STRUCTURAL AND COMPOSITIONAL PROPERTIES OF IRON CORES IN NATIVE AND RECONSTITUTED BULLFROG TADPOLE FERRITINS

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Iron biomineralization is well demonstated in the study of a protein known as ferritin. Ferritins found in all five living kingdoms have a major role in iron storage, transport and detoxification. At physiological pH, Fe(III) is extremely insoluble, precipitating as polynuclear iron complexes. Ferritin prevents these iron complexes from precipitating aggregates by confining such complexes within a soluble protein shell. Ferritin (1) consists of a hollow protein shell, apoferritin, of 24 subunits surrounding an iron oxide core which has a structure similar to that of the mineral ferrihydrite($5Fe_2O_3 \cdot 9H_2O$).

Ferritin isolated from bullfrog tadpole blood consists of 3 different subunit types (H-, M- and L-chains) instead of two subunit types (H - and L-chains) known in all other ferritins (1). Some differences in the primary structure of tadpole ferritin subunits are reported. Considering the fact that the protein plays an active role in iron core formation, comparative studies on the iron core of tadpole ferritin brings about our interest. In particular, since mineralization in ferritin are not very strictly controlled it can be influenced to a considerable range by surrounding environments such as Fe and/or phosphate concentrations, Fe/apoferritin ratio, pH, redox or other factors (2). Thus ferritin provides a good model to study biomineralization through protein and core modifications.

In this study, ferritin cores isolated from bullfrog tadpole blood have been analyzed by chemical analysis, electron microscopy and electron diffraction to determine the structural and compositional properties of native and reconstituted tadpole ferritins.

Preparation of proteins

Tadpole blood ferritin was purified as previously described (3). Apoferritin was prepared by chemical reduction using 1 % thioglycolic acid (pH 5.0) and dialized against 0.1 M sodium bicarbonate (pH 8.0) followed by 20 mM MOPS buffer (pH 7.0). Apoferritin was reconstituted by addition of Fe under controlled condition to give calculated loading of 2000 Fe atoms/molecule. Reconstituted cores were obtained by adding iron in the absence of phosphate to tadpole apoferritin. This was performed in 20mM MOPS buffer (pH 7.0) by the addition of ferrous ammomium sulphate solution to give a loading of 1000 Fe atoms/molecule. After 2 h, a further 1000 Fe atoms/molecule were added and the solution was kept at 4 °C for 18 h followed by dialysis against the buffer.

ICP (Inductively Coupled Plasma) spectrometry

Chemical analysis of ferritins was made by using a ICP AES spectrometer. Horse spleen ferritin (Sigma) was also determined as a control. The analysis showed that the native ferritin contains ca. 1200 Fe atoms/molecule of protein which is significantly less than other animal ferritins. The iron contents of horse spleen ferritin is above 2000 Fe atoms per molecule (2). The ferritin shows comparable amounts of phosphate content ($Fe:P_i$ = 5.2) compared to those reported for mammalian ferritins which have $Fe:P_i$ = 4.5 and 21 (2, 4). Although there is not a direct correlation between phosphate content and crystallinity, higher levels of phosphate as in bacterioferritin ($Fe:P_i$ =1.7) are known to have an influence on crystallinity (2, 5).

Electron microscopy

Unstained samples of native and reconstituted tadpole ferritin were prepared for electron microscopy by air-drying small drops of solution onto Formvar-coated copper grids. These were examined in a Jeol 2000 FX II transmission electron microscope operating at 100 keV. Dense area of ferritin cores were selected for electron diffraction patterns. Particle size determinations were made by measurement of 50 iron cores. Protein shells were negatively stained

with 2 % phosphotungstic acid (pH 6.0) for 5 min.

Image of unstained protein molecules showed discrete electron dense cores that were generally spherical (Fig. 1). The core size of native ferritin ranges 4.6 - 8.4 nm and shows limited crystallinity, resulting in diffuse diffraction lines. This corresponds to those of ferrihydrite (6) (Table 1). Stained sample of native ferritin showed the presence of apoferritin molecules. Reconstituted cores were also spherical in shape and appeared to be densely aggregated. The cores showed significant increase in size (8.0 - 11.9 nm) although the iron content increased less than 2 times to native ferritin. The increase in size may reflect that the reconstituted tadpole ferritins contain highly hydrated, low-density cores (3). The reconstituted cores show diffuse diffraction lines, implying the crystallinity similar to native ferritin cores. Thus, our *in vitro* reconstitution study suggests that the protein does not have a role in crystallochemical specificity for core formation at the interface at which the mineral grows.

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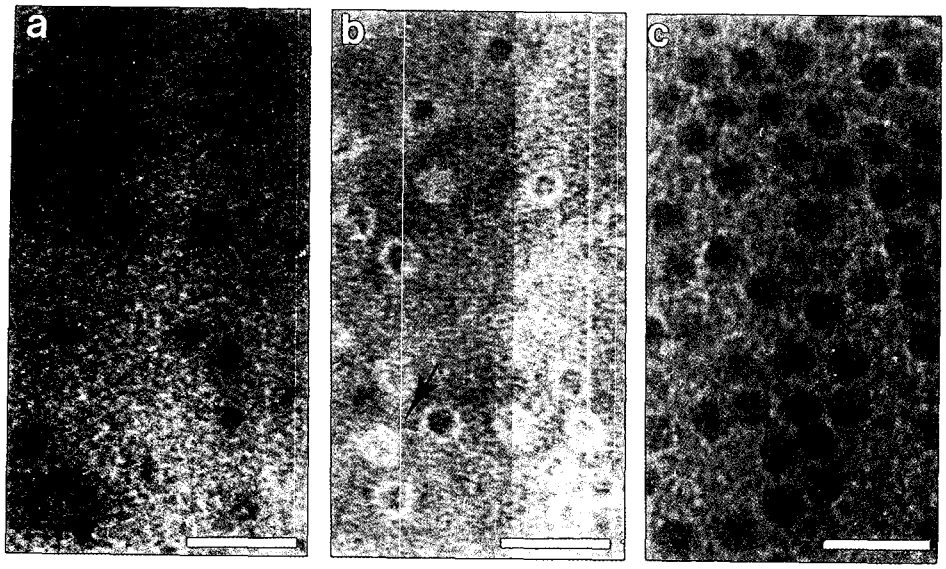

Fig. 1. Transmission electron micrographs of native and reconstituted bullfrog tadpole ferritins. (a) unstained iron cores of native ferritin. (b) negatively stained native ferritin with 2 % phosphotungstic acid. Arrow indicates an apoferritin molecule. (c) unstained iron cores of reconstituted ferritin. Scale bars represent 20 nm in all micrographs.

Table 1. Electron diffeaction data (d-spacings) for native and reconstituted tadpole ferritins.

Ferrihydrite		native		rTF	
d(Å)	I	d(Å)	I	d(Å)	I
2.52	s	2.44	w	2.47	vw
2.25	m	2.10	s	2.09	s
1.97	m				
1.72	vw				
1.48	s	1.20	m	1.18	m

rTF, reconstituted bullfrog tadpole ferritin;
I, Intensity; w, weak; vw, very-weak; s, strong;
m, medium