Effects of Trehalose on the Swelling Behavior of Hydrogel —Visualization of the Preferential Hydration of Disaccharides—

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We study effects of trehalose on the swelling behavior of hydrogel, exemplified here by agarose. Its volume increase on immersion into water is suppressed in the presence of disaccharides, the extent of which lies in the order, sucrose < maltose < trehalose. Such tendency is attributable to the relatively higher hydration ability of trehalose.

Trehalose occurs widely in the so-called anhydrobiotic organisms such as yeast, brine shrimp, nematodes, and tardigrades, which fall into a state of suspended animation when dried, although they revive on rehydration.[1] Throughout many in vitro studies with purified proteins, enzymes, or biomimetic membranes, there has been growing consensus that desiccation tolerance abilities of these organisms originate in vitrification of endogenous trehalose[2] and/or its role as the substitute for bound water of body components.[3] Recently we have successfully obtained direct evidence that vitrification is essential for anhydrobiosis of larvae of an African chironomide, *Polypedirum vanderplanki*,[4] which increased the endogenous trehalose, finally up to 18 wt % of their dried bodies, when they were slowly dehydrated.[5] Now toward entire clarification of the anhydrobiotic mechanism, it is necessary to gain insight into the intermediate situations on going from the body water-rich to the almost completely dehydrated state. Actually, it remains unclear what physicochemical effects are exerted by trehalose on biological tissues and organs when the disaccharide is concentrated, for example, to 20 wt % or higher in aqueous media. At the current stage there is no available approach suitable for such investigation to allow in situ measurements of intact tissues just in the course of falling into the anhydrobiosis. In the present work, instead, effects of trehalose have been comparatively studied on the swelling behavior of a typical natural hydrogel, agarose, which has been often used as a model of intact tissues due to its similarity of hydration characteristics in terms of ^{1}H-magnetic relaxation.[6] We found that swelling of the hydrogel was more effectively suppressed in the presence of trehalose as compared with sucrose or maltose, especially when the disaccharide concentration of the aqueous solution was more than 1 M.

α,α-trehalose dihydrate was a kind gift from Hayashibara Biochem. Lab. Inc. (Okayama, Japan). Sucrose, maltose monohydrate, and agarose were purchased from Sigma Chemical Co. The agarose was Type I-A (low electroendosmosis (EEO), A0169), whose number average molecular weight was estimated to be as high as 10^5.[7] An aqueous solution with agarose concentration of 3% on a weight basis was at first prepared by dispersing a necessary amount of agarose in deionized water. The solution was preheated at 105 °C for 10 min using an autoclave, followed by further heat at 110 °C for 30 min to ensure the complete dissolution of the agarose. The obtained hot solution was immediately poured into a capillary tube used as a mold to get a cylindrically shaped gel. The tube was stored for a day in a refrigerator (2–5 °C) to allow the aqueous agarose to undergo a transition to a gel state. The prepared gel was released from the tube and placed into an empty cell (1 cm × 1 cm × 5 cm), followed by mixing with an aqueous disaccharide (trehalose, maltose, or sucrose), whose concentration was changed from 0 to 2 mol/L. It took 24 h to achieve equilibrium swelling at room temperature. The equilibrium diameters of each gel, d and d_o, were measured with a calibrated microscope. We calculated the swelling ratio V/V_o from the value of $(d/d_o)^3$, where V and V_o are the equilibrium swelling volumes of the gel. The subscript of d_o and V_o represents the absence of disaccharides, respectively.

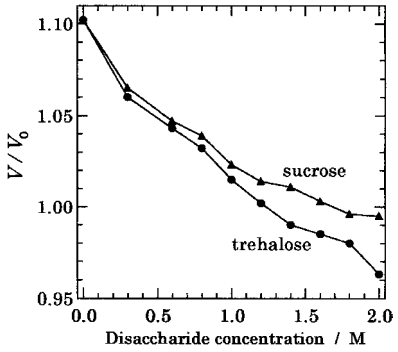

Figure 1. The disaccharide concentration dependence of the swelling ratio of equilibrium volumes, V/V_o.

As shown in Figure 1, the swelling ratio of equilibrium volumes, V/V_o, decreases with increasing the concentration of a given disaccharide. The magnitude of the change in V/V_o is larger in aqueous solutions of trehalose than in aqueous sucrose, and such a tendency becomes remarkable in higher concentration ranges. Maltose exhibited almost the same effects as did sucrose (data not shown). To thermodynamically interpret such gel-swelling behavior, we attempted to apply the well-known theory of gel network systems,[8,9] where the effective polymer–solvent interaction parameter χ is given by[10]

$$\chi = (1/2)(N_c v_1/N_A V_o)\{[f + (1/2)](V_o/V) - (V_o/V)^{1/3}\}/\phi^2 - [\phi + \ln(1-\phi)]/\phi^2 \quad (1)$$

$$2\chi = \Delta F/k_B T = (\Delta H/T - \Delta S)/k_B \quad (2)$$

where N_A, N_c, v_1, f, and ϕ represent Avogadro's number, the total number of segments in the polymer network, the molar volume of the solvent, the number of dissociated counter ions per segment, and the volume fraction of the polymer network in the gel, respectively. Since $V_o/\phi = V/\phi_o$, $\phi = \phi_o/(V/V_o)$, where ϕ_o is the volume fraction of the polymers before its

