THERMODYNAMICS OF CARBOHYDRATE-LIPID INTERACTIONS

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ABSTRACT Thermodynamic analyses of carbohydrate-lipid interactions were performed by investigating the effects of a series of carbohydrates, including monosaccharides, disaccharides, and trisaccharides, on the phase-transition properties of aqueous dispersions of 1,2-dipalmitoyl phosphatidylcholine (DPPC). The temperature of the lipid's main phase transition from the gel to liquid-crystalline phase is essentially unchanged in the presence of carbohydrate. The change in the free energy (ΔG) of the transition is zero when a carbohydrate is added to aqueous dispersions of DPPC, while the enthalpy (ΔH) and the entropy of the melting of DPPC are decreased. The thermodynamic information was used to examine carbohydrate-lipid interactions. Such interactions were elucidated according to our knowledge of the specific properties of carbohydrates in aqueous solutions and the previously proposed hydrophobic interaction involving hydrocarbon tails of the lipid in aqueous dispersions.

INTRODUCTION

In our previous studies (Chen, 1980; Chen and Berns, 1978; Chen and Berns, 1977), quaternary ammonium salts (including tetraalkylammonium and azoniaspiroalkane salts) and a variety of alcohols having known free-energy and enthalpy pairwise-interaction coefficients (Okamoto et al., 1978; Savage and Wood, 1976) were used to examine the theromdynamics of solute-solute hydrophobic interaction. A change in hydrophobicity due to the changes in alkyl-chain length and in the molecular shape of these hydrophobic species is responsible for a change in their hydrophobic interaction with protein. Evaluation of thermodynamic parameters for the interactions of protein with cyclohexanol and quaternary ammonium salts revealed that characteristics of solute-solute hydrophobic interaction are that the enthalpy (ΔH) is positive, the free energy (ΔG) is negative, and ΔH and ΔG are opposite in sign (Chen, 1980).

The interactions between the above hydrophobic species and aqueous dispersions of lipid were also investigated (Chen, 1981). The order of the abilities of these salts and alcohols to lower the phase-transition temperature and to affect the ΔH and the entropy (ΔS) of the melting of aqueous dispersions of 1,2-dipalmitoyl phsophatidylcholine (DPPC) from the gel to liquid-crystalline phase was found to parallel their order for hydrophobic interaction with protein. Current knowledge of hydrophobic interaction could aid in the interpretation of the physicochemical nature of the phase-transition process of aqueous dispersions of DPPC (Chen, 1981).

Sucrose and glucose decrease the solubility of aromatic amino acids and peptides in aqueous solution (Lakshmi and Nandi, 1976). This decrease has been interpreted as a result

of an increase in hydrophobic interaction between nonpolar groups of amino acids and peptides in sugar solutions. Sugars and polyols also stabilize proteins against thermal and urea denaturations (Back et al., 1979; Gersma and Stuer, 1972; Simpson and Kauzmann, 1953). The magnitude of the stabilizing effect depends on the nature of the sugar or polyol and of the protein. Such stabilization is believed to be due to the effects of sugars and polyols on hydrophobic interactions within proteins (Back et al., 1979). Carbohydrates are hydrophilic species. Their properties in aqueous solutions have been explained in terms of stepwise solution equilibrium (Stokes and Robinson, 1966), pairwise solute-solute interaction (Kozak et al., 1968), and hydration sites and relative conformations (Franks et al., 1972; Tait et al., 1972).

Few theromdynamic analyses of carbohydrate-lipid interactions have been published. Such analyses could provide information on the influence of carbohydrates on the ordering of the hydrocarbon chains in lipid, and could thus provide an insight into the nature of the interaction forces involved in the melting of these hydrocarbon chains. In an extension of previous studies (Chen, 1981), we used differential scanning microcalorimetry to elucidate the interactions of carbohydrates with lipid. The effects of a series of sugars, including monosaccharides, disaccharides, and trisaccharides, on the phase-transition properties of aqueous dispersions of DPPC were investigated. The information on the observed carbohydrate-lipid interactions, considered in connection with the specific properties of carbohydrates in aqueous solution, will help to elucidate the previous proposal that knowledge of hydrophobic interaction could help in the interpretation of the physicochemical nature of the phase transition property of aqueous dispersions of DPPC (Chen, 1981).

