Biological Science

10C/2009G571

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

Yoji INOKO*[1], Ryouta HASEGAWA[1], Akira OGATA[1], Yasushi WATANABE[2]
[1]Division of Biophysical Engineering, Graduated School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan
[2]National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan

Introduction

Ferritin is an iron storage and detoxification protein assembled from 24 subunits (in most mammals) forming a hollow protein shell. Its central cavity has a capacity for up to 4500 iron atoms which are deposited in a mineralized form. We have been studying the structure of intermediate states of iron core formed during iron uptake by apo-ferritin *in vitro* using techniques of anomalous scattering and contrast variation small-angle X-ray scattering (SAXS). The data so far obtained showed that the size and density of the iron core increases with iron loading (beyond $2\sim3x10^2$ atoms) but that on initial loading the formation of the core in the cavity cannot be confirmed. This results suggest the possibility that iron atoms incorporated into apo-ferritin at an initial stage of uptake reaction may be accommodated in the interior or/and on the inner surface of the protein shell. We simulated the experimental scattering curves of ferritins having low iron contents with the models based on the above mentioned hypothesis.

Experimental

SAXS experiments were carried out on the solution X-ray scattering camera at BL-10C. Scattering patterns were recorded by using R-AXIS VII 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. The actual iron content in reconstituted ferritin was determined spectrophotometrically. Simple models were used for calculations, in which protein shell is represented by a hollow sphere with an inner radius of 39 A and an outer radius of 63 A and crystallites of hydrated iron oxide by spheres with 1-3 A radius depending on the degree of crystallization.

Results and Discussion

Fig.1A shows scattering profiles from the solutions of reconstituted ferritins containing 70-300 irons per molecule and of apo-ferritin. The experimental curve of apo-ferritin reveals, outside a central maximum, two distinct subsidiary maxima. This characteristic profile can be well simulated with a spherical shell with an outer radius of 63A and an inner radius of 39A as shown in Fig.1B. With increasing the amount of iron incorporated into apo-ferritin, the troughs between maxima shallow gradually, keeping the heights of the maxima constant.

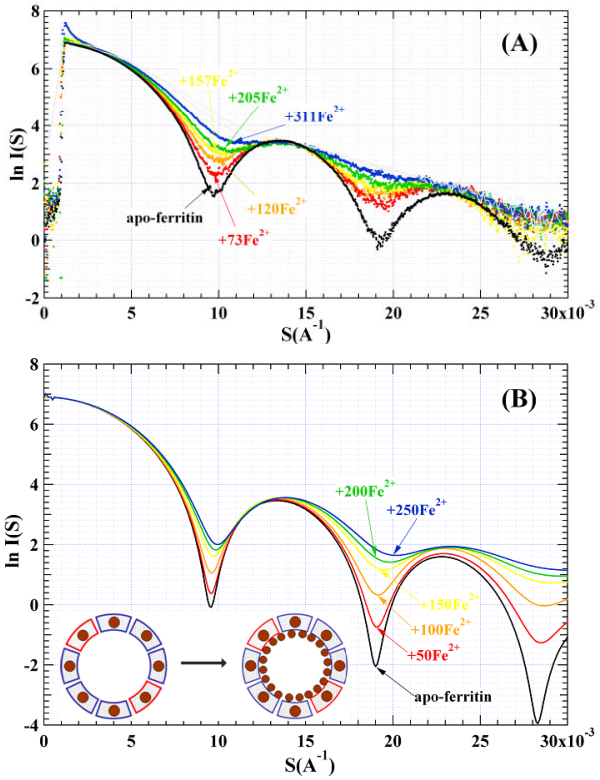

Fig.1 Scattering intensity profiles from solutions of apo-ferritin and of reconstituted ferritin containing amount of 50 to 300 iron atoms per protein molecule. (A) Experimental curves. (B) Calculation curves and models with low iron contents. The I (0) of each profile is normalized to 10^3.

Fig.1B depicts the theoretical scattering of models, in which iron atoms incorporated into apo-ferritin are placed in the interior or/and on the inner surface of the protein shell as shown in the inset in Fig.1B. The size and position of iron crystallites were made to vary with restrictions. The scheme that the small crystallites of iron oxide move from the interior to the inner surface of the shell with the progress of iron loading well reproduces the experimental curves.

*inoko@bpe.es.osaka-u.ac.jp