Structural study of ferritin iron core by a combination of anomalous X-ray scattering and contrast variation techniques

Yoji INOKO*[1], Yasushi WATANABE[2], Katsumi KOBAYASHI[3]
[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
[3] KEK-PF, Tsukuba, Ibaraki 305-0801, Japan

Introduction

Ferritin is an iron-storage protein and its 24 subunits form a roughly spherical protein shell providing a cavity in which up to 4500 iron atoms are stored as a hydrous ferric oxide. We have been studying the structure of intermediate states of iron core formed during iron uptake by apoferritin using a technique of anomalous small-angle X-ray scattering (SAXS) [1-3]. The anomalous SAXS data has provided the size of growing iron core for iron-rich ferritin, but has not been able to determine accuracy core size for low iron-content ones since the contribution of anomalous scattering intensity from iron atoms to total intensity is relatively small. In this project, a contrast variation technique has been combined with anomalous scattering measurement to eliminate the obstructive scattering coming from the protein shell.

Experimental

SAXS experiments were carried out on the solution X-ray scattering camera at tunable beamline BL-10C at a wavelength of 1.488A for normal scattering measurements and various wavelengths near the ferric iron K-absorption edge (1.741A)[1]for anomalous scattering measurements. Scattering patterns were recorded by using a PSPC with an efficient detection length of 180mm. 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 obtain the mean scattering density of the protein shell, the zero-angle scattering intensities from apoferritin were measured in 0.1M Hepes buffer containing different amount of sucrose in the range of 0 to 45 wt%. Figure 1 shows a plot of the square-root of zero-angle intensities derived from small angle scattering curves of various sample solutions against sucrose concentration. The zero intensity intercept of the regression line reveals that the zero contrast between the mean scattering densities of the solvent and protein shell is attained at 60 wt% sucrose, corresponding to an electron density of about $0.42e^-/A^3$. This value of mean scattering density obtained for apoferritin is significantly higher than those ($0.39\text{-}0.40e^-/A^3$) reported for most of other globular proteins.

Reconstituted ferritin containing different iron content have been studied in buffered 60 wt% sucrose solutions at which the scattering of the protein shell of ferritin approximately vanishes in the small angle region. The scattering measurements were done firstly at a wavelength of 1.488A to evaluate the core size of iron atoms deposited in various intermediate ferritin molecules by the Guinier analysis of their small angle scattering data. The apparent radius of gyration of iron core gradually increases from about 20A for 500 iron atoms/protein to about 28A for 3000 iron atoms/protein.

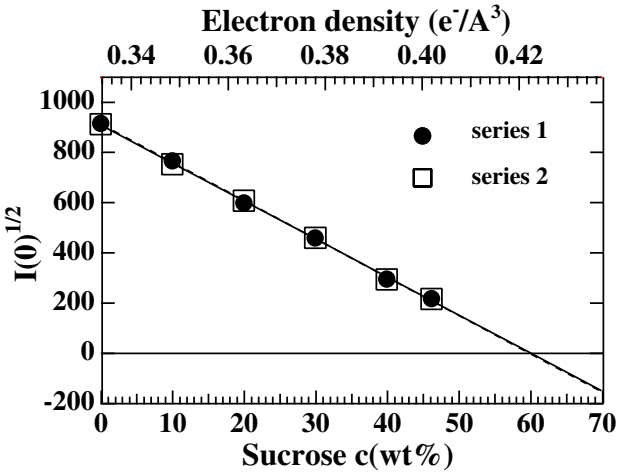

Figure 1. The square-root of zero angle intensities of horse spleen apoferritin solutions(0.076mg/ml) are plotted *versus* the electron density (top axis) and concentration (bottom axis) of the sucrose solvent.

Next, the scattering intensity from various intermediate in 60 wt% sucrose solutions was recorded at wavelengths in the vicinity of the K-edge (1.741A) of ferric iron. The anomalous scattering part due to iron atoms was decomposed from a set of the total scattering profile measured at different wavelengths. The distribution of ferric iron atoms is to be calculated and compared with the size of the iron core obtained directly from the zero contrast measurements as described above.

References

[1] Y. Inoko et al., PF Activity Rep.**12**, 219(1994)
[2] Y. Inoko et al., PF Activity Rep.**15**, 55(1997)
[3] Y. Inoko et al., PF Activity Rep.**16**, 237(1998)

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