determined, our preliminary results suggest that the core mineral is amorphous metal oxides(Fig. 1b).

Images of other unstained protein molecules which were adsorbed by two, three or four mixed-metal ions show discrete electron dense cores that are generally spherical in morphology. The protein cores adsorbed with different metal ions generally exhibited diffuse diffraction lines implying low structural order. EDXA spectra show that the cores contained adsorbed metal ions and resulting cores are nanometer particles. The study reported here demonstrates that all the metals examined are adsorbed by ferritin under the experimental condition. The Al^{3+} adsorbed by ferritin *in vitro* is reported for the first time in this study.

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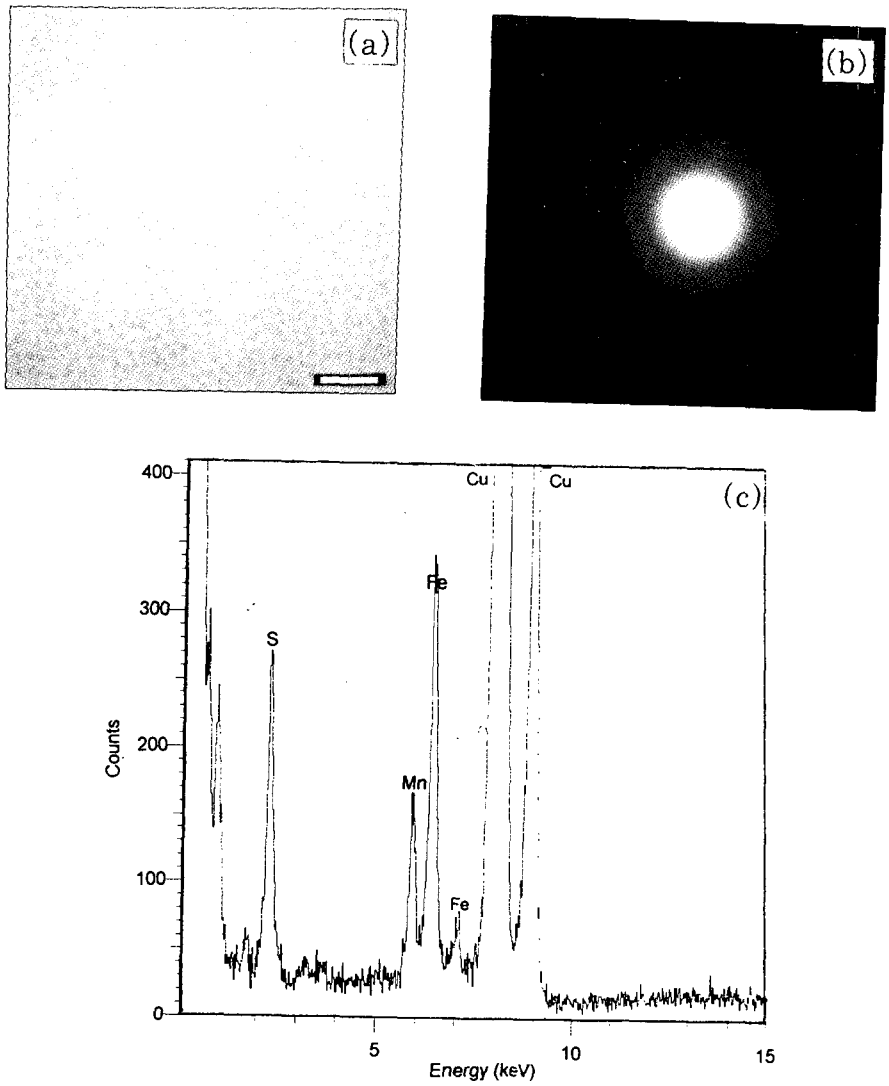

Fig. 1. (a) and (b) A TEM micrograph and the corresponding electron diffraction pattern of nanoparticle metal(1000Fe+ 1000Mn atoms per ferritin molecule) oxide cores encapsulated within the protein cage of ferritin. The scale bar is 40nm. (c) EDXA spectrum of the image shown in (a).