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Increased Thermal Stability of Proteins in the Presence of Sugars and Polyols†

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ABSTRACT: Sugars and polyols stabilize proteins against heat denaturation. Scanning calorimetry was used to obtain a quantitative estimate of the degree of stabilization. Solutions of ovalbumin, lysozyme, conalbumin, and α-chymotrypsinogen were heated at a constant rate, and the temperature of the maximum rate of denaturation was estimated (T_m). Addition of a sugar or polyol raised T_m. The magnitude of the stabilizing effect (ΔT_m) depended on both the nature of the protein and the nature of the sugar or polyol, ranging from 18.5 °C for lysozyme at pH 3 in the presence of 50% (w/w) sorbitol to 0 °C for conalbumin at pH 7 in 50% glycerol solution. It is argued that this stabilization is due to the effects of sugars and polyols on hydrophobic interactions. The strength of the hydrophobic interaction was measured in model systems in sucrose and glycerol solutions. Sucrose and glycerol strengthened the pairwise hydrophobic interaction between hydrophobic groups; however, they reduced the tendency for *complete* transfer of hydrophobic groups from an aqueous to a nonpolar environment. The extent of stabilization by different sugars and polyols is explained by their different influences on the structure of water. The difference between the partial molar volume of the sugar or polyol and its van der Waals volume was used as a rough quantitative measure of the structure-making or structure-breaking effect. There was a linear relationship between this quantity and ΔT_m.

It has been known for many years that sugars may protect proteins against loss of solubility during drying and may inhibit heat coagulation (Ball et al., 1943). Simpson & Kauzmann (1953) observed that the extent of denaturation of ovalbumin in urea solutions was reduced in the presence of sucrose. More recently, Gerlsma & Stuur (1972) showed that polyhydric alcohols raised the thermal transition temperatures of lysozyme and ribonuclease and Donovan (1977) observed the stabilizing effect of sucrose on the proteins of egg white.

The technique of scanning calorimetry has been increasingly applied over the last 10 years in studies of thermal transitions in proteins [reviewed by Privalov (1947)] and in investigations of stability changes of proteins caused by specific interaction with metal ions (Donovan & Ross, 1975), by complex formation (Donovan & Ross, 1973; Donovan & Beardslee, 1975), and by change in conformation (Donovan & Mapes, 1976). We have used scanning calorimetry in this investigation to measure the increase in denaturation temperature of several proteins in the presence of various sugars and polyols—an effect apparently related to changes in solvent properties and of opposite sign to the destabilization caused by the addition of monohydric alcohols or urea to protein solutions.

Lakshmi & Nandi (1976) showed that sucrose and glucose decrease the solubility of phenylalanine, tyrosine, and tryptophan in aqueous solution and suggested that this was owing to increased hydrophobic interaction. A similar stabilization of hydrophobic interactions was postulated for proteins in sugar solutions. We have, therefore, also investigated the effects of sucrose and glycerol on hydrophobic interactions in two model systems—ion pair formation by long-chain alkyltrimethylammonium carboxylates (Oakenfull & Fenwick, 1977) and the critical micelle concentrations of alkyltrimethylammonium bromides (Emerson & Holtzer, 1967). These model systems distinguish between two types of hydrophobic interaction which are influenced in different ways by added solutes (Oakenfull & Fenwick, 1979)—pairwise interaction in which hydrophobic groups associate but otherwise remain surrounded by water and complete transfer of a hydrophobic group from an aqueous to a nonpolar environment.

Our results, presented here, indicate a general phenomenon which might be used to throw more light on the relation between solvent structure and the stability of proteins and suggest that reactivity and turnover of proteins in vivo may often be

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regulated by the presence of apparently inert polyhydroxy compounds.

