Biochimica et Biophysica Acta 821 (1985) 165–168
Elsevier

BBA 70251

BBA Report

An X-ray diffraction and differential scanning calorimetric study on the effect of sucrose on the properties of phosphatidylcholine bilayers

Jürgen Stümpel, Winchil L.C. Vaz and Dieter Hallmann

Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen-Nikolausberg (F.R.G.)

(Received July 16th, 1985)

Key words: Phosphatidylcholine bilayer; Sucrose effect; Phase transition; Calorimetry; X-ray diffraction

The influence of sucrose, between 0 and 70% in the aqueous phase, upon multilamellar liposomes of dimyristoylphosphatidylcholine was examined by differential scanning calorimetry and X-ray diffraction analysis. Increasing concentrations of sucrose increase the temperatures of both the main transition and the pretransition of the lipid. The effect is greater on the pretransition than on the main transition. At 35°C the interlamellar spacing in the multilamellar liposomes is reduced by increasing sucrose concentration in the aqueous phase and no significant effects are seen in the chain lattice of the bilayers. This result is interpreted as a dehydrating effect of sucrose upon the bilayer-water system at 35°C. At 5°C the interlamellar spacing is increased and this increase is, at high (70%) sucrose concentrations, attributable to an untilting of the lipid acyl chains with no change in the thickness of the aqueous layers in the multilamellae.

The influence of water-soluble polyols upon the phase transition behaviour of phospholipid-water systems has received some attention in the recent literature [1–7]. Particularly, the influence of sugars, in solution in the aqueous phase, upon the lipid bilayer structure and phase behaviour is important from a biological point of of view since cell membranes often have a considerable amount of sugars associated with their surfaces as glycoproteins and glycolipids. In a recent high sensitivity differential scanning calorimetric study, Chowdhry et al. [7] have shown that upto 1 M sucrose in the aqueous phase increases the main and pretransition temperatures of multilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes. These authors concluded that the calorimetric results indicated a solid phase miscibility and a liquid phase immiscibility of sucrose in the phospholipid-water system. In this work we report our results on the effects of sucrose upon the phase transition behaviour, studied by differential scanning calorimetry, and the structural characteristics, studied by X-ray diffraction analysis, of dimyristoylphosphatidylcholine (DMPC) multilamellar liposomes.

DMPC was from Fluka Feinbiochemica, Buchs, Switzerland, and sucrose was of the best commercial quality available from E. Merck, Darmstadt, F.R.G. The concentrations of sucrose solutions were determined by measuring the refractive index at 20°C and comparing with tables [8]. To prepare multilamellar liposomes, the lipid was dissolved in chloroform/methanol (2 : 1 v/v) and the solvent was evaporated in a test tube by blowing a stream of nitrogen over the solution. The residue on the walls of the tube was freed of solvent traces by leaving it in a vacuum overnight at room temperature. Hydration was done by adding 2 ml (for 10 mg lipid) of the desired aqueous solution of sucrose in doubly distilled water containing 0.02% sodium azide, warming at 35°C for 30 min and then vortexing vigorously to suspend the lipid. The suspensions were allowed to stand at 35°C for 16 h and were then centrifuged at $10000 \times g$ for 10

min. The lipid pellet or pellicle was then used for calorimetry or X-ray diffraction. Differential scanning calorimetry (DSC) was done using a Perkin-Elmer DSC-2 calorimeter. Heating or cooling was done at 1.25 Cdeg/min, in the sensitivity range of 1 mcal/s. The sample volume was approx. 70 μl and contained approx. 7 mg of lipid. Before recording the calorimetric scans, the sample was passed through one heating and cooling cycle between about 0 and about 50°C.

Diffraction studies were carried out using a Guinier camera the details of which are described elsewhere [9]. Lipid samples were sealed in glass capillaries (2 mm diameter, 0.01 mm wall thickness, obtained from Fa. Hilgenberg, Malsfeld, F.R.G.). Measurements were performed using a thermostated sample holder in the temperature range between 2 and 40°C. Before each diffraction experiment the sample was equilibrated for at least 2 h at the new temperature. Diffraction spectra were collected with a photon counting system including a position-sensitive proportional counter (TEC model 205) designed for the detection of low-energy X-rays (5–20 KeV). The operating voltage for the best signal to noise ratio was 1700 V and the sample to detector distance was 12 cm. The signal from the photon counter was fed into an electronic modulator system for amplification and digitalisation. A multichannel analyser (EG& G Ortec Model 7100) connected with a Hewlett-Packard HP86 microcomputer was used for storage and analysis of the diffraction data. Spectra in the low and wide angle regions were detected separately to avoid parallax problems. Detection times were usually 2 min for spectra in the low angle region and 3 min in the wide angle region.

