Sugars can protect hydration shell of proteins and stabilize their native structures in crowded molecular environment: clarified by complemental use of X-rays and neutrons

The denaturation and/or deactivation of proteins can be prevented by additives such as sugars and polyols. These additives are widely applied to the storage of living cells, food preservation, and so forth. On the other hand, organisms tolerant to adverse environmental conditions such as desiccation and freezing have been shown to temporarily produce and/or accumulate stress proteins and/or sugars to stop all measurable metabolic processes until environmental conditions return to being favorable. This metabolic state is called cryptobiosis. In particular, the protective actions of sugars and polyols on protein structures have been considered to be related to specific bindings of these additives, changes in solvent viscosity and surface tension, and free energy changes upon addition of proteins into these additive solutions. Clearly, from a previous report, hydration is the key determinant of the isothermal and concentration-dependent effects on protein equilibria, since the dynamics of proteins are surely coupled with and/or governed by surrounding water molecules. Nevertheless, direct observation or characterization of the protein structure and its solvation in solutions where large quantities of additives such as sugars and polyols exist have not been obtained and clarified yet. This is because the crowded molecular environment of these additives significantly interferes with the acquisition of high quality statistical data of a protein owing to a large decrease in the difference between the average scattering densities of the solute and the solvent, so-called contrast.

By the complemental use of small- and wide-angle X-ray scattering (SWAXS) and small- and wide-angle neutron scattering (SWANS), we succeeded in quantitatively clarifying the effect of sugars (mono- and disaccharides) on protein hydration [1] and structural stability against chemical and thermal denaturation [2]. SWAXS experiments were carried out at SPring-8 **BL40B2**. We used glucose and fructose as the monosaccharides, and sucrose and trehalose as the disaccharides. Figure 1 shows examples of the SWAXS curves of myoglobin as a function of sugar concentration (panel 1A, trehalose; panel 1B, glucose). Note that the SWAXS curve covers all structures at different hierarchal levels of the myoglobin structure. Namely, the observed q regions of q < ~0.2 Å^{-1}, ~0.25 Å^{-1} < q < ~0.5 Å^{-1}, ~0.5 Å^{-1} < q < ~0.8 Å^{-1}, and ~1.1 Å^{-1} < q < ~1.9 Å^{-1} respectively correspond to the tertiary structure, the inter- and intradomain

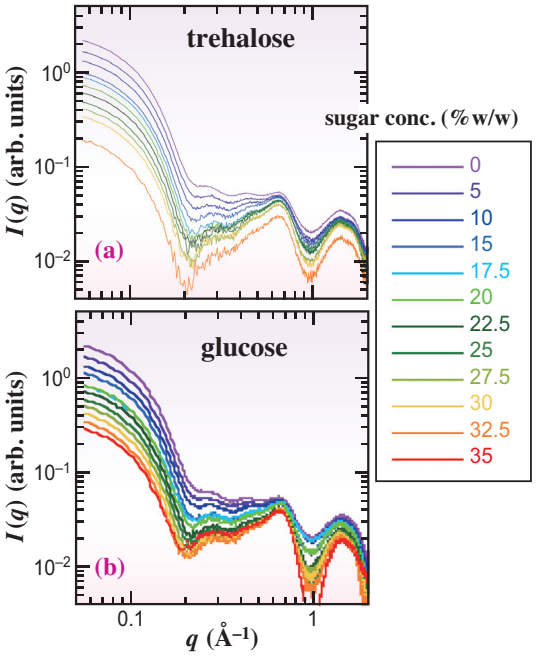

Fig. 1. SWAXS curves of myoglobin as a function of sugar concentration (%w/w). **(a)** trehalose; **(b)** glucose.

structures, and the secondary structure including the closely packed side chains [2]. By comparing experimental data with data obtained by theoretical simulation based on three different solvation models (Model 1, preferential solvation model; Model 2, neutral (non-preferential) solvation model; Model 3, preferential exclusion (preferential hydration) model), we quantitatively describe the observed changes in the zero-angle scattering intensity ($I(0)^{1/2}$) and the radius of gyration (R_g), as shown in Fig. 2. Results indicate that sugar molecules are preferentially or weakly excluded from the protein surface to preserve the native protein hydration shell, and the preferential exclusion shifts gradually to neutral solvation as the concentration increases. The hydration-shell density directly determined by the inverse contrast matching method of neutron scattering using deuterated sugars strongly supported the WAXS results [1]. Owing to such action of sugars, guanidinium chloride-mediated denaturation and thermal denaturation were found to be significantly suppressed by the presence of sugars [3]. Similar protective action of glycerol was observed [4]. On the other hand, myoglobin is known to undergo an amyloidogenic reaction under denaturated conditions. The SWAXS and dynamic