Materials and Methods

Proteins. Ovalbumin was prepared by the method of Sørensen & Høyrup (1915–1917) from eggs less than 48 h old and recrystallized twice from half-saturated ammonium sulfate at pH 4.7. Lysozyme was crystallized directly from egg white containing 5% NaCl (Alderton & Fevold, 1946) and recrystallized twice from 5% NaCl at pH 4.5. Iron-free conalbumin was obtained from Sigma Chemical Co. and α-chymotrysinogen (bovine pancreas, A grade) from Calbiochem.

Other Materials. Sucrose, glucose, glycerol, and ethylene glycol were analytical reagent grade; xylitol (food grade) was a gift from Roche Products; Dextran 10 and Ficoll are Pharmacia products; and other sugars and polyols were from Calbiochem and BDH Biochemicals.

Scanning Calorimetry. The temperature (T_m) of the maximum rate of denaturation (peak temperature) was measured in the adiabatic scanning calorimeter described by Smith & Rose (1975), subsequently modified to accept sample cells made from 7-mm glass tubing of 0.5-mm wall thickness. Stock protein solutions at a concentration of 50 or 20 g/L were made up in 0.05 ionic strength phosphate (pH 7.0) or glycine hydrochloride (pH 3.0) buffers, the pH was adjusted if necessary, and the sugar or polyol was added by weight. From 0.5 to 1.0 g of the mixture was weighed into the sample tube, and an equal weight of the mixture without the protein was placed in the reference tube. Both tubes were flame-sealed at a final length of 80 mm. The scanning rate was 30 °C/h, and the cell temperature and the differential heating rate required to keep the sample and reference at the same temperature were recorded.

T_m could be estimated to ±0.25 °C and was found to be independent of protein concentration over the range studied. Some estimates were made of the heat of denaturation (ΔH) by measuring the area between the transition peak and a base line fitted by inspection. This was not independent of concentration (ΔH for ovalbumin at pH 7 changed from 18.5 to 26.0 J/g as the concentration was varied from 40 to 10 g/L), and in the presence of 50% w/w sugar or polyol base line curvature was great enough to preclude estimates of ΔH. When reducing sugars, such as glucose, were used, the "browning" reaction with protein amino groups resulted in an exothermic heat change with a corresponding shift in the base line. However, this reaction occurred above the T_m (possibly being governed by the exposure of amino groups on denaturation) and did not affect the precision of measuring T_m.

Ion Pair Formation by Alkyltrimethylammonium Carboxylates. Conductance measurements were made with a series of long-chain decyltrimethylammonium carboxylates in aqueous solutions of sucrose and glycerol. The details of the method have been given in a previous paper (Oakenfull & Fenwick, 1974).

Critical Micelle Concentration. The critical micelle concentrations (cmc) of a series of alkyltrimethylammonium bromides were measured by following the fluorescence intensity of *N*-phenylnaphthylamine with change in detergent concentration (Fisher & Oakenfull, 1979). Dye solubilization methods may give misleading results, but the fluorescence method uses a very low concentration of dye (10^{-5} mol/L), and the results obtained in water were in excellent agreement with literature data (see Table V).

Partial Molar Volumes. Partial molar volumes of those sugars and polyols for which information was not available from the literature were obtained from the densities of solutions of accurately known concentrations. Solutions were prepared usually within the range 0.2–1.0 mol/L, with concentration precise to $\pm 10^{-4}$ mol/L. Densities were measured to a precision of $\pm 4 \times 10^{-6}$ g/mL with an Anton Paar precision density meter (DMA 02C) standardized against air and water. The temperature was maintained at 25 ± 0.005 °C. Apparent molar volumes (ϕ_v) were calculated from the expression

$$\phi_v = \frac{M_r}{d_0} = \frac{1000(d - d_0)}{cd_0} \quad (1)$$

where d and d_0 are the densities of the solution and solvent, M_r is the molecular weight, and c is the concentration (mol/L) of solute. Values of ϕ_v were linear with concentration, and the partial molar volume ($\bar{V}_2$) was obtained by using a least-squares procedure to extrapolate ϕ_v to zero concentration.

