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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 500Fe^{2+}/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 100Fe^{2+}/molecule.

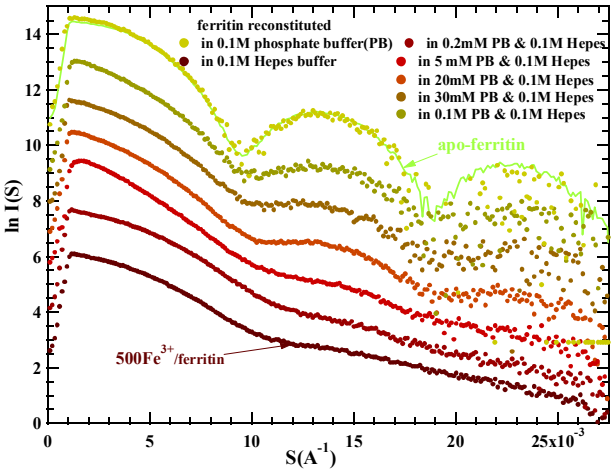

Fig.1 Scattering intensity profiles from solutions of apo-ferritin and of ferritin reconstituted in 0.1M Hepes in the presence of phosphate.

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 Fe^{2+} 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

[1] Y. Inoko et al., PF Activity Rep.**26**, 207(2008)

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