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Effect of Trehalose on Protein Structure Stabilization

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1 Introduction

It is well known that some organisms that have considerable tolerances against extreme environmental conditions, produce stress proteins and/or accumulate sugars in their cells. This phenomenon is called cryptobiosis, i.e., the ability of an organism to tolerate environmental changes without having to actively adapt to them. In particular, trehalose has been drawing attention relevant to cryptobiosis under external stress such as high or low temperature, drying, and osmotic pressure. The action of trehalose to afford an organism to tolerate environmental changes has been thought to result from the restriction of the intra-and/or-inter-molecular movement by vitrification or from the replacement of water molecule by trehalose. It has been pointed out that the structure of water is an important factor. By the complementary use of synchrotron radiation wide-angle X-ray scattering (SR-WAXS) and small-angle neutron scattering (SANS), we have studied the effect of trehalose and other sugars on protein structure and its hydration-shell against thermal and chemical denaturation.

2 Experiment

The protein measured was myoglobin from horse skeletal muscle, purchased from SIGMA Chemical Co. (USA) and used without further purification. Trehalose (crystalline di-hydrated powder) from HAYASHIBARA were used as sugars. The buffer solvent used was 10 mM HEPES (*N*-(2-hydroxymethyl) piperazine-*N*′-(2-ethane-sulfonic acid)) at pH 7.1 (pD 6.7) at 50 mM NaCl. The myoglobin solution with 5 % w/v and the trehalose solutions with the concentrations from 10 to 60 % w/w were prepared as the stock solutions. The protein and sugar stock solutions were mixed by 1:4 volume ratio, and used as sample solutions. Just before the scattering measurements, these solutions were filtered to remove some large aggregates by using a Millipore membrane filter. The final protein concentrations of the solutions were determined by ultraviolet-visible spectrophotometry measurements.

SR-WAXS measurements were done by using the the BL-10C spectrometer at KEK, Tsukuba, Japan. The X-ray wavelength and the sample-to-detector distance were 1.55 Å, 23.9 cm at BL-10C. The X-ray scattering intensity was recorded by the PILATUS3 2M detector. The exposure time was 30 seconds at BL-10C. The temperature of the solutions contained in the sample cells was controlled by the temperature controller mK2000 of INSTEC Co. While the measurements, the sample solutions were slowly oscillated to avoid some radiation damages.

3 Results and Discussion

Figure 1 depicts the temperature dependence of the WAXS curves of myoglobin, where (A): in pure solvent, (B): in 20 % w/w trehalose solution. The observed WAXS curve in each q-region reflects the protein structure at a different hierarchal structure level, namely, the tertiary structure (q < ~0.2 Å^{-1}), the internal structure (~0.25 Å^{-1} < q < ~0.8 Å^{-1}), and the secondary structure (~1.1 Å^{-1} < q < ~1.9 Å^{-1}), respectively. As we observed the whole hierarchal structure, the stabilization effect of trehalose on the protein structure can be clarified in detail. The results have been already published [1-3].

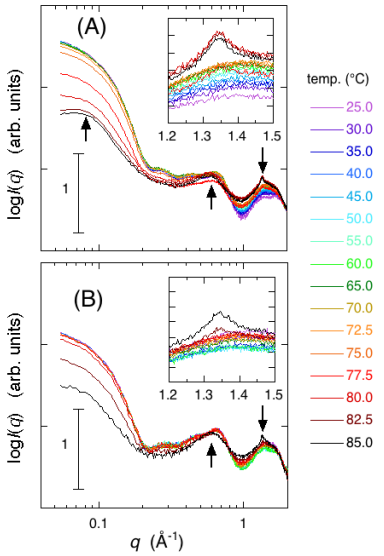

Fig. 1: Temperature dependence of the WAXS curve of myoglobin. (A) In water solvent, (B) In 20 % w/w trehalose. The insets enlarge the WAXS curves in the secondary structure region. The arrows indicate the typical features in the scattering curve appearing at the initial process of amyloid transition, namely, the pleated-beta-sheet stacking and the helix-to-sheet (cross-beta-sheet) transition.

References

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[3] S. Ajito, M. Hirai *et al.*, *Physica B: Condensed Matter* **551**, 249 (2018).

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