METHODS

Chemicals

Arabinose, sucrose, and raffinose were obtained from Fisher Scientific Co., Pittsburgh, Pa.; lactose from J. T. Baker Chemical Co., Phillipsburg, N.J.; glucose from Mallinkrodt Inc., St. Louis, Mo.; fructose, galactose, mannose, rhamnose, and ribose from Sigma Chemical Co., St. Louis, Mo.; and xylose from Pfanstiehl Laboratories Inc, Waukegan, Ill. These reagent-grade carbohydrates were used without further purification. Pure synthetic DPPC was used as purchased from Koch-Light Laboratories, Ltd., Colnbrook, Buckhinghamshire, England.

Lipid Aqueous Dispersions

Aqueous dispersions of the lipid-carbohydrate mixture were prepared as follows: 5.0 mg of DPPC was added to a small trap containing 1.80 ml of the appropriate concentration of carbohydrate in double-distilled water. Nitrogen was passed through and allowed to remain in the trap before sonication. The DPPC was dispersed by sonicating the lipid suspension at ~45°C for 90 min in a 50-W Cole-Parmer ultrasonicator (Cole-Parmer Instrument Co., Chicago, Ill.). To ensure that the DPPC dispersions were in the gel state, they were refrigerated for 2 h and then kept at 5°C overnight.

Microcalorimetry

A highly sensitive differential scanning microcalorimeter was constructed according to Ross and Goldberg (1974) with some minor modifications. Its instrumentation has been described previously (Chen, 1981). It was designed to measure heat capacities and heat effects in dilute solution with a sensitivity of ~48 μV/mW. The absolute temperature determination was accurate within ±0.05°C.

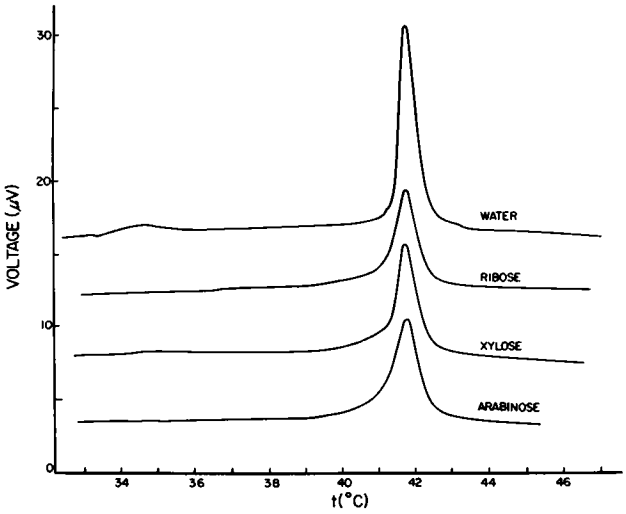

FIGURE 1 Effects of 0.20 M pentose monosaccharide on the phase transition of aqueous dispersions of DPPC.

Measurements

The sample and reference cells were removed from the 5°C cold room, placed in the cooled (<0°C) calorimeter, and allowed to stand for ~1 h for thermal equilibrium. The calorimeter was then heated to >50°C at a rate of 0.15°C/min. This low heating rate was necessary to achieve great accuracy and reproducibility. The area under the endothermic transition peak was measured with a Science Accessories Corp. (Southport, Conn.) sonic digitizer and converted into thermal units. The precision of the heat of transition measurements was ~3%.

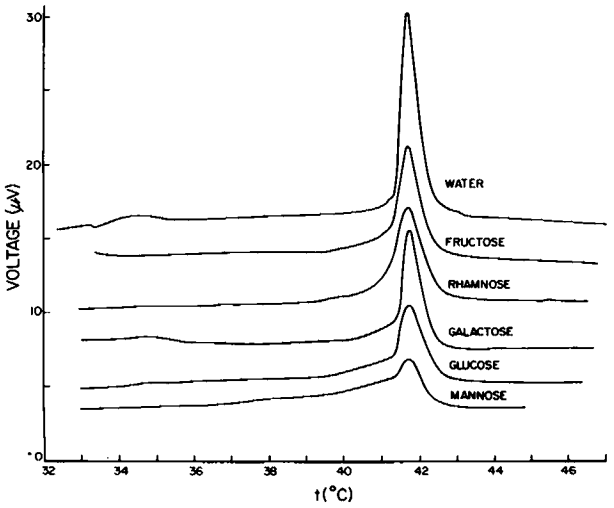

FIGURE 2 Effects of 0.20 M hexose monosaccharide on the phase transition of aqueous dispersions of DPPC.

