Biological Science

10C/2007G570

# X-ray solution scattering study of ferritin iron up-take and iron core in various buffer solutions

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## Introduction

Mammalian ferritin is an intracellular iron storage protein assembled from 24 subunits forming a spherical shell of an outer diameter of ~13nm and an inner diameter of ~8nm. Its central cavity has a capacity for up to 4500 atoms of ferric iron. The iron loading by ferritin *in vitro* has been shown to be dependent upon the presence of a Good's buffer and fails in the presence of phosphate. On the other hand, phosphate is also a major component of core in variable amounts. Horse spleen ferritin has a Fe:P ratio of ~10 and bacterial ferritin has contains a much higher ratio of Fe:P up to 1:1. The significance of phosphate in the core is not well-understood. In this report, we describe small-angle X-ray scattering (SAXS) study on the structures of iron core of native ferritin containing phosphate and of phosphate-free reconstituted.

## 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 up-take by ferritin were done by incubating apoferritin (horse spleen) and ferrous ammonium sulfate at various mole ratios in 0.1M Hepes buffer and the actual iron content of reconstituted ferritin was determined spectrophotometrically.

## Results and Discussion

To examine the effect of phosphate on the structure of iron core, we investigated the core size and density of native ferritin (n-ftn) and of reconstituted ferritin (r-ftn) using contrast variation technique. Fig.1 shows the results as a plot of the square-root of zero angle scattering intensity of ferritin against the sucrose concentration (0 to 60 wt%) in solvent. The averaged electron density, $\rho_{ferritin}$, of whole ferritin is given from the extrapolation to zero scattering intensity as shown in Fig.1. Also, the radius of gyration, $Rg_{ferritin}$, of whole ferritin at each sucrose concentration was obtained from the Guinier analysis of scattering intensity data. At a matching point where the electron density of solvent containing 57.5 % sucrose is equal to that of the protein shell, $\rho_{shell}$ (~0.42$e^-/A^3$), the scattering intensity profiles of iron cores were recorded for n-ftn and r-ftn solutions, following by an evaluation of radius of gyration, $Rg_{core}$, of iron cores. From the experimentally obtained parameters ($\rho_{shell}$, $Rg_{shell}$, $\rho_{ferritin}$, $Rg_{ferritin}$, $Rg_{core}$), the averaged electron densities, $\rho_{core}$, of iron cores were directly determined. The radius of gyration and electron density of the whole and core part of ferritin molecule are summarized in Table 1.

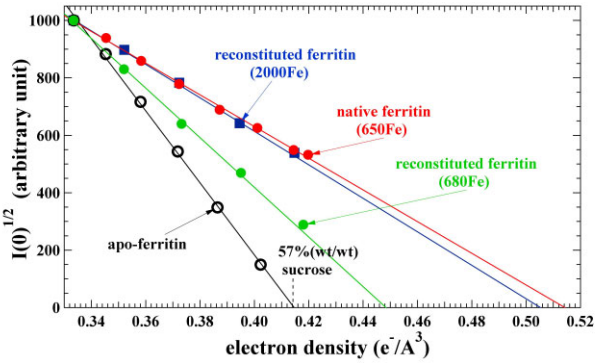


**Fig.1** The square-root of zero angle scattering intensity from native and reconstituted ferritin solutions is plotted against the electron density of the sucrose solvent.

**Table 1** omparison of structural parameters of native and reconstituted ferritin molecules

| | n-ftn 650Fe | | r-ftn 680Fe | | r-ftn 2000Fe | |
|---|---|---|---|---|---|---|
| | core | whole | core | whole | core | whole |
| Rg(A) | 31.2 | 41.5 | 23.8 | 44.5 | 27.3 | 40.6 |
| ρ($e^-/A^3$) | 1.517 | 0.515 | 0.756 | 0.449 | 1.433 | 0.505 |

The results of the contrast variation SAXS experiments shows that, despite of same iron amount, there are significant differences in the core size $Rg_{core}$ and density $\rho_{core}$ between n-ftn and r-ftn; rather, the values of $Rg_{core}$ and $\rho_{core}$ of n-ftn are close to those of the core in iron-rich ferritin (2000Fe). This difference may be explained by the difference in chemical composition of the core or may be originated from the difference of iron loading process between *in vivo* and *in vitro*. At present, the reconstitution of ferritin in the presence of phosphate at various concentrations in Hepes buffer is being attempted to distinct two possibilities.

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