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# Effect of Osmotic Stress on Protein Structure

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## Introduction

Hydration of biological macromolecules plays an important role in their structural stability and functions. As shown by inelastic neutron scattering studies and molecular dynamics simulation studies, the functional properties and dynamics of proteins are coupled with the behavior of water molecules surrounding proteins. The hydration shell densities of proteins are also known to subject to the electrostatic properties of their local surface topography. On the other hand, the mechanism by which proteins fold into their native structures has been one of the essential problems in biology for a long time. However, the role of the hydration shells on protein folding is still ambiguous in spite of many experimental and theoretical investigations of protein folding issues.

In the present study, we have carried out small-angle X-ray scattering (SAXS) experiments to clarify the effect of the change of osmotic pressure on the protein unfolding and refolding. We previously demonstrated that the wide-angle X-ray scattering (WAXS) method enables us to observe the whole hierarchical structure of proteins from their quaternary or tertiary structures to secondary ones in solutions [1] and that the details of the unfolding–refolding process of proteins can be analyzed on all hierarchical structure levels and on structural transition cooperativity among them [2]. We also found the collapse of the hydration shell of hen egg-white lysozyme (HEWL) prior to its thermal unfolding [3]. In the present experiments, we have studied the effect of osmotic pressure on protein hydration and unfolding-refolding process of different types of proteins.

## Experimental

Small-angle scattering (SAXS) measurements were also performed by using the synchrotron radiation small-angle X-ray scattering spectrometer installed at BL10C beam port of the 2.5 GeV synchrotron radiation source (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The X-ray wavelength used was 1.49 Å and the sample-to-detector distances were 80 cm and 190 cm.

Proteins used for measurements were myoglobin and apomyoglobin from horse skeletal muscle, hemoglobin from bovine, and lysozyme from chicken egg white, which were all purchased from Sigma Chemical Co. and used without further purification. The proteins were dissolved in 20 mM sodium citrate buffer or 10 mM Hepes (*N*- (2-hydroxymethyl)piperazine-*N'*-(2-ethane-sulfonic acid)) buffer adjusted an appropriate pH pH 3, 4, 5, 7, 8). The osmotic pressure was varied from 0 to $4.16 \times 10^5$ N/m$^2$ by adding the polyvinylpyrrolidone (PVP) in the range from 0 to 25% w/v.

## Results and Discussion

Fig 1 shows the PVP concentration dependence of the radius of gyrations ($R_g$) of myoglobin (2.5% w/v) and lysozyme (2.5% w/v) at different pH at 25°C. In the case of lysozyme, the $R_g$ value shows a decreasing tendency at every pH. The decrement becomes to be smaller above PVP ≧ 15%. Whereas, in the case of myoglobin, according to increase of the PVP concentration, the $R_g$ value becomes to increase after showing a minimum around PVP = 15% at pH 7, and PVP = 10% at other pH. 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. The increase of the $R_g$ value is ascribed to the oligomerization of the proteins. Namely, even at a low osmotic pressure, the shrinkage of the dimension of the protein is occurred for both different types of proteins, suggesting that the collapse of the hydration shell surrounding the protein surface can be induced by the depletion of hydrated water molecules with the osmolytes. Such a change in hydration shell affected the thermal unfolding and refolding processes for both proteins. The detail of the results and discussion will be published.

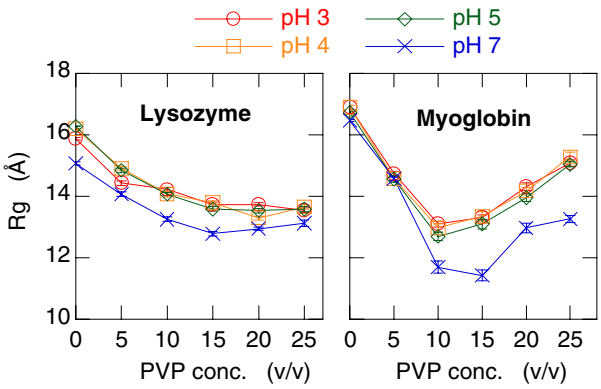


Fig. 1. Change of radius of gyration observed by SAXS depending on osmotic pressure (PVP concentration).

## References

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