X-ray structural study on formation of ferritin iron core and its iron(II) auto-oxidation in the presence of phosphate

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Introduction
The iron loading by ferritin in vitro has been shown to be dependent upon the presence of a Good’s buffer and to fail in the presence of phosphate. However, phosphate is also a major component of iron core, though in variable amounts. Horse spleen ferritin has a Fe:Pi ratio of ~10 :1 and bacterial ferritin has contains a much higher ratio of Fe:Pi up to 1:1. The significance of phosphate in the core is not well-understood. In the previous report [1], we described the results of small-angle X-ray scattering (SAXS) study on the structures of iron cores of native ferritins containing phosphate and of phosphate-free reconstituted ferritins. Here, we report structural study on reconstituted ferritins prepared in buffers containing various concentrations of phosphate.

Experimental
SAXS experiments were carried out on the solution X-ray scattering camera at BL-10C. Scattering patterns were recorded by using a PSPC for a camera length of 2m. Reactions of iron loading by apo-ferritin were done by incubating apo-ferritin (horse spleen) and ferrous ammonium sulfate in 0.1M Hepes buffer containing various concentrations of phosphate (molar ratios of Fe:Pi=1:2~1000). In all samples, irons were added to apo-ferritin solutions at a molar ratio of 500Fe2+/molecule. The actual iron content in reconstituted ferritin was determined spectrophotometrically.

Results and Discussion
When apo-ferritins are incubated with various concentrations of ferrous iron in phosphate-free Hepes buffer, all soluble iron atoms are accumulated into the molecules at a mixing molar ratio of iron/protein. On the other hand, in pure phosphate buffer, apo-ferritin or ferritin has no ability of iron loading. We examined a reactivity of iron loading by apo-ferritin in 0.1M Hepes buffer in the presence of phosphate. Fig.1 shows scattering profiles from the solutions of ferritin prepared with phosphate at different concentrations. Apo-ferritin reveals, outside a central maximum, two distinct subsidiary maxima. This characteristic profile is well simulated with a spherical shell with an outer radius of 63A and an inner radius of 39A. With increasing the concentration of phosphate in the buffers, the troughs between maxima shallow gradually, indicating a lowering of iron content. As can be seen from a comparison with the profile of ferritin in the absence of phosphate, ferritin reconstituted in the presence of 0.2mM phosphate is able to accommodate iron atoms almost at a mixing ratio of 500Fe2+/molecule. However, ferritins reconstituted in the presence of higher phosphate concentrations have lower iron contents. In the presence of 0.1M phosphate, iron content in ferritin is about 100Fe2+/molecule.

The dependence of iron loading ability on the concentration of phosphate in Hepes buffer may be interpreted by the facts that phosphate anions form complexes with Fe2+ions in competition with Hepes ions, resulting in an inhibition of the incorporation of iron atoms into apo-ferritin and that phosphate ions in pre-loaded ferritin also suppress significantly a final amount of accumulated irons.

References

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