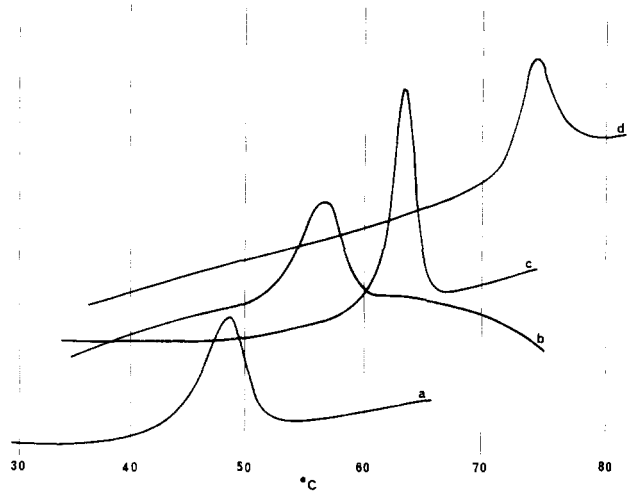

FIGURE 1: Representative curves (thermograms) of differential heating rate vs. temperature. Curves a and b are α-chymotrypsinogen in glycine buffer at pH 3 with no sugar and with 50% w/w sucrose, respectively. Curves c and d are conalbumin in phosphate buffer at pH 7 with no sugar and with 50% w/w glucose, respectively.

Table I: Stabilization of Different Proteins[a]

protein	pH	T_m (°C)	ΔT_m (°C) sucrose	glucose	sorbitol	glycerol
ovalbumin	7.0	77.0	11.0	15.5	14.0	1.5
lysozyme	3.0	66.5	15.0	17.0	18.5	8.5
	7.0	71.0	14.5	15.0	17.0	8.5
conalbumin	7.0	63.5	9.0	11.0	11.0	0
α-chymotrypsinogen	3.0	48.5	8.0	9.0	11.0	3.0

[a] Temperature of peak maximum (T_m) with no added sugar or polyol and temperature increase (ΔT_m) in the presence of 50% w/w sugar or polyol.

Results

Scanning Calorimetry. Some typical curves of differential heating rate vs. temperature are shown in Figure 1. The effects of two sugars (sucrose and glucose) and two polyols (sorbitol and glycerol) at a concentration of 50% w/w on the denaturation temperature, T_m, of ovalbumin (pH 7), lysozyme (pH 3 and 7), conalbumin (pH 7), and α-chymotrypsinogen (pH 3) are shown in Table I. The magnitude of the stabilizing effect (ΔT_m) varies with the protein and with the sugar or polyol. Lysozyme (at both pH values) is stabilized to a greater extent than ovalbumin, and with all four stabilizing agents the least effect is observed with conalbumin and α-chymotrypsinogen. The relative stabilizing effect is much the same for each protein—glucose and sorbitol are about equally effective,

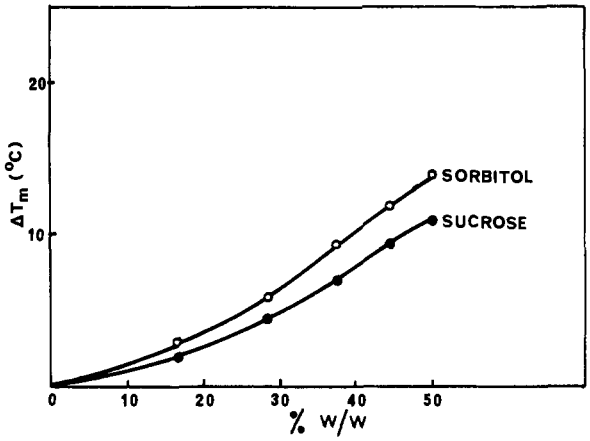

FIGURE 2: Effects of sucrose and sorbitol concentration on the change in denaturation temperature (ΔT_m) for ovalbumin in phosphate buffer at pH 7.

