How do sugars affect protein structure and its stability?

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1. Introduction

Structural stability of proteins in aqueous solutions not only depends on physical environment such as temperature and pressure but also on the presence of osmolytes. It has been known for a long time that protein denaturation and enzyme deactivation can be prevented by the addition of sugar or polyol. Several factors, such as specific binding between protein and additives and changes in solvent viscosity, have been considered. Proteins are preferentially hydrated in the presence of sugars, and extensive hydrogen bonding of water in the hydration shell around proteins in the presence of sugars has been considered to be a major driving force toward their stabilizing effect. However, the experimental basis for the discussion so far is mostly the studies using spectroscopic methods such as densitometry, calorimetry, circular dichroism, NMR, etc. There are few studies utilizing X-ray or neutron scattering method that is applicable to observe direct evidences for the effect of sugars on protein structure and hydration. From biological points of view, under external stress such as high or low temperature, drying, osmotic pressure, and so on, some organisms that have tolerances against extreme environment produce stress proteins and/or accumulate sugars in cells. It has been pointed out that the structure of water is an important factor. Therefore, we have studied the effect of glucose on protein structure and its hydration-shell by the complementary use of small-angle neutron scattering (SANS) and synchrotron radiation wide-angle X-ray scattering (SR-WAXS). The experimental results obtained by SANS and SR-WAXS can be discussed in detail using the theoretical scattering function simulations.

2. Experiment

SANS measurements were carried out by using the BL15 TAIKAN spectrometer at MLF at J-PARC, Tokai, Japan. The neutron wavelength was 1.0 – 7.8 Å. The sample solutions were contained in the quartz cells with 1 mm or 2 mm path length. The exposure time was around 1 - 3 hours. In the SANS measurements, we employed the inverse contrast variation method. This method using deuterated materials is known to avoid or minimize the artificial effect on the scattering curves caused by the presence of co-solute molecules. We have also done synchrotron radiation wide-angle X-ray scattering (SR-WAXS) measurements by using the BL-10C spectrometer at KEK, Tsukuba, Japan. We have succeeded to observe only a protein structure by mostly diminishing the effect of mimic-cell environment. The protein measured was myoglobin from horse skeletal muscle purchased from SIGMA Chemical Co. (USA). Non-deuterated glucose and 97%-atom-deuterated glucose from SIGMA were used as sugars. All other chemicals used were of analytical grade. The buffer solvent used was 10 mM HEPES (*N*-(2-hydroxymethyl) piperazine-*N*′-(2-ethane-sulfonic acid)) at pH 7.0 (pD 6.6) at 50 mM NaCl. The concentration of myoglobin was 2 % w/v, and the sugar concentration was varied from 0 to 30 % w/w.

3. Results

Figure 1 shows the observed neutron scattering curve depending on the concentration of the glucose mixture ([h-glucose]/[d-glucose] = 0.706/0.294), where the insert shows the square-root of the zero-angle scattering intensity, $I(0)^{1/2}$, depending on the glucose concentration. As shown in the insert, the magnitude of $I(0)^{1/2}$ kept constant, indicating that the effect of the presence of glucose on the contrast is mostly eliminated. In Figure 6, the solid lines show the theoretical scattering functions obtained by fitting experimental data using the CRYSON program, where the number of harmonics we used for the calculations was 50. The CRYSON program was also

developed by Svergun et al. to simulate or fit neutron scattering functions based on the same architecture and principle of the CRYSOL program. As shown in Figure 1, the CRYSON fitting has been done very effectively since the contrast of the protein is constant at every glucose concentration under the present neutron scattering measurement condition. The □-square values of the fitting in Figure 1 were in the range from 1.0 to 1.6. The theoretical scattering functions describe well the experimental ones. In Figure 2 (A) shows the distance distribution function, $p(r)$, obtained from the observed SANS curves in Figure 6 by using Equation 1. The maximum diameter, D_{max}, of the particle shows a slightly decreasing tendency from 47.8 Å to 44.7 Å. In Figure 2 (B), the contrast of the hydration-shell obtained by the CRYSON fitting and the R_g value are shown. It clearly indicates that the hydration shell density is almost constant in spite of the increase of the glucose concentration. The hydration-shell contrast preserves the value of ~0.58 x10^{10} cm^{-2} (corresponding to ~9.1 % higher than the average scattering-density of D_2O) up to 30 % w/w glucose. The above results strongly support the WAXS results that sugar molecules are preferentially excluded from the hydration-shell region of the protein.

