THERMODYNAMICS OF PHOSPHOLIPID-SUCROSE INTERACTIONS

B. Z. CHOWDHRY, G. LIPKA, AND J. M. STURTEVANT
Department of Chemistry, Yale University, New Haven, Connecticut 06511

ABSTRACT The effect of 0–1.0 M sucrose on the phase-transition properties of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (1,2-DPPC) was examined by high-sensitivity differential scanning calorimetry at a scan rate of 0.1 K min^{-1}. Increasing the concentration of sucrose caused a small, but experimentally significant, increase in the temperature (T_m) of maximal excess apparent specific heat (C_{max}) and in $\Delta T_{1/2}$ (the transition width at ½ C_{max}), a reduction in C_{max}, and a small decrease (~8–10% at 1.0 M sucrose compared with 0 M sucrose) in the calorimetric enthalpy (ΔH_{cal}) of the gel-to-liquid crystalline transition. The calorimetric parameters of the pretransition of 1,2-DPPC were not significantly affected by sucrose in the concentration range examined, except there was a 1.0°C increase in the temperature (T_p) of maximal excess apparent specific heat in the presence of 1.0 M sucrose. The results are discussed in terms of the possible molecular mechanisms that could have caused the observed changes and are contrasted with the results obtained by C. -H. Chen et al. (1981, *Biophys. J.*, 36:359–367).

INTRODUCTION

The effects of sugars (1, 2) and polyols (3, 4) on the physical properties of phospholipids have been examined using a variety of physical techniques. It has been reported that in the presence of carbohydrates, (monosaccharides [pentoses and hexoses] and dissaccharides as well as trisaccharides), the temperature (T_m) of maximal excess apparent specific heat (C_{max}) of 1,2-DPPC is "essentially unchanged" and the calorimetric enthalpy (ΔH_{cal}) is decreased (1). Surface pressure-area and surface potential-area measurements obtained from monolayers of 1,2-DPPC indicate that at low-surface pressures (10–30 mNm^{-1}), sucrose (≥ 1.5 M) causes the monolayer to become more liquid-expanded i.e., increases the molecular area, which makes the film more liquid in character (2).

In this brief communication, we report sucrose's effect on the phase-transition properties of 1,2-DPPC. The results differ from those reported by Chen et al. (1).

MATERIALS AND METHODS

1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (Avanti Polar Lipids Inc., Birmingham, AL) was found by analytical thin-layer chromatography (TLC) (Uniplate silica gel HL; Analtech, Inc., Newark, DE), using 100–400 $\mu g\ cm^{-1}$ of phospholipid dissolved in chloroform with $CHCl_3$/MeOH/7 N NH_3 (230:90:15; vol/vol/vol) or CH_3Cl_3/MeOH/H_2O (65:25:4; vol/vol/vol) as the eluent, and by differential scanning calorimetry to be >99.5% pure. Ultra-pure sucrose (Becton Dickinson Immunodiagnostics, Orangeburg, NY; Schwarz/Mann Div.) and doubly deionized water were used.

Method A: Multilamellar lipid vesicles were prepared (5) by adding 1–10 mg of 1,2-DPPC to 2.0 ml of a 0–1.0 M sucrose solution, heating to 50°C, vortexing for 20 s, and repeating this procedure three times. Method B: Multilamellar lipid vesicles were also prepared (Maggio, B., personal communication) by dissolving lipid in 0.2 ml of spectroscopic grade chloroform, subjecting the solution to rotary evaporation, which caused a thin film to form in the round bottom flask, and then further drying in a vacuum oven at 50°C for 30 min. The appropriate amount of sucrose solution was added and the mixture was incubated for 1 h at 50°C, vortexed for 1 min, and then left at room temperature for 1 h before calorimetric examination.

High-sensitivity differential scanning calorimetric experiments were performed with a DASM-1M differential microcalorimeter (v/o Mashpriborintorg 121200 Moscow, Union of Soviet Socialist Republics) (6) using a scan rate of 0.1 K min^{-1}. Scans of H_2O vs. H_2O or sucrose solution vs. sucrose solution were horizontal in the temperature range 5°–80°C. Initial and final baselines for all samples were also horizontal and there were no significant permanent changes in excess apparent specific heat (C). Calorimetric parameters (T_m or T_p [the temperature of maximal excess apparent specific heat], $\Delta T_{1/2}$ [the transition width at ½C_{max}], ΔH_{cal}, and C_{max}) were obtained as previously reported (7).

RESULTS AND DISCUSSION

1,2-DPPC suspensions prepared by methods A and B showed identical calorimetric properties in both the absence and presence of sucrose, and showed complete reversibility upon rescanning after being cooled to 20°C in the calorimeter. 1,2-DPPC suspensions prepared by the method of Chen et al. (1) displayed different phase-transition properties compared with multilamellar liposomes prepared by methods A and B (Table I). It is possible that lipid suspensions prepared by the method of Chen et al. (1) consist of a heterogeneous mixture of small and large unilamellar and multilamellar vesicles.