Table II: Stabilization of Ovalbumin by Different Sugars and Polyols[a]

stabilizer	ΔT_m[b] (°C) 28%	50%
ribose	1.5	
arabinose	4.5	
glucose	7.0	15.5
galactose	7.0	
fructose		12.0
mannose		12.5
rhamnose, H_2O	0.5	
sucrose	4.0	11.0
maltose		10.0
raffinose	4.0	
melezitose, H_2O	4.0	
Dextran 10		6.5
Ficoll	0	
glycerol		1.5
erythritol		9.0
ribitol		11.5
xylitol		13.0
sorbitol	5.5	14.0
mannitol	6.5	
ethylene glycol[c]	−9.0	

[a] Ovalbumin at pH 7.0. [b] Temperature increase in the presence of 28% w/w or 50% w/w of stabilizer. [c] Ethylene glycol lowers T_m.

sucrose is slightly less so, and the least stabilization is observed with glycerol. The effects of the concentration of sucrose and sorbitol on ΔT_m for ovalbumin at pH 7 are shown in Figure 2.

The relative stabilizing effects of a more extensive series of sugars and polyols were investigated by using ovalbumin at pH 7. These results are given in Table II. Table II contains values of ΔT_m for 50% solutions (w/w) of eleven sugars or polyols with values ranging from 15.5 °C for glucose to 1.5 °C for glycerol. Table II also contains results for a further eight sugars or polyols which are less soluble. In this series, which also includes ethylene glycol, the concentration of sugar or polyol was 28% (w/w). Again, glucose was the most effective stabilizer and the least effective was Ficoll, a polymeric material prepared from sucrose. Ethylene glycol had a destabilizing effect.

Effect of Sucrose and Glycerol on the Pairwise Hydrophobic Interaction. Figure 3 shows the results of a typical set of conductance measurements for a series of long-chain decyltrimethylammonium carboxylates. The equivalent conductance was always less than that predicted by the Onsager equation (the broken line in Figure 3), indicating the formation

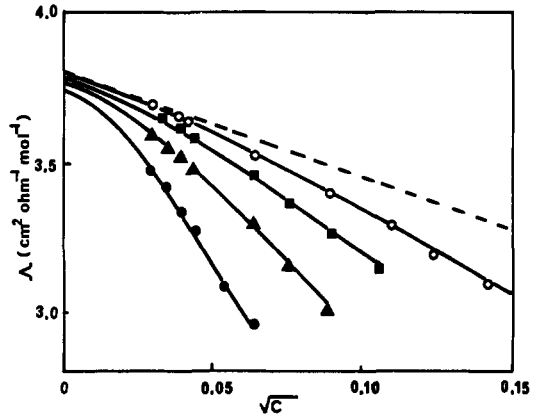

FIGURE 3: Equivalent conductance (Λ) of decyltrimethylammonium carboxylate solutions in 2 M sucrose at 25 °C plotted against $c^{1/2}$, where c is the molar concentration: (●) hexanoate, (▲) heptanoate, (■) octanoate, (○) nonanoate. The broken line indicates the Onsager slope in the absence of ion pair formation.

Table III: Free Energy of Ion Pair Formation in Sucrose and Glycerol Solutions[a]

soln	ΔG_{HI}° (kJ/mol)[b]	ΔG_{elec}° (kJ/mol)[c]
water	−1.53 ± 0.09	
1 M sucrose	−1.88 ± 0.17	6.2 ± 0.9
2 M sucrose	−2.34 ± 0.07	8.6 ± 0.5
3 M glycerol	−2.02 ± 0.07	5.7 ± 0.6
6 M glycerol	−2.63 ± 0.29	12.2 ± 2.2

[a] From conductance measurements of association constants of decyltrimethylammonium carboxylates at 25 °C. [b] Hydrophobic contribution. [c] Electrostatic contribution.

