Biological Science

10C/2005G306

Time-resolved X-ray solution scattering study for nucleation and growth of iron core in ferritin cavity

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Introduction

Ferritin is an iron storage and detoxification protein assembled from 24 subunits (in most mammals) 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 iron atoms which are deposited in a mineralized form. The crystal structures of protein shell from many species have been revealed but the structural aspect of a hydrous ferric oxide mineral particle, so-called an iron core, remains obscure. We have been studying the structure of intermediate states of iron core formed during iron uptake by apoferritin using techniques of anomalous scattering and contrast variation small-angle X-ray scattering (SAXS). In this report, we describe the structures of intermediate state of iron core revealed by contrast variation X-ray scattering.

Experimental

SAXS experiments were carried out on the solution X-ray scattering camera at BL-10C at a wavelength of 1.488A. Scattering patterns were recorded by using a PSPC for a camera length of 2m. Intermediates were prepared by incubating apoferritin (horse spleen) and ferrous ammonium sulfate at various mole ratios and the actual iron content of reconstituted ferritin was determined spectrophotometrically.

Results and Discussion

In order to derive the structural information on the iron core in a hollow cavity, the structures of horse spleen ferritin differing in their iron-content were investigated by contrast variation X-ray scattering. The averaged electron density, $\rho_{ferritin}$, and radius of gyration, $Rg_{ferritin}$, of ferritin with various iron-contents (~500 to ~2000 Fe^{3+}) were derived from the scattering data obtained varying sucrose concentration (0 to 60 wt%) in sample solutions (Figs.1 and 2). At a matching point where the electron density of buffer containing 57.5 % sucrose is equal to that of the protein shell, ρ_{shell} (~0.42e^-/A^3)[1], the scattering intensity profiles of iron cores were recorded for ferritin varying iron-content, following by an evaluation of radius of gyration, Rg_{core}, of iron cores (Fig.1). From these experimentally obtained parameters (ρ_{shell}, Rg_{shell}, $\rho_{ferritin}$, $Rg_{ferritin}$, Rg_{core}), the averaged electron densities, ρ_{core}, of iron cores were directly determined (Fig.2). The apparent radius of gyration of iron core Rg_{core} gradually increases from about 22A for 500 Fe^{3+}/protein to about 28A for 2000 Fe^{+3}/protein, but not in proportion to the cube root of the number of iron atom (broken line in Fig.1).

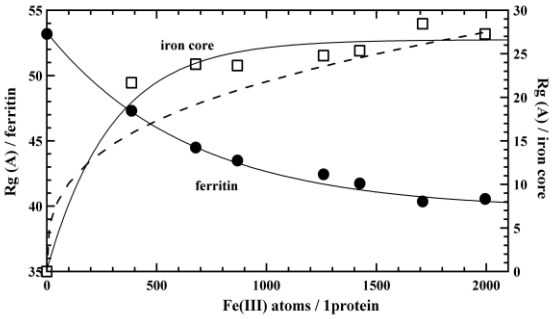

Fig.1 Radii of gyration of ferritin molecules with various iron contents and of their iron cores.

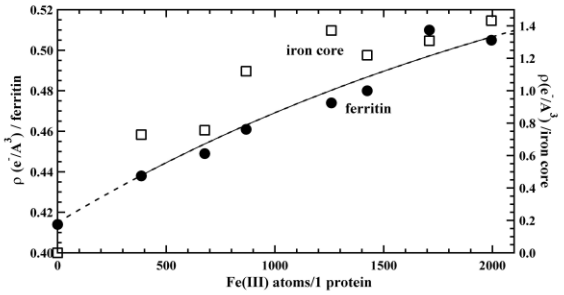

Fig.2 Mean electron densities of ferritin molecules with various iron contents and of their iron cores.

The value of ρ_{core} was 1.2 – 1.4e^-/A^3 for ferritin containing more than 1000 Fe^{3+} and ~0.8e^-/A^3 for low Fe^{3+} content (<700Fe^{3+}). The iron core in iron-rich ferritin was found to have the electron density close to that of ferrihydrite (FeOOH). These results deny the "crystal growth" model [2] as a model of growing iron core. The explanation of our results is that the iron core is composed of dispersed crystalline of ferrihydrite.

References

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