In the presence of sucrose (0.2–1.0 M), the calorimetric

TABLE I
PHASE TRANSITION PROPERTIES OF 1,2-DPPC IN THE PRESENCE AND ABSENCE OF SUCROSE

Sucrose concentration	T_p	$\Delta T_{1/2}$	ΔH_{cal}	C_{max}
M	°C	°C	*kcal mol*$^{-1}$	*cal* K^{-1} g^{-1}
Pretransition	(± 0.02 SE)	(± 0.02 SE)	(± 0.2–0.4 SE)	(± 0.5–2.0 SE)
0.0	34.8	1.00	1.35	1.90
0.5	35.3	0.95	1.30	1.80
1.0	35.8	1.10	1.20	1.7
0.0*	34.2	2.40	0.65	0.52
Gel-to-liquid crystalline transition				
0.0	41.51	0.09	7.6	78.0
0.2	41.63	0.10	7.4	69.0
0.4	41.74	0.11	7.3	61.0
0.6	41.86	0.12	7.2	55.0
0.8	41.95	0.13	7.1	52.0
1.0	42.11	0.13	6.9	49.5
0.0*	41.35	0.43	9.5	24.3

*Prepared by the method of Chen et al. (1) using lipid concentrations of 2.8 mg ml^{-1}. All other results are for multilamellar suspensions of 1,2-DPPC at lipid concentrations of 1 mg ml^{-1}. Phospholipid concentrations of 2–5 mg ml^{-1} gave the same results. The scan rate employed was 0.1 K min^{-1} for all experiments.

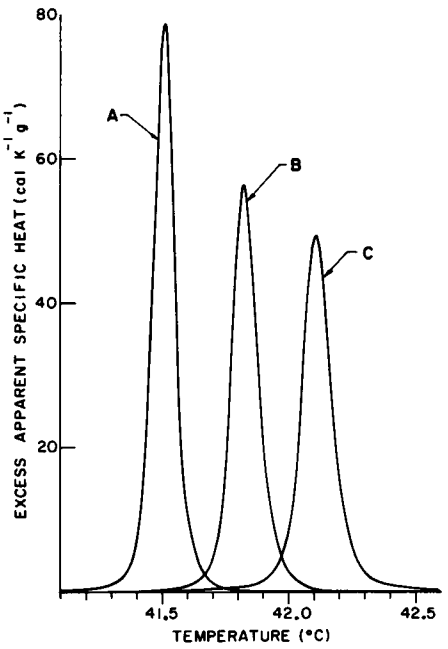

FIGURE 2 The variation of excess apparent specific heat with temperature during the gel-to-liquid crystalline transition of 1,2-DPPC suspensions (*A*) in the absence of sucrose, (*B*) in the presence of 0.5 M sucrose, and (*C*) in the presence of 1.0 M sucrose.

parameters of 1,2-DPPC suspensions prepared by methods A and B were altered in a small but experimentally significant and systematic manner compared with suspensions containing no sucrose. The pretransition temperature increased in the presence of sucrose until, at 1.0 M sucrose, it was 1.0°C higher than in the absence of sucrose. Other calorimetric parameters, $\Delta T_{1/2}$, ΔH_{cal}, and C_{max}, of the pretransition were not significantly affected (Fig. 1, Table I). However, Chen et al. (1) found that the pretransition is greatly reduced in the presence of 0.2 M sucrose. When the sucrose concentration was increased, the gel-to-liquid crystalline transition underwent systematic changes in T_m, $\Delta T_{1/2}$, and C_{max}, as well as having a small change in ΔH_{cal}. T_m and $\Delta T_{1/2}$ increased while C_{max} decreased. In going

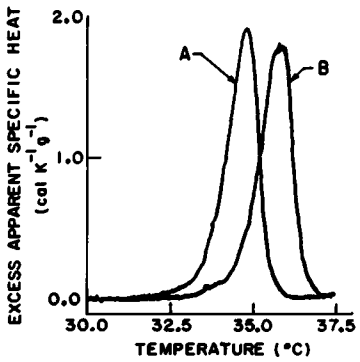

FIGURE 1 Tracings of the differential scanning calorimetric (DSC) curves of excess apparent specific heat vs. temperature for the pretransition of 1,2-DPPC (*A*) in the absence of sucrose, and (*B*) in the presence of 1.0 M sucrose.

from 0 M sucrose to 1.0 M sucrose, ΔH_{cal} decreased by ~8–10% (Fig. 2, Table I). The addition of sucrose raised T_p and T_m, suggesting that in both cases, this solute is more soluble in the low-temperature phase involved in the transition than in the high-temperature phase. For the gel-to-liquid crystalline transition, adding an ideal solute that increases T_m by 0.6 K and that is insoluble in both the liquid crystalline and aqueous phases would increase $\Delta T_{1/2}$ to >1 K (8). The transition remained relatively sharp in 1.0 M sucrose probably because the sucrose concentration in the lipid phases is buffered by the relatively enormous amount of the solute in the aqueous phases (9), and perhaps also because sucrose is somewhat soluble in the liquid crystalline phase.