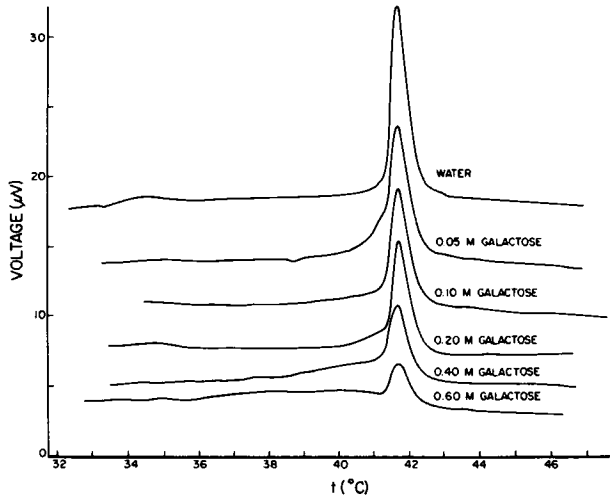

FIGURE 3 Effects of 0.20 M disaccharides and trisaccharide on the phase transition of aqueous dispersions of DPPC.

RESULTS

The effects of monosaccharides, disaccharides, and trisaccharides on the phase transition of aqueous dispersions of DPPC are shown in Figs. 1–3 respectively. In the presence of carbohydrate the pretransition peak at 34.2°C is greatly reduced or disappears. The lipid's main phase transition at a temperature (t) of 41.7°C is essentially unchanged, but the area under the endothermic peak of the main transition is significantly decreased. In most cases the main transition peak is no longer symmetric and has a longer tail on the lower temperature side.

The values of t, ΔH, and ΔS for the melting of DPPC from the gel to liquid-crystalline phase in the presence of carbohydrate are listed in Table I. Typical measurements on the dependence of the phase-transition properties of DPPC on carbohydrate concentration (C) are illustrated in Fig. 4, where the carbohydrate is galactose. For C = 0.05, 0.10, 0.20, 0.40, and 0.60 M, ΔH is 7.22, 6.13, 4.87, 4.71, and 4.31 kcal/mol, and ΔS is 22.9, 19.5, 15.5, 15.0, and 13.7 entropy units, respectively. The value of t, however, is basically independent of the galactose concentration and remains essentially at 41.7°C. The degree of perturbation of the lipid thermogram by galactose is increased as the concentration of galactose increases from 0.05 to 0.60 M.

Aqueous dispersions of DPPC have a well defined enthalpy and entropy of the main phase transition. During this transition an equilibrium equation can be written as follows: gel phase $\rightleftharpoons$ liquid-crystalline phase. The free energy change (ΔG) of the transition is defined as

$$\Delta G = G_{lp} - G_{gel}, \quad (1)$$

where G_{lp} and G_{gel} are the Gibbs free energies of aqueous dispersions of DPPC in liquid-crystalline and gel phases, respectively.

When a carbohydrate is added to the solution, a new phase-transition temperature may be

TABLE I

ΔH AND ΔS OF AQUEOUS DISPERSIONS OF DPPC IN 0.20 M CARBOHYDRATE SOLUTIONS*

Carbohydrate	t	ΔH	ΔS	$-\{AA\}_g$‡	$-A_{2\,min}$§
	(°C)	(Kcal/mol)	(e.u.)	($j \cdot kg \cdot mol^{-2}$)	($cm^3\,mol^{-1}$)
None	41.7 ± 0.1	7.22 ± 0.17	22.9 ± 0.5		
Monosaccharide (pentose)					
Arabinose	41.6 ± 0.1	6.61 ± 0.40	21.0 ± 1.2		
Ribose	41.6 ± 0.1	5.57 ± 0.36	17.7 ± 1.1		
Xylose	41.7 ± 0.1	5.86 ± 0.37	18.6 ± 1.1		
Monosaccharide (hexose)					
Glucose	41.8 ± 0.1	5.07 ± 0.32	16.1 ± 1.0	62	
Fructose	41.7 ± 0.1	6.19 ± 0.32	19.7 ± 1.0		200
Galactose	41.8 ± 0.1	4.87 ± 0.06	15.5 ± 0.2		
Mannose	41.7 ± 0.1	4.93 ± 0.39	15.7 ± 1.2		
Rhamnose	41.6 ± 0.1	5.66 ± 0.23	18.0 ± 0.7		
Disaccharide					
Sucrose	41.8 ± 0.1	4.46 ± 0.02	14.2 ± 0.1	174	558
Lactose	41.8 ± 0.1	6.77 ± 0.56	21.5 ± 1.8		
Trisaccharide					
Raffinose	41.8 ± 0.1	5.05 ± 0.19	16.0 ± 0.6	330	950

*Each datum is an average of two–four measurements.