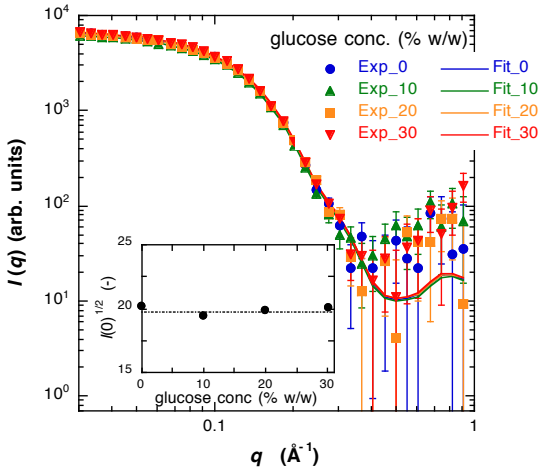

Figure 1: SANS curve of myoglobin Neutron scattering curve depending on the glucose concentration (100 % D_2O, 10 mM Hepes, 50 mM NaCl, pD 6.7 (= pH 7.1) at 25 °C)). Glucose is the mixture of [h-glucose]/[d-glucose] = 70.6/29.4. The insert shows the zero-angle scattering intensity, $I(0)^{1/2}$. The solid lines show the theoretical scattering functions obtained by fitting experimental data using CRYSON program.

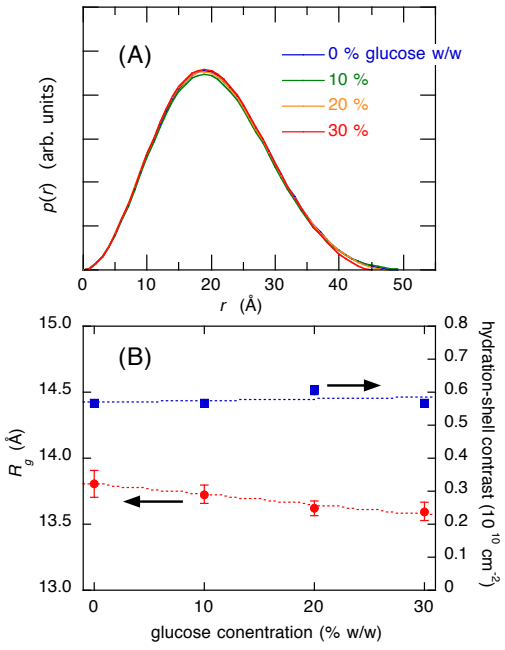

Figure 2: (A), distance distribution function; (B), contrast of the hydration-shell evaluated by the fitting and the experimental R_g values obtained from Figure 1.

4. Conclusion

The neutron scattering results using the inverse-contrast variation method strongly support the preferential exclusion of sugar and the preservation of the hydration-shell of the protein. At the sugar concentration higher than ~25 % w/w, the present results suggest the possibility of the preferential solvation of sugars that will be partially occurring on the protein surface. From the methodological point of view, it should be noted that the complementary use of X-ray and neutron scattering would have a high availability or superiority to afford us a direct evidence of the effect of various osmolytes on protein structures and on those hydration properties under molecular crowding environments. The detailed results and discussion will be shown soon [1].

References

[1] S. Ajito, M. Hirai et al., Physica B, doi.org/10.1016/j.physb.2018.03.040.