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Structural Properties of Proteins Embedded in Sugar-Glass and/or Sugar-Rubber

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1 Introduction

Insects such as tardigrades, rotifers, and polypedilum vanderplanki are well known as animal resistance in extreme environments (cryptobiosis "non-metabolic state"), all of which are found in extreme environments such as desiccation and freezing conditions. They survive by accumulating sugar (trehalose) as a "compatible solute" that protects biological components and cell membranes instead of water and stopping metabolic activity (hibernation, anhydrobiosis). It is also known that amphibians can survive by creating a hyperglycemic state when living conditions deteriorate such as low temperature and dryness (extreme heat). Such environmental resistance mechanisms have been explained by the "glass state theory" (protection of higher-order structures such as cell membranes and proteins associated with vitrification by sugar) or the "water substitution theory" (direct hydrogen by sugar acting as a substitute for bound water). However, the details of the mechanism are still unknown because there are few experimental studies at the molecular structure level.

Recently, by the complementary use of X-ray and neutron scattering techniques, we have provided direct evidence that the protein hydration (solvation) and structural stability against chemical and thermal denaturation significantly depend considerably on the sugar species and glycerol.[1-3] The sugar and glycerol molecules tend to be preferentially or weakly excluded from the protein surface, which preserves the native protein hydration shell; however, the preferential exclusion (preferential hydration) shifts gradually toward the non-preferential solvation (replacement of the hydrated water by sugar or glycerol) as the concentrations of these molecules increase. Owing to the protective action of these molecules on the protein hydration shell, the protein structure is stabilized against chemical (guanidinium chloride) and thermal denaturation. The protective action depends on the sugar species.[3] In addition, we also found that intermolecular interactions between sugar molecules are essentially repulsive interactions. However, we have observed that the correlation distance between sugar molecules varies significantly depending on the type of sugar, and it depends on the hydration structure and dynamic properties of sugars.[4]

On the other hand, carbohydrates are one of the three major components of foods along with lipids and proteins. At the same time, depending on changes in temperature and water content during food processing, carbohydrates undergo various physical property changes (crystallization, melting, glass transition, inclusion complex formation, etc.), which determines the quality and preservation of food. Currently, the importance of glass transition (T_g) is recognized from the scientific point of view on freezing, storage, and thawing of foodstuffs. Therefore, if a relatively easy storage temperature can be set by increasing T_g by adding sugar, the storage period and quality can be extended. T_g varies depending on the type and concentration of sugars. Thus, for long-term stable storage, it is important to search for the optimum sugar vitrification state conditions that can stably retain the structures of proteins and lipids based on nanoscale-level structural analysis.

Thus, the purpose of this research is to elucidate the structural characteristics of proteins trapped in extremely low moisture conditions, such as sugar glassy and sugar rubbery states, which are unresolved issues in the fields of extremophile biology and food science.

2 Experiment

The proteins measured were horse skeletal muscle myoglobin, egg white lysozyme, bovine serum albumin, horse hemoglobin, which were purchased from Sigma-Aldrich. We used four different types of sugars: disaccharides (trehalose, sucrose) and monosaccharides (fructose, glucose). The proteins were dissolved in the water solvent (0.5 mM HEPES, pH 7.1) to be the concentration of 3 % w/v. The sugars were dissolved in the water solvent to be the concentration of 30 % w/w. These protein and sugar solutions were used as the stock solutions, respectively. After mixing the protein solution and the sugar solution at a ratio of 3/1, contained in the disc-shaped sample cell, and dried in a constant temperature bath for 4 days. The water contents of the samples served for the scattering measurement were evaluated from the weight measurement using an electronic balance. The water contents of the samples were in the range from 3.2 % w/w to 12 % w/w. The small- and wide-angle X-ray scattering (SWAXS) measurements were carried out by using the BL10C spectrometer at PF. The X-ray wavelength and the

sample-to-detector distance was 1.5 Å for 24 cm, and 1.5 Å for 203 cm. The temperature of the he samples et in the sample cell holder was controlled by the model mK2000 temperature controller (Instec, Inc.).

3 Results and Discussion

Fig. 1 shows the WAXS curves of proteins embedded sugar rubber at 20°C. The water contents in Fig.1 were around 10% w/w. The scattering curves below q = ~0.4 Å^{-1} in Fig.1 reflect well characteristic scattering curves depending on the structural properties of each protein at native state. The values of the radius of gyration (R_g) of proteins are known to depend on the difference between the average scattering densities of the solute particle and the solvent matrix, so-called contrast. The R_g values estimated from Fig. 1 almost agrees with the theoretical value considering contrast change due to excessive sugar environment.

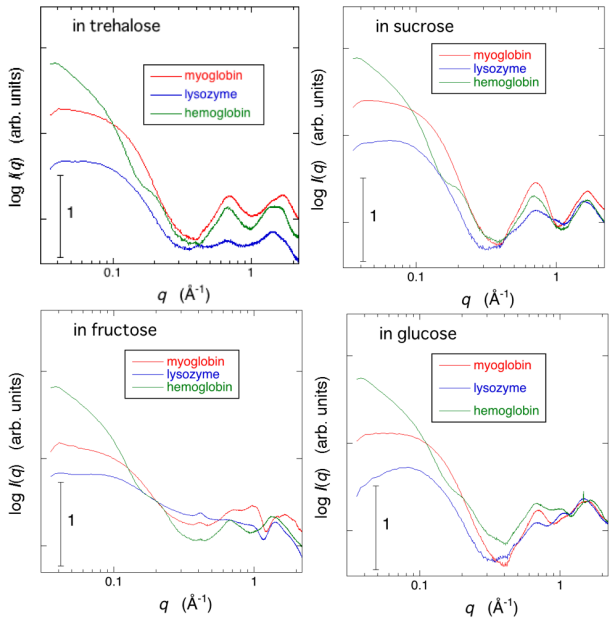

Fig. 1: WAXS curves of proteins embedded in different types of sugar rubber at 20°C.

The difference in the scattering curves above q = ~0.5 Å^{-1} is attributable to the sugar matrix depending on the sugar type as we already reported in the results of highly concentrated sugar solutions. The detailed results will be seen soon.

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