Although Chen et al. (1) presented data only for 0.2 M sucrose, their findings are significantly different from those reported here. They found a substantial decrease (~40%) in ΔH_{cal} for 1,2-DPPC in the presence of 0.2 M sucrose. Also, there was a significant difference in excess apparent specific heat before and after the gel-to-liquid crystalline transition of 1,2-DPPC that occurred when sucrose was present (Fig. 4 in reference 1), which was not found in the present study. Chen et al. (1, 10) hypothesized that during 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." They attributed the differences they observed in the phase transition properties of 1,2-DPPC in the presence and absence of carbohydrates to "the smaller difference in icelike water in the two phases in

the presence of carbohydrate." The assumption that melting of icelike water is a major contribution to the enthalpy of the gel-to-liquid crystalline transition has been questioned (11). Certainly the effects of sucrose observed in our experiments cannot be explained in terms of the mechanism postulated by Chen et al. (1) Other workers have suggested that certain carbohydrates may stabilize membranes at low-water activities (11). Our experiments were, however, conducted at high-water activity. It has also been suggested that certain carbohydrates may act as substitutes for H_20 at macromolecular surfaces (12) and that OH-containing compounds may alter membrane fluidity through hydrogen bonding with the polar head groups of the phospholipid (13). The mechanisms proposed for the effect of sucrose on proteins (14) and the mechanism proposed by MacDaniel et al. (4) to explain the effect of glycerol on 1,2-DPPC may help explain the effect of sucrose on aqueous suspensions of 1,2-DPPC liposomes. Data from other techniques such as nuclear magnetic resonance (NMR), Raman spectroscopy, and Fourier transform infrared (FT-IR) spectroscopy, in which phospholipid suspensions are prepared in a defined manner, will be needed before any definite conclusions can be drawn concerning the molecular mechanism of the interaction of sucrose with phospholipids.

Finally, we propose that full calorimetric data, i.e., $\Delta T_{1/2}$, T_m, C_{max} and ΔH_{cal}, be reported to allow valid comparisons of calorimetric data obtained in different laboratories. An examination of the calorimetric literature relating particularly to model biological systems shows that such complete data are usually not reported.

This research was aided by grants from the National Institutes of Health (GM04725) and the National Science Foundation (PCM81-17341).

Received for publication 10 February 1984 and in final form 16 April 1984.

REFERENCES

1. Chen, C. -H., D. S. Berns, and A. S. Berns. 1981. Thermodynamics of carbohydrate-lipid interactions. *Biophys. J.* 36:359–367.
2. Maggio, B., and J. A. Lucy. 1978. Interactions of water-soluble fusogens with phospholipids in monolayers. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 94:301–303.
3. Cadenhead, D. A., and R. J. Demchak. 1969. Observations and implications of glycerol-monomolecular film interactions. *Biochim. Biophys. Acta.* 176:849–857.
4. McDaniel, R. V., T. J, McIntosh, and S. A. Simon. 1983. Nonelectrolyte substitution for water in phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 731:97–108.
5. Mabrey, S., P. L. Mateo, and J. M. Sturtevant. 1978. High sensitivity scanning calorimetric study of mixtures of cholesterol with dimyristoyl- and dipalmitoylphosphatidylcholines. *Biochemistry.* 17:2464–2468.
6. Privalov, P. L. 1980. Scanning microcalorimeters for studying macromolecules. *Pure Appl. Chem.* 52:479–498.
7. Velicelebi, G., and J. M. Sturtevant. 1979. Thermodynamics of the denaturation of lysozyme in alcohol-water mixtures. *Biochemistry,* 18:1180–1186.
8. Sturtevant, J. M. 1982. A scanning microcalorimetric study of small molecule-lipid bilayer mixtures. *Proc. Natl. Acad. Sci. USA.* 79:3963–3967.
9. Sturtevant, J. M. 1984. The effects of water soluble solutes on the phase transitions of phospholipids. *Proc. Natl. Acad. Sci. USA.* 81:1398–1400.
10. Chen, C. -H. 1982. Interactions of lipid vesicles with solvent in heavy and light water. *J. Phys. Chem.* 86:3559–3562.
11. Crowe, J. H., L. M. Crowe, and R. Mouradian. 1983. Stabilization of biological membranes at low water activities. *Cryobiology.* 20:346–356.
12. Webb, S. J. 1965. Bound Water in Biological Integrity. Charles C. Thomas, Publisher, Springfield, IL. 53–89.
13. Buckingham, J. H., and L. A. Staehelin. 1969. The effect of glycerol on the structure of lecithin membranes. A study of freeze-etching and x-ray diffraction. *J. Microsc. (Ox.).* 90:83–106.
14. Gekko, K., and T. Morikawa. 1981. Preferential hydration of bovine serum albumin in polyhydric alcohol-water mixtures. *J. Biochem. (Tokyo).* 90:39–50.