‡$\{AA\}_g$ is the coefficient of pairwise free energy interaction of A with A (Okamoto et al., 1978).

§A_{2min} is the coefficient of minimum pair interaction (Simpson and Kauzamann, 1953).

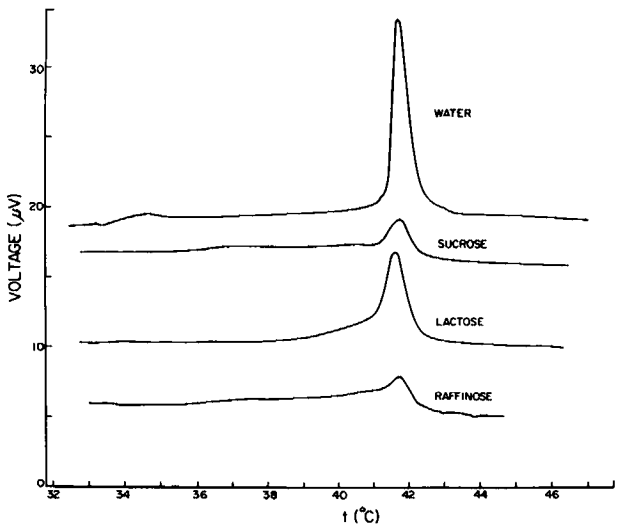

FIGURE 4 Typical measurements showing the dependence of the phase transition of aqueous dispersions of DPPC on carbohydrate (galactose) concentrations.

created, but the ΔG of transition is still zero. Therefore,

$$\left(\frac{\partial \Delta G}{\partial T}\right)_C dT + \left(\frac{\partial \Delta G}{\partial C}\right)_T dC = 0, \tag{2}$$

where C is the concentration of the added carbohydrate and T is the temperature in degrees Kelvin. Since

$$\left(\frac{\partial \Delta G}{\partial T}\right)_C = -\Delta S = -(S_{lp} - S_{gel}), \tag{3}$$

rearrangement of Eq. 2 gives

$$T - T_0 = \frac{\left(\frac{\partial \Delta G}{\partial C}\right)_T dC}{\Delta S}, \tag{4}$$

where T and T_0 are the main phase-transition temperature (°K) in the presence and absence of carbohydrate. Values of ΔS are always positive, since the transition is an endothermic process. The presence of carbohydrate does not affect the lipid's phase transition temperature, i.e.,

$$T - T_0 = 0. \tag{5}$$

Combining Eqs. 4 and 5 gives

$$\left(\frac{\partial \Delta G}{\partial C}\right)_T = 0. \tag{6}$$

This means that the change in ΔG of the transition is zero when a carbohydrate is added.

In the presence of a carbohydrate (Table I) the enthalpy and entropy of the melting are decreased; i.e., $\Delta H - \Delta H_0 < 0$, and $\Delta S - \Delta S_0 < 0$, where ΔH, ΔH_0, ΔS, and ΔS_0 are the enthalpies and the entropies of the main phase transition of aqueous dispersions of DPPC in the presence and absence of carbohydrate.

DISCUSSION

Current knowledge of hydrophobic interaction has been used to help in interpretating the physicochemical nature of the phase-transition process in aqueous dispersions of DPPC (Chen, 1981). In the phase-transition process, there is a melting of "icelike" water that reflects the difference in the amount of icelike water around the hydrocarbon tails between gel and liquid-crystalline phases. Such a melting is a major contribution to the observed endothermic ΔH. Since sugars can cause an increase in hydrophobic interaction between nonpolar groups in proteins, peptides, and aromatic amino acid (Lakshmi and Nandi, 1976; Back et al., 1979; Gerlsma and Stuer, 1972), the presence of carbohydrate may decrease the difference in the amount of icelike water around the lipid hydrocarbon tails between the two phases. The smaller difference in icelike water in the two phases in the presence of carbohydrate results in the melting of less icelike water, which can account for the observed lower endothermic ΔH for DPPC, as shown in Table I.