Typical calorimetric scans with water and 70% sucrose as the aqueous phase are shown in Fig. 1 and the calorimetric results for several concentrations of sucrose in the aqueous phase are shown in Fig. 2. It is seen that with increasing sucrose concentration, the lipid bilayer main transition and pretransition temperatures are increased. This rate of increase with sucrose concentration is greater for the pretransition than it is for the main transition so that the difference in temperature between the two transitions is only approx. 3 Cdeg in the presence of 70% sucrose in comparison with approx. 11 Cdeg in water. Small but noticeable

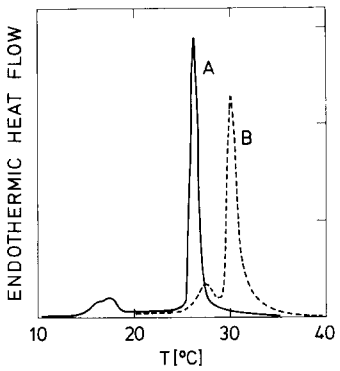

Fig. 1. Differential scanning calorimetric traces for DMPC (approx. 8 mg) in approx. 70 μl of deionized water (A), and approx. 70 μl of 70% sucrose (B). The heating rate was 1.25 Cdeg/min in the sensitivity range of 1 mcal/s.

effects are also seen in the transition widths but no significant changes in the transition enthalpy are observable. Except for this last observation our results are in agreement with those recently published by Chowdhry et al. [7] for dipalmitoylphosphatidylcholine and disagree with the results of Chen et al. [6].

In order to obtain an understanding of what was occurring at the structural level of the DMPC bilayers we undertook an X-ray diffraction study of the system. Typical diffraction results for DMPC

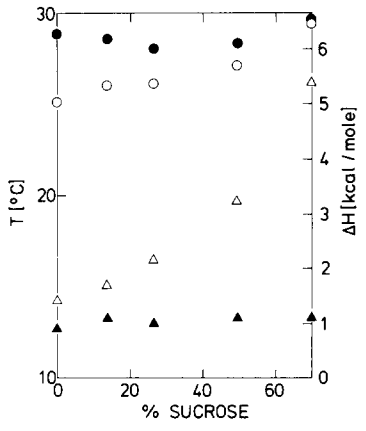

Fig. 2. Effect of sucrose in the aqueous phase upon the phase transition characteristics of DMPC multilamellar liposomes. ○, main lipid bilayer phase transition temperature; △, pretransition temperature; ●, enthalpy change for the main lipid bilayer phase transition; ▲, enthalpy change for the pretransition.

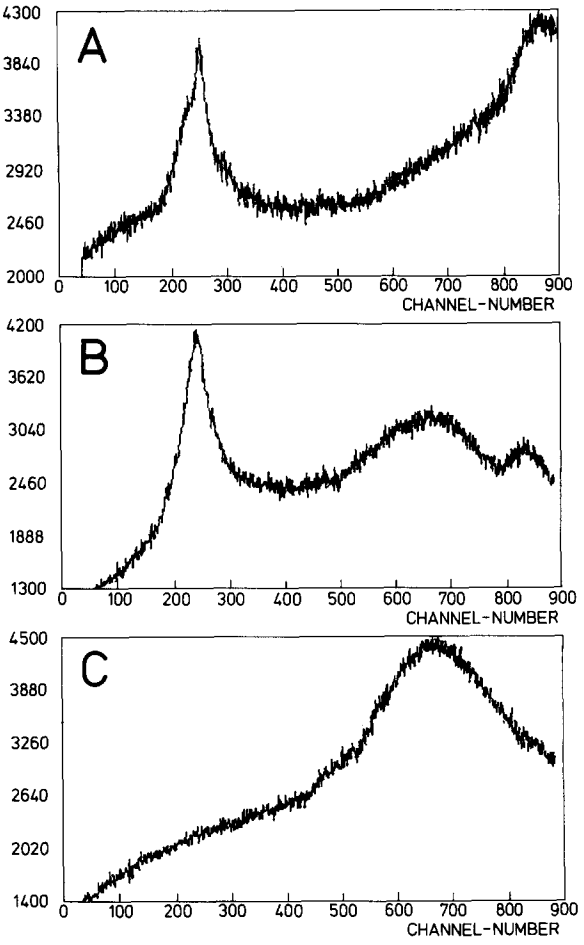

Fig. 3. X-ray diffraction pattern in the wide-angle region of (A) DMPC in deionized water; (B) DMPC in 70% sucrose; (C) 70% sucrose. The temperature was 5°C.