of ion pairs (Robinson & Stokes, 1955). Ion pair association constants (K_a) were calculated from these data by the method of Davies (1933, 1962). The standard free energy of ion pair formation (ΔG_{ip}°) was then given by

$$\Delta G_{ip}^\circ = -RT \ln K_a \quad (2)$$

Since the electrostatic interaction between the charged groups (ΔG_{elec}°) is effectively independent of the lengths of the alkyl chains, then for a series of measurements such as that shown in Figure 3 the hydrophobic contribution to ΔG_{ip}° is given by

$$\Delta G_{ip}^\circ = \Delta G_{elec}^\circ + n\Delta G_{HI}^\circ \quad (3)$$

where ΔG_{HI}° is the free energy of hydrophobic interaction per pair of interacting CH_2 groups and n is the number of interacting pairs of CH_2 groups (i.e., the hydrocarbon chain length of the carboxylate ion which differs for each member of the series). Values of ΔG_{HI}° and ΔG_{elec}° (with their standard errors) can therefore be estimated by a least-squares regression analysis of values of ΔG_{ip}° from conductance data.

The results obtained in sucrose and glycerol solutions are summarized in Table III. Sucrose and glycerol strengthen hydrophobic interaction (ΔG_{HI}° is more negative) at concentrations at which these substances stabilized proteins to heat denaturation. Sucrose (1 and 2 mol/L) has a slightly less effect though on ΔG_{HI}° than glycerol (3 and 6 mol/L) whereas the effect of glycerol on the temperature of the maximum rate of denaturation (T_m) is less than that of sucrose for all the proteins studied. This possibly reflects the very different temperatures at which the two sets of measurments were made. T_m was measured between 48 and 77 °C whereas ΔG_{HI}° was measured at 25 °C. Ideally, ΔG_{HI}° should have been measured at a temperature within the range of T_m. This was prevented by the lack at higher temperatures of reliable values

Table IV: Critical Micelle Concentrations in Sucrose and Glycerol Solutions[a]

soln	cmc (mM)[b] C_{10}	C_{12}	C_{16}	ΔG_{hg}° (kJ/mol)[c]	ΔG_{trans}° (kJ/mol)[d]
water	12 ± 1	1.46 ± 0.08	0.040 ± 0.005	−2.34	2.25
water (lit.)	16[e]	1.46[f]			
1 M sucrose	10 ± 1	1.8 ± 0.1	0.070 ± 0.005	−2.23	1.71
2 M sucrose	7 ± 1	1.1 ± 0.2	0.030 ± 0.005	−2.25	1.65
6 M glycerol	22 ± 1	4.4 ± 0.1	0.18 ± 0.01	−1.98	1.38

[a] For alkyltrimethylammonium bromides at 25 °C and ionic strength 0.5 M (sodium bromide). [b] Critical micelle concentrations for three chain lengths. [c] Head group contribution. [d] Contribution from transfer from aqueous to nonpolar environment. [e] From Geer et al. (1971). [f] From Emerson & Holtzer (1967).

of the physical constants needed to calculate ion pair association constants (Robinson & Stokes, 1955). The effect of temperature on ΔG_{HI}° in 0.1 mol fraction ethanol–water was measured by Oakenfull & Fenwick (1977), and it was shown that hydrophobic interaction became stronger with increasing temperature in the range 5–55 °C. However, although this general effect may hold here, it is likely that each solvent mixture has a different temperature coefficient.