Carbohydrate has no effect on the lipid-phase transition temperature (t), which corresponds to no change in ΔG (Eq. 6). That is, the free energy change in the gel phase is canceled out by that in the liquid-crystalline phase. Alternatively, if the entropy effect $T\Delta S$ just compensates ΔH in the presence of carbohydrates, ΔG of the melting will remain the same, and t will be unchanged. The observations that carbohydrates mainly affect hydrogen-bond formation between solute and solvent, rather than solvent and solvent (Walrafen, 1966; Taylor and Rowlinson, 1966; Sturtevant, 1941; Franks, 1973) may suggest that carbohydrates might not cause a stronger effect on $T\Delta S$ than ΔH, and ΔH and $T\Delta S$ could be in balance. It should be noted, however, that the situation here is complicated and other information is needed before a definite interpretation of the present results can be reached.

The free-energy pairwise-interaction coefficient $\{AA\}_g$ is a measure of self-interaction of a solute in aqueous solution. The higher the value of a negative $\{AA\}_g$ for solute, the stronger is its tendency for pairwise interaction between its molecules and the greater its ability for hydrophobic interaction. The values of $-\{AA\}_g$ for carbohydrates are in the order raffinose > sucrose > glucose (Okamoto et al., 1978). The ΔH values of DPPC aqueous dispersions (Table I) with these three carbohydrates are rather close: 5.05 for raffinose, 4.46 for sucrose, and 5.07 kcal/mol for glucose. This closeness indicates that the presence of a carbohydrate molecule with a stronger tendency for pairwise interaction between its molecules does not have a greater effect on the ΔH of the lipid's melting. Comparison of Figs. 2 and 3, however, reveals that the thermogram of the lipid's phase transition in the presence of raffinose has the longest tail on the lower temperature side of the phase transition. The degree of perturbation caused by these three carbohydrates on the thermogram is in the order raffinose > sucrose> glucose. This order may be taken as an indication that the degree of perturbation by a carbohydrate on the lipid thermogram is correlated with its $\{AA\}_g$. As expected, raffinose, a trisaccharide, and sucrose, a disaccharide, cause a larger perturbation than do the monosaccharides (Figs. 1–3). Lactose, a disaccharide, is an exception; More $\{AA\}_g$ data for carbohydrates are needed to examine this matter further.

Values of the minimum attraction contribution (Kozak et al., 1968), $-A_{2\text{min}}$, to the second osmotic virial coefficients of some carbohydrates in water are also listed in Table I. These values, which are a measure of the minimum pairwise attraction between two carbohydrate molecules embedded in water, have the same meaning as those of $-\{AA\}_g$. Although raffinose, sucrose, and fructose have significantly different $-A_{2\text{min}}$ values, their effects on the ΔH of the lipid melting are not significantly different. There is, however, a correlation between the degree of the perturbation on the thermogram by these carbohydrates and their value for $-A_{2\text{min}}$.

The shape of a solute molecule can affect its interaction with surrounding water molecules. A six-member ring is a favored one (Hatsuho and Hisashi, 1969). The hydration number of six-member ring glucose is higher than that of five-member ring ribose (Tait et al., 1972). The ΔH values (Table I) in the presence of some hexose monosaccharides (fructose and rhamnose) do not differ significantly from those in the presence of pentose monosaccharides (ribose, xylose, and arabinose). However, ΔH values in the presence of the other hexose monosaccharides (galactose, glucose, and mannose) are significantly lower. Furthermore, the degree of perturbation on the thermograms of DPPC dispersions (Figs. 1 and 2) is greater in the presence of galactose, glucose, and mannose.

The hydration properties of carbohydrates are not simply related to the number of OH groups or oxygen atoms. The number of equatorial OH groups is also important (Franks et al. 1972; Tait et al., 1972). Arabinose and xylose have an identical chemical formula, and each forms a six-member ring; but arabinose contains two equatorial and two axial OH groups, whereas xylose contains three equatorial groups and one axial OH group. In the presence of arabinose and xylose, the thermogram patterns of DPPC dispersions are similar (Fig. 1), and the values of ΔH of the lipid's melting have about the same magnitude within experimental error (Table I). The ΔH of the lipid's melting also has the same magnitude in the presence of glucose (with three equatorial groups and one axial OH group) or galactose (with two equatorial and two axial groups), yet glucose causes the larger perturbation on the thermograms.