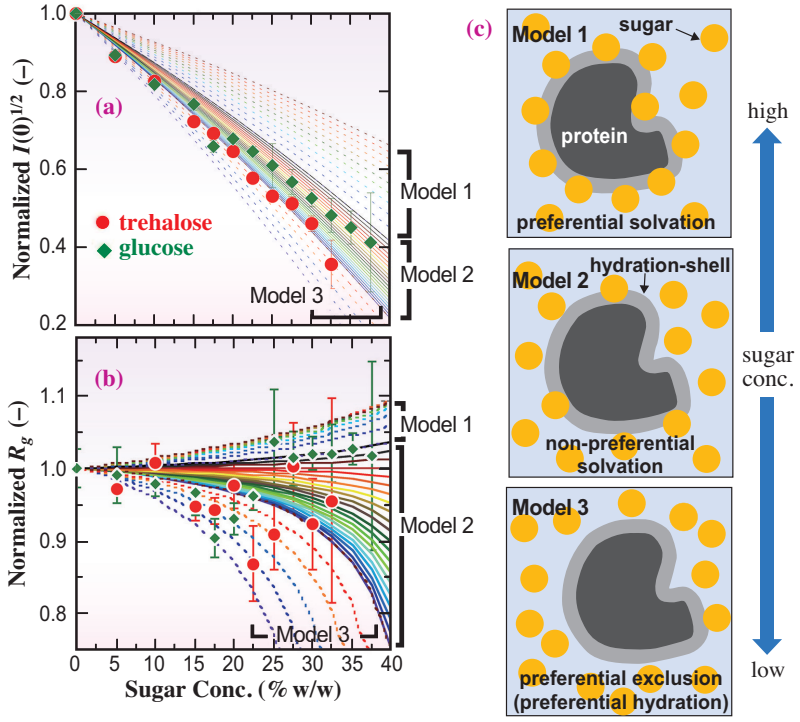

Fig. 2. **(a)** Zero-angle scattering intensity ($I(0)^{1/2}$) and **(b)** radius of gyration (R_g) as a function of sugar concentration. The frames depict the theoretical values in the different solvation models shown in **(c)**. From the top in (c), preferential solvation by sugar (Model 1); neutral (non-preferential) solvation (Model 2); preferential exclusion (preferential hydration) (Model 3).

light scattering measurements indicated that acid-denatured myoglobin in the initial process of the amyloidogenic reaction (helix-to-sheet transition followed by oligomerization) was substantially restored to its native structure by trehalose [5]. Figure 3 shows the restoration of the protein structure from the amyloid state to the native state with increasing trehalose concentration. The hump at q = ~0.58 Å^{-1} and the peak at q = ~1.34 Å^{-1} are the characteristic features emerging during the early stages of amyloid formation, (i.e., the pleated-beta-sheet stacking) and the helix-to-sheet (cross-beta-sheet) transition, respectively. These characteristic profiles reflecting amyloid formation disappeared and returned to those of the native ones.

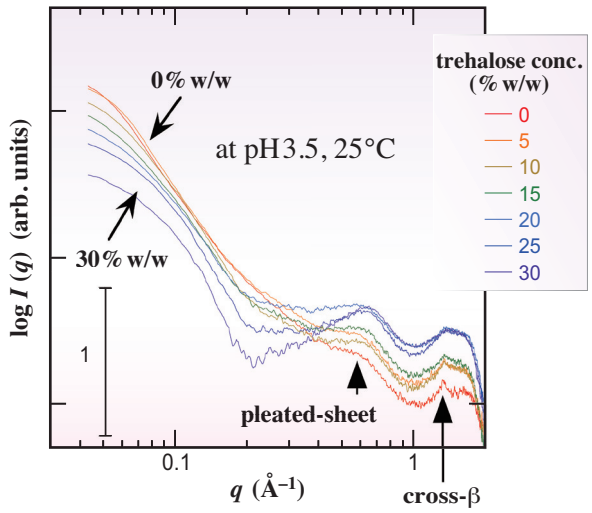

Fig. 3. SWAXS curves showing the restoration of acid-denatured myoglobin (amyloid state) to its native structure by trehalose.

The series of SWAXS and SWANS experiments on the effect of sugars on the protein structure and stability are expected to provide new insight into the molecular mechanism underlying the intrinsic function of sugars in cryptobiosis and amyloidosis.

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