Effects of Sucrose and Glycerol on the Complete Transfer of Hydrophobic Groups from an Aqueous to a Nonpolar Environment. The free energy change when one more detergent ion is added to a micelle of most probable size is given by (Emerson & Holtzer, 1965; Fisher & Oakenfull, 1977)

$$\Delta G_m^{\circ} = RT \ln \text{cmc} \quad (4)$$

As in the case of ion pair formation by double long-chain electrolytes, described in the previous section, ΔG_m° can be split into its electrostatic and hydrophobic components

$$\Delta G_m^{\circ} = \Delta G_{hg}^{\circ} + n\Delta G_{trans}^{\circ} \quad (5)$$

where ΔG_{hg}° is the free energy of the interaction of the charged head group of the extra detergent ion with the charged groups that already form the outside layer of the micelle, n is the number of CH_2 groups of the alkyl chain of the detergent ion, and ΔG_{tran}° is the free energy of transfer of a CH_2 group from the aqueous to the micellar environment. Thus, if cmc values are measured for a homologous series of detergents, ΔG_{hg}° and ΔG_{trans}° can be calculated. Values of the cmc of decyl–, dodecyl–, and hexadecyltrimethylammonium bromides in sucrose and glycerol solutions are given in Table IV. All measurements were made at 25 °C at an ionic strength of 0.5 mol/L (adding appropriate concentrations of sodium bromide). A least-squares regression analysis of the data was used to estimate values of ΔG_{hg}° and ΔG_{trans}° which are also given in Table IV. These results show that both sucrose and glycerol reduce the driving force for transfer of a hydrophobic group from an aqueous to a nonpolar environment (ΔG_{trans}° becomes *less*).

van der Waals and Partial Molar Volumes of Sugars and Polyols. The van der Waals and partial molar volumes of the sugars and polyols are given in Table V. The van der Waals volumes were calculated from the group contributions given by Bondi (1964). The partial molar volumes were either obtained directly from the literature, calculated from published tables of densities of sugar solutions (Norrish, 1967), or, when published data were not available, calculated from experimentally determined densities of aqueous sugar or polyol solutions (see Materials and Methods).

Table V: van der Waals Volumes and Partial Molar Volumes of Sugars and Polyols[a]

	$\bar{V}_2$ (mL/mol)	V_w (mL/mol)[b]	$100(\bar{V}_2 - V_w)/V_w$
ribose	95.3[c]	74.7	27.6
arabinose	93.0 ± 0.5	74.7	24.5
glucose	111.9[c]	89.5	25.0
galactose	110.7[c]	89.5	23.6
fructose	110.8 ± 0.7	89.5	23.8
rhamnose	113.6 ± 0.8	84.9	33.8
sucrose	211.5[d]	168.2	25.8
Ficoll[e]	0.679 ± 0.007	0.508	33.6
glycerol	73.5[f]	51.4	43.1
erythritol	86.7 ± 0.4	66.2	31.0
ribitol	100.6 ± 0.9	81.0	24.2
xylitol	100.1 ± 0.2	81.0	23.6
sorbitol	118.2 ± 0.3[g]	95.8	23.4
mannitol	118.6 ± 0.1[h]	95.8	23.8
ethylene glycol	54.7 ± 0.1	36.5	49.6

[a] In water at 25 °C. [b] Calculated from the group contributions given by Bondi (1964). [c] From Franks et al. (1972). [d] From Garrod & Herrington (1970). [e] Calculated as specific volume (mL/g) since the molecular weight is indeterminate. [f] From Herskovitz & Kelly (1973). [g] Edward et al. (1977) reported 118 ± 0.3 mL/mol. [h] Edward et al. (1977) reported 118.8 ± 0.5 mL/mol.

Discussion

Factors Which Influence the Stability of Protein Structures. Proteins are stabilized generally by a combination of hydrogen bonding, electrostatic interactions, and hydrophobic interactions, with additional contributions in particular proteins from cross-linking, metal complexing, and specific binding of ions and cofactors. In discussing the effects of sugars and polyols on the thermal stability of proteins, we have to consider the effects of sugars and polyols on these various forces and interactions.