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REFERENCES

Back, J. F., D. Oakenfull, and M. B. Smith. 1979. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* 18:5191–5196.

Chen, C-H. 1981. Thermodynamics of solute-solute hydrophobic interaction: interactions of hydrophobic species with lipid. *J. Phys. Chem.* 85:603–607.

Chen, C-H. 1980. Thermodyanmic studies of protein-small molecule interaction. III. Solute-solute hydrophobic interaction of phycocyanin with alcohols. *J. Phys. Chem.* 84:2050–2053.

Chen, C-H., and D. S. Berns. 1978. Thermodynamic studies of protein-salt interaction. 2. Effects of geometrical configuration in alkyl-substituted quaternary ammonium salts on their interactions with phycocyanin. *J. Phys. Chem.* 82:2781–2786.

Chen, C-H., and D. S. Berns. 1977. Thermodynamic studies of protein-salt interaction. Phycocyanin-tetrabutylammonium bromide and tetraethylammonium bromide. *J. Phys. Chem.* 81:125–129.

Franks, F., J. R. Ravenhill, and D. S. Reid. 1972. Thermodynamic studies of dilute aqueous solutions of cyclic ethers and simple carbohydrates. *J. Solution Chem.* 1:3–16.

Franks, F. 1973. The solvent properties of water. *In* Water, A Comprehensive Treatise. F. Frank, editor. Plenum Publishing Corp., New York. 2:1–54.

Gerlsma, S. F., and E. R. Stuer. 1972. The effect of polyhydric and monohydric alcohols on the heat-induced reversible denaturation of lysozyme and ribonuclease. *Int. J. Pept. Protein Res.* 4:377-383.

Hatsuho, U., and U. Hisachi. 1969. Activity coefficients of aqueous xylose and maltose solutions. *Bull. Chem. Soc. Jpn.* 42:2137–2140.

Kay, R. L., and D. F. Evans. 1966. The effect of solvent structure on the mobility of symmetrical ions in aqueous solution. *J. Phys. Chem.* 70:2325–2335.

Kozak, J. J., W. S. Knight, and W. Kauzmann. 1968. Solute-solute interactions in aqueous solutions. *J. Chem. Phys.* 48:675–690.

Lakshmi, T. S., and P. K. Nandi. 1976. Effects of sugar solutions on the activity coefficients of aromatic amino acids and their N-acetyl ethyl esters. *J. Phys. Chem.* 80:249–252.

Okamoto, B. Y., R. H. Wood, and P. T. Thompson. 1978. Freezing points of aqueous alcohols. Free energy of interaction of the CHOH, CH_2, CONH and C – C functional groups in dilute aqueous solutions. *J. Chem. Soc. Faraday Trans.* I, 74:1990–2007.

Ross, P. D., and R. N. Goldberg. 1974. Scanning microcalorimeter for thermally induced transitions in solution. *Thermochim. Acta.* 10:143–151.

Savage, J. J., and R. H. Wood. 1976. Enthalpy of dilution of aqueous mixtures of amides, sugars, urea, ethylene glycols, and pentaerythriol at 25°C: enthalpy of interaction of the hydrocarbon, amide, and hydroxyl functional groups in dilute aqueous solution. *J. Solution Chem.* 5:733–750.

Simpson, R. B., and W. Kauzmann. 1953. The kinetics of protein denaturation. I. The behavior of the optical rotation of ovalbumin in urea solutions. *J. Am. Chem. Soc.* 75:5139–5152.

Stokes, R. H., and R. A. Robinson. 1966. Interactions in aqueous nonelectrolyte solutions. I. Solute-solvent equilibria. *J. Phys. Chem.* 70:2126–2131.

Sturtevant, J. M. 1941. Calorimetric investigations of organic reactions. II. A new calorimeter. The mutarotation of α and β-d-glucose. *J. Phys. Chem.* 45:127–147.

Tait, M. J., A. Suggett, F. Franks, S. Ablett, and P. A. Quickenden. 1972. Hydration of monosaccharides: A study by dielectric and nuclear magnetic relaxation. *J. Solution Chem.* 1:131–151.

Taylor, J. B., and J. S. Rowlinson. 1955. Thermodynamic properties of aqueous solutions of glucose. Trans. Farad. Soc. 51:1183–1192.

Walrafen, G. E. 1966. Raman spectral studies of the effects of urea and sucrose on water structure. *J. Chem. Phys.* 44:3726–3727.