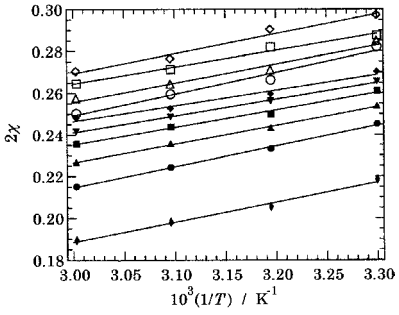

Figure 2. Plot of 2χ versus $1/T$. ◆: 0, ●: 0.3, ▲: 0.6, ■: 0.8, ▼: 1.0, ○: 1.4, and □: 1.8 M.

gel-network development. In eq 2 k_B and T are the Boltzmann constant and absolute temperature, respectively, and ΔF, ΔH, and ΔS represent changes in free energy, enthalpy, and entropy, respectively, when the interaction occurs between polymer and solvent, instead of polymer–polymer and solvent–solvent interactions. In a good solvent the thermal energy k_BT is larger than ΔF, i.e $\chi < 0.5$, where the polymer and networks are swollen.[10] In the current study, to evaluate semiquantitatively the interaction parameter χ, the following approximations were adopted. The molar volume of pure water was used for v_1 of aqueous sugars. Since for agarose gels the volume fraction ϕ_o is nearly equal to its weight fraction,[11] $\phi_o = 0.03$ here. f was taken to be zero since agarose is virtually uncharged macromolecule. Cross-linking points in agarose gels can be described as a kind of junction zones of zippers, each of which is constitutive of parallel links.[12] Suppose that N_z and N_l represent the number of zippers per total mass of the gel and the number of parallel links in a single zipper, respectively, N_c in eq 1 can be provided approximately by the product $N_z \cdot N_l$, which is as high as 10^{20} for hydrogels of 2–4 wt % agarose with molecular weight of ca. 10^5 when 1 M sucrose coexists, although each value of N_z and N_l slightly depends on the concentrations of the added sugar.[13] For simplicity N_c was approximated to be constant, 10^{20}. The calculated values of 2χ became larger with increasing disaccharide concentration at each temperature studied, as exemplified by the trehalose–agarose case (Figure 2), suggesting that the hydrogel had less affinity for water molecules under the disaccharide-rich conditions. In addition the values of 2χ exhibited a linear increase with $1/T$, from which the numerical data of ΔH and ΔS can be estimated (eq. 2). ΔH values are almost independent of the disaccharide concentrations, C_{di}, for all the disaccharides tested here, although somewhat scattering at their higher concentrations. Now we give just the averaged value of ΔH at $C_{di} < 1.4$ M; 1.2×10^{-17}, 1.0×10^{-17}, and 0.9×10^{-17} erg for aqueous trehalose, maltose, and sucrose, respectively. On the other hand, ΔS values depend on C_{di}, decreasing monotonously from as high as 1.0×10^{-17} erg K^{-1} down to -1.6×10^{-17} erg K^{-1} with increasing C_{di}.

The theory of gel networks, eqs 1 and 2, is based upon the mean field approach, which is in general more useful to apply to chemically cross-linked polymers rather than to physically cross-linked ones such as agarose. Thus it may be difficult to expect quantitatively rigorous evaluation of the above thermodynamic parameters in the current case. However it is noteworthy that ΔH values have positive sign for all of three aqueous disaccharides studied here, indicating that the addition of such a disaccharide makes the interaction less attractive between water and the agarose, with keeping the order of trehalose > maltose > sucrose. This relative effect is in parallel with the hydration abilities of these disaccharides, trehalose > maltose > sucrose.[14] On the basis of this, the present result can be rationalized in terms of the so-called preferential hydration of the added disaccharide. That is, many water molecules, although maybe not every, in the hydration shell of agarose are attracted to the disaccharide and thereby make it unfavorable for the hydrogel to swell.

Finally we speculate on effects of trehalose on biological tissues in water-remaining states. The steep decrease in V/V_o with increasing trehalose concentration (Figure 1) suggests that the presence of intermediately concentrated trehalose suppresses the swelling of hydrogels. Such physicochemical effects would contribute to stabilization of biological tissues and organs in temporary states when the body water is gradually replaced by trehalose. This seems to be one of the mechanical backgrounds necessary for successful anhydrobiosis.

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