(*1*) *Hydrogen Bonding.* The endothermic transitions observed in the scanning calorimeter are indicative of the "melting" of a cooperative hydrogen-bonded structure, in which water successfully competes as both donor and acceptor with backbone and side-chain groups in the protein. The individual hydroxyl groups of sugars and polyols may also compete, but no more effectively than water, as indicated by the minor stabilizing effect of glycerol compared with sorbitol at the same concentration of hydroxyl groups. It is also unlikely that new cooperative structures are formed since transition heats appear to remain the same as in the absence of stabilizer. Specific cooperative bonding with groups on the outside of the protein molecule, displacing water, has been suggested by Giles & McKay (1962). They used a refractometric method to observe complex formation between gelatin or bovine plasma albumin and sugars and polyols. All the pentoses examined, some hexoses, *meso*-inositol, and mannitol showed evidence of complex formation, but the disaccharides did not. The effect appears to be more specific than the general increase in T_m shown in Tables I and II.

(*2*) *Electrostatic Interactions.* Those sugar or polyol solutions for which data are available have lower dielectric constants than pure water (Akerlof, 1932). Thus, electrostatic interactions should be stronger in these solutions than in water.

However, this contribution to the stabilizing effect must be relatively small. The fact that the stabilizing effects of sucrose or glycerol on lysozyme are almost the same at pH 7 as at pH 3 (see Table I) suggests that any electrostatic contribution to the stabilizing effect must be minimal.

(*3*) *Hydrophobic Interactions.* Hydrophobic interactions are generally considered to be the major single factor in stabilizing the three-dimensional structure of proteins (Fersht, 1977). Oakenfull & Fenwick (1979) have shown that in aqueous–organic mixed solvents hydrophobic interaction depends on solvent structure, with maximum hydrophobic interaction occurring in those solvent mixtures in which the three-dimensional hydrogen-bonded structure of water was most developed. The effects of sugars and polyols on hydrophobic interaction and consequently on the thermal stability of proteins should also depend upon how they affect the structure of water. We have shown that hydrophobic interactions between pairs of hydrophobic groups are stronger in sucrose or glycerol solutions than in pure water. It seems likely therefore that this is the mechanism by which sugars and polyols in general may stabilize proteins to heat denaturation. The next question then is to what extent do the other sugars and polyols strengthen hydrophobic interaction?

(*4*) *Effects of Sugars and Polyols on the Structure of Water.* Evidence derived from both spectroscopy and thermodynamics shows that sugars and polyols interact with water to an extent which depends upon their molecular structure (Franks et al., 1972; Tait et al., 1972). Glucose behaves differently from sucrose, for example, and mannitol behaves differently from sorbitol (Stern & O'Connor, 1972). Tait et al. (1972) have proposed a "specific hydration model" to explain these effects. Sugar molecules induce structure in the water molecules, surrounding them if the orientation of OH groups is such that some of the O–O spacings correspond with the O–O distance of 4.86 Å of the water lattice.

For this reason, in comparing the effects of different sugars and polyols on T_m, we used equal concentrations on a weight basis rather than a molar basis. The effect we are investigating seems not to be a colligative property of the sugar or polyol but a property related to the OH groups. It is in any case clearly inappropriate to compare equal molar concentrations of, for example, glucose and high molecular weight polymeric materials such as Ficoll or Dextran 10.

There is no completely unambiguous numerical measure of "structure making" or "structure breaking" by a solute, but since a change in the extent to which water is structured is accompanied by a volume change (Franks & Reid, 1973), the partial molar volume of the solute provides a *rough* measure of the effect of that solute on the structure of water. The partial molar volume is the intrinsic volume of the solute molecule plus a contribution from whatever volume change results from the interaction of the solute with the surrounding solvent. Thus, a measure of the structure-making or structure-breaking effect of a solute is given by the quantity

$$\frac{(\bar{V}_2 - V_w)}{V_w} \qquad (6)$$

where V_w is the van der Waals volume of the solute. Since this quantity is dimensionless, it can be compared for different solutes. Values of $\bar{V}_2$, V_w, and $(\bar{V}_2 - V_w)/V_w$ for the sugars and polyols used in this work are given in Table V.

