タンパク質の構造転移と広角散乱：浸透圧下における折畳みの研究
Structural transition of protein observed wide-angle scattering: Study of protein folding under osmotic pressure

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[Introduction]

Hydration of biological macromolecules is assumed to have an important role in their structural stability and functions. As suggested by inelastic neutron scattering experiments, the functional properties and dynamics of proteins in solutions are coupled with the dynamic behaviour of the surrounding water molecules. Molecular dynamics simulation studies also suggested that the variation in the first hydration shell density is subject to the electrostatic properties of the protein surface and local surface topography. On the other hand, the mechanism by which proteins fold into their native structures is one of the essential problems in biology and is under extensive studies. To clarify the mechanism of protein folding, various methods have been used to observe detailed features of the folding‐unfolding processes of proteins. Hydration of proteins is assumed to greatly contribute not only to their structural stability but also their functions. However, the role of the hydrated water shells on protein folding is still the subject of many experimental and theoretical investigations.

By using synchrotron radiation (SR) wide-angle X-ray scattering, we have been studying the thermal unfolding‐refolding process of hen egg-white lysozyme (HEWL) at various pH values under stepwise heating and cooling conditions. Recently, we demonstrated that the wide-angle X-ray scattering (WAXS) method using high-intensity X-rays from a third-generation synchrotron-radiation source enables us to observe directly the whole hierarchical structure of proteins including quaternary, tertiary, domain and secondary structures in solution (1), and we successfully analyzed the details of the reversible unfolding‐refolding process of HEWL covering all the hierarchical structures from tertiary to secondary structure, and showed that the thermal structural transition of HEWL characterized by its hierarchical structures and the cooperativity between them (2). In addition, we found the collapse of the hydration shell prior to the unfolding of HEWL (3).

In the present study, we have carried out SR-WAXS experiments to clarify the effect of the change of osmotic pressure on the protein unfolding and refolding. We have used polyvinylpyrrolidone (PVP) (Mt 40,000) to vary the osmotic pressure.

[Experimental]

The spectrometer used for SR-WAXS experiments was a X-ray scattering spectrometer installed at BL-40B2 of the 8 GeV synchrotron radiation source of the Japan Synchrotron Radiation Research Institute (JASRI), Harima, Japan. The sample-to-detector distances were 50 cm and 4000 cm for 1.0 Å X-ray. The scattering intensity was recorded by an RAXIS VII imaging plate system from RIGAKU. A sample cell composed of a pair of thin quartz windows with 1 mm path length was used. The exposure time to the X-rays was 30 s for each measurement. Other details of the WAXS measurements are given in the previous reports (Hirai et al., 2002, 2004, 2007). The proteins measured are myoglobin and lysozyme at different pH and PVP concentration.

[Results]

Fig. 1 shows the PVP concentration dependence of the WAXS curve of myoglobin (2.5 % w/v) at pH 5 at 25 ° C, where the PVP concentration was varied from 0 to 25 % w/v. The scattering curve

reflecting the intramolecular structure, mostly holds below PVP = 20 % w/v. However, the radius of gyration obtained from Fig. 1 shows the minimum value around PVP = 5-10 % w/v. This suggests that the addition of small amount of PVP destroys the hydration shell without affecting the intramolecular structure.

Fig. 2 shows the temperature dependence of the WAXS curve of the myoglobin at pH 5 at PVP = 5 % w/v. The presence of PVP tends to lower the transition temperature. At high temperature the peak around q = 1.34 Å^{-1} becomes to be evident, reflecting the α-to-β transition, namely the formation of cross beta structure of amyloid. We are now carried out further analyses to clarify the effect of PVP, namely the effect of osmotic pressure on the unfolding and refolding processes of myoglobin and lysozyme at various pH values and PVP concentrations.

Although the above experiments were done by using the third generation synchrotron source, we should mention that such WAXS measurements are essentially important for structural biology and are executable even for the second generation synchrotron source when we can improve the optics and the efficient 2-D detector of the present SAXS spectrometers at PF.

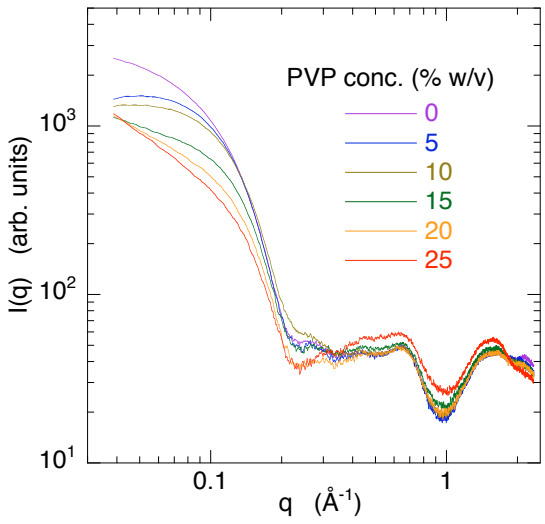

Fig. 1 PVP concentration dependence of WAXS profile of myoglobin (2.5 % w/v, pH 5, 25 °C).

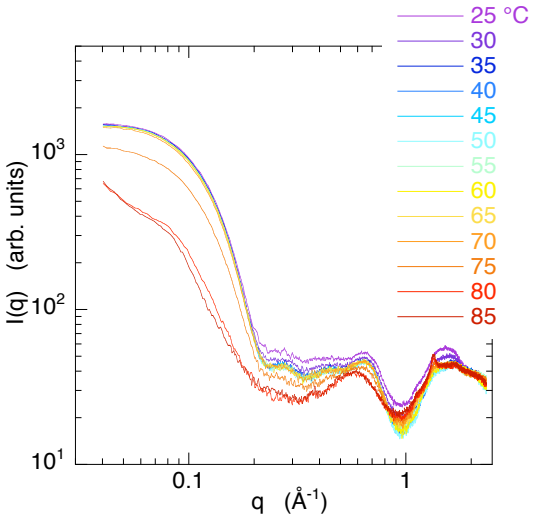

Fig. 2 Thermal unfolding of myoglobin (2.5 % w/v, pH 5) under osmotic pressure (PVP conc. = 5 % w/v).

[References]
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