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Development of resonant X-ray scattering method of metallo-proteins in solutions

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1. Introduction

Small-angle X-ray scattering technique of solutions is a useful method for observing in-situ structural information of solute-particles such as radii of gyration and size distributions by analyzing data sets in small-angle scattering regions. In addition, recent improvements in X-ray beam brightness and detection enable us to obtain high-statistic data with a wide real-space resolution ranging from ~0.2 nm to ~250 nm, even in standard experimental set-ups. Due to such improvements, we successfully discussed about whole hierarchal structures of proteins and those transition phenomena in detail [1-5].

However, another characteristic of synchrotron sources, namely selectivity of incident X-ray energy, has been rarely used for solution scattering of proteins. Therefore, by the combination of anomalous dispersion and wide-angle scattering of X-ray, we have tried to apply resonant (anomalous dispersion) small- and wide-angle X-ray scattering (A-SWAXS) to determine internal structures of metallo-proteins in solutions. We have studied the structures of metallo-proteins in solutions. The thermal unfolding-refolding process of lysozyme with mercury atom isomorphous replacement was also studied.

2. Experimental

As metallo-proteins, hemoglobin (HM) from bovine blood, ferritin from horse spleen, and hen-egg white lysozyme with Hg-atom isomorphous replacements (Hg-LY) were used. A-SWAXS measurements were performed by using the BL10C and BL15A2 spectrometers at PF. The energy of incident X-ray has been varied at near Hg-L3 absorption edge and at Fe-K absorption edge. Namely, from 12.27 KeV to 12.31 KeV for Hg-L3 edge, and from 7.118 KeV to 7.134 KeV for Fe-K edge. The temperature range was from 25 °C to 85 °C controlled by a water-bath circulator. The sample-to-detector distances were ~200 cm and ~24 cm. X-ray scattering intensity was recorded by PILATUS3 2M detector (Dectris Co.). The exposure time was 30 seconds. Sample cells composed of a pair of thin-quartz windows with 1 mm path length were used.

3. Results and Discussion

Fig. 1 shows the WAXS curve of thermal unfolding and refolding process of Hg-label lysozyme (5 % w/v) observed at different X-ray energy, where (A), (B), and (C) correspond to X-ray energies of 12.28 KeV, 12.29 KeV, and 12.30 KeV, respectively. As shown in Fig. 1, this thermal transition holds the thermal reversibility. By the difference of the scattering intensity, we can extract the contribution of only-Hg atoms, namely four s-s bonds in the protein. Fig. 2 shows the X-ray energy dependence of the WAXS curve of Hg-label lysozyme at 25 °C & pH 2.9 normalized by that of native lysozyme at 25 °C & pH2.9. Although the statistic of the data is not enough, we can recognize two peaks at ~0.29 $Å^{-1}$ and 0.48 $Å^{-1}$ in the middle q-region that correspond to the distance correlation of the s-s bounds replaced by Hg within the lysozyme molecule. Thus, the present results suggest the potential of the application of resonant X-ray scattering to the observation of internal structures of proteins in solutions. The detailed discussion and results will be shown elsewhere.

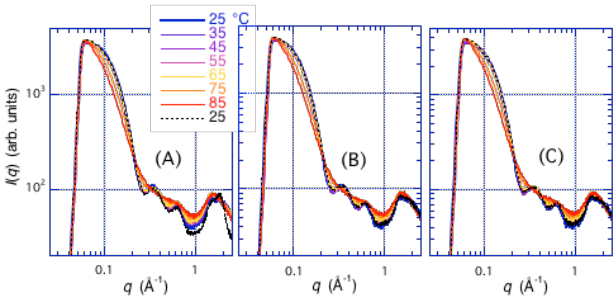

Fig. 1. Thermal unfolding and refolding of Hg-label lysozyme at pH 2.9 observed at near Hg-L3 absorption edge. (A), 12.28 KeV; (B), 12.29 KeV; (C), 12.30 KeV.

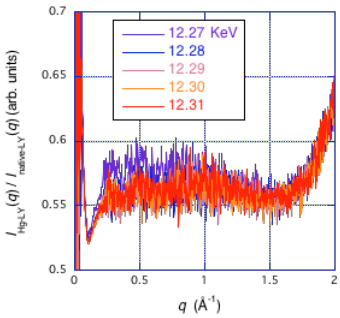

Fig. 2. Incident X-ray energy dependence of WAXS curve of Hg-label lysozyme at pH 2.9 normalized by that of native lysozyme.

References

[1] M. Hirai et al., *J. Sync.Rad.*, **9**, 202 (2002).

[2] M. Hirai et al., *Biochemistry*, **43**, 9036 (2004).

[3] M. Hirai et al., *J. Appl. Cryst.* **40**, s17 (2007).

[4] M. Hirai et al., *J. Appl. Cryst.* **40**, s184 (2007).

[5] M. Hiari et al., *Thermochimica Acta,* **532**, 15 (2012).

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