There is an inverse relationship between $(\bar{V}_2 - V_w)/V_w$ and the structure-making effect of the sugar or polyol (Oakenfull & Fenwick, 1979). Thus, there should be a corresponding relationship between $(\bar{V}_2 - V_w)/V_w$ and the extent to which

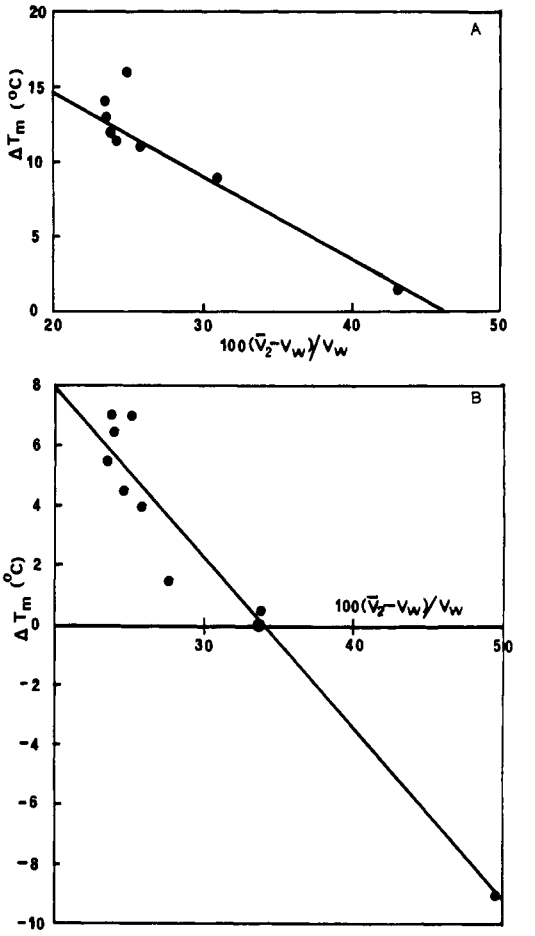

FIGURE 4: Stabilization of ovalbumin at pH 7 (ΔT_m) plotted against $100(\bar{V}_2 - V_w)/V_w$ (eq 6) for the sugar or polyol. (A) 50% sugar or polyol. (B) 28% sugar or polyol.

the sugar or polyol strengthens hydrophobic interactions. Figure 4 shows plots of $(\bar{V}_2 - V_w)/V_w$ against ΔT_m measured in 50% solutions of sugar or polyol and 28% solutions of less soluble sugars and polyols. Both plots contain data points for glucose, sucrose, and sorbitol, but otherwise they represent two different and independent series of sugars and polyols. In each case there is a roughly linear relationship between ΔT_m and $(\bar{V}_2 - V_w)/V_w$ (correlation coefficients of 0.9840 and 0.9623, respectively) with the exception that ΔT_m for glucose is ~30% greater than that predicted by the regression line. There is no obvious reason for glucose to behave differently from the other sugars and polyols. However, it should be recognized that we are comparing volume changes in solution at 25 °C with stabilizing effects at temperatures above 75 °C, where mutarotation changes for glucose (and galactose, arabinose, and ribose) could alter the proportions, and hence the partial molar volumes, of the mixtures of anomers. It is significant that the slopes of the two lines of best fit are the same, within the limits of error (−0.56 ± 0.05 and −0.57 ± 0.07, respectively) even though the two plots represent predominantly different series of sugars and polyols.

These results strongly support the hypothesis that the dominant mechanism by which sugars and polyols stabilize proteins to heat denaturation is through their effect on the structure of water, which, in turn, determines the strength of hydrophobic interactions. An explanation of the differences in stabilizing effect between isomeric sugars and polyols will obviously require a more rigorous approach than the one we have used, while the differences in response between proteins are most likely related to the fine detail of protein structure

involving contacts between nonpolar side chains (Privalov & Khechinashvili, 1974).

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