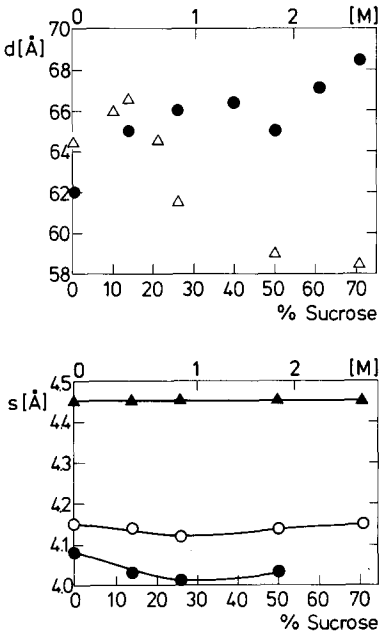

Fig. 4. Summary of X-ray diffraction results for DMPC multilamellar liposomes as a function of sucrose concentration in the aqueous phase. Upper panel: Long spacings (low-angle region); Lower panel: Short spacings (wide-angle region). The circles are for results obtained at 5°C and the triangles are for results obtained at 35°C. The errors are ±0.5 Å and ±0.01 Å in the low-angle and wide-angle regions, respectively.

multilamellar liposomes in water and in 70% sucrose are shown in Fig. 3. In Fig. 3A the diffraction pattern for DMPC/water at 5°C with a sharp reflection at 4.15 Å and a shoulder at 4.08 Å, characteristic of the $L_{\beta'}$ phase, is seen. In the presence of 70% sucrose (Fig. 3B) only a sharp reflection at 4.15 Å is observed indicating that the acyl chains in DMPC/70% sucrose are not tilted. The broad reflection at approx. 10 Å (approximately channel No. 650 in Fig. 3B) is attributable to the sucrose (see Fig. 3C).

The results for various concentrations of sucrose in the aqueous phase are summarized in Fig. 4. In the upper panel of this figure the effect of aqueous phase sucrose upon the interlamellar spacing (low-angle region) in the DMPC multilamellae is shown for temperatures of 5 and 35°C. At 5°C an increase in the interlamellar distance is observed between 0 and 70% sucrose. The value at approx. 50% sucrose falls reproducibly outside of an otherwise monotonic increase. At 35°C a decrease in the interlamellar spacing with increasing sucrose concentration in the aqueous phase is evident. Again, the value at approx. 14% sucrose falls reproducibly outside of an otherwise monotonic decrease. A similar result was obtained by Le Neveu et al. [3] for L_α phase egg yolk phosphatidylcholine in excess water (aqueous sucrose). In the lower panel of Fig. 4 we show the short spacings (wide angle region) at 5 and 35°C. At 35°C the short spacings are constant (4.45 Å) at all concentrations of sucrose. At 5°C two reflections are seen, a sharp reflection and a shoulder, which is typically interpreted as being due to tilted acyl chains [10,11]. At 70% sucrose only one sharp reflection (4.15 Å) is seen, indicating that the chains are no

longer tilted. In the region between 14 and 50% sucrose the tilt attains a maximum at approx. 30% sucrose. The difference between the long spacings at 0% sucrose (62 Å) and at 70% sucrose (68.5 Å) could be explained as being due to an untilting of the acyl chains with no change in the thickness of the aqueous layer between the lipid layers. However, the behaviour between these two extremes is not uniquely explicable by this argument since the tilt reaches a maximum at approx. 30% sucrose and it may be expected that under these conditions the long spacings should decrease. At 35°C the simplest explanation for the decrease in the long spacings is a dehydration of the multilamellae provoked by sucrose in the bulk aqueous phase.

This work was supported in part (J.S.) by the Deutsche Forschungsgemeinschaft through grant No. Ei78/4-2.

References

1 Moucharafieh, N. and Friberg, S.E. (1979) Mol. Cryst. Liq. Cryst. 49, 231–238
2 Cadenhead, D.A. and Demchak, R.J. (1969) Biochim. Biophys. Acta 176, 849–857
3 Le Neveu, D.N., Rand, R.P., Parsegian, V.A. and Gingell, D. (1977) Biophys. J. 18, 209–230
4 McDaniel, R.V., McIntosh, T.J. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 97–108
5 Maggio, B. and Lucy, J.A. (1978) FEBS Lett. 94, 301–303
6 Chen, C.H., Berns, D.S. and Berns, S.A. (1981) Biophys. J. 36, 359–367
7 Chowdhry, B.Z., Lipka, G. and Sturtevant, J.M. (1984) Biophys. J. 46, 419–422
8 CRC Handbook for Chemistry and Physics, 52nd Edn., p. E-205, The Chemical Rubber Co., Boca Raton, Fl
9 Stümpel, J., Eibl, H. and Nicksch, A. (1983) Biochim. Biophys. Acta 727, 246–254
10 Tardieu, A., Luzzati, V. and Reman, F.C. (1972) J. Mol. Biol. 75, 711–733
11 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575–4580