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EFFECT OF SUCROSE ON THE OPTICAL PROPERTIES OF DIPALMITOYLPHOSPHATIDYLCHOLINE

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The gel to liquid crystal phase transition of dipalmitoylphosphatidylcholine (DPPC) has been followed by the change in absorbance at 400 nm; this change is due to the change in lipid light scattering properties during the transition. The effect of sucrose on the change in absorbance during the transition of DPPC has been investigated. It has been shown that the presence of sucrose or glycerol in the multilamellar liposome suspension increases the change in absorbance due to the main transition, decreases the total absorbance, and decreases the change in absorbance due to the pretransition. This effect of sucrose and glycerol is shown to be an optical effect which is correlated with the solvent index of refraction.

Introduction

We are involved in a systematic study of the effects of alcohol on the thermotropic behavior of synthetic phospholipids. In these studies we have made use of the change in light scattering properties of lipid suspensions to follow phase transitions [1]. In order to stabilize the suspension during the course of the experiments several additives were used to increase the density of the solutions to prevent settling. In the course of this work we found that some additives, particularly sucrose, have optical effects on the observed phenomena which are not accounted for by the change in solvent density. The present report describes these optical effects.

Materials and Methods

Materials. DPPC was purchased from Calbiochem and found to be free of impurities by thin-layer chromatography in chloroform/-methanol/water (35:65:5, v/v) on Silica gel G plates. Water was deionized, passed over a charcoal filter, and then distilled. Organic solvents were distilled.

Lipid samples were handshaken multilamellar liposomes prepared according to the method of Bangham et al. [2]. The chloroform solution of lipid was shell dried under a stream of nitrogen, and warm buffer (0.05 M Tris, pH 7.4, 0.001 M EDTA, and 0.15 M NaCl) was added and the sample vortexed. The suspensions were incubated above the transition temperature for 2 h, with intermittant vortexing. Individual samples prepared from stock suspensions by addition of sucrose or glycerol were incubated above the transition temperature for 1 h. The suspensions ranged from 0.13 mg/ml to 0.6 mg/ml. Lipid concentrations of stock solutions were determined by phosphorus analysis according to the method of Bartlett [3]. It

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine.

is difficult to prepare samples of reproducible absorbance, even when using the same stock suspension and preparing the samples at the same time. For the experiments described here where the absolute value of the absorbances of different samples are compared, the samples were prepared in the spectrophotometer cells. The stock suspension was placed in the sample and control cuvettes and the absorbance checked to make sure they were the same; the additive stock solution was then added. A parallel control sample was used in each experiment and the corrections for dilution were determined from it, since Beer's law does not necessarily hold for these suspensions.

Spectrophotometry. The work described here was performed using the Cary 219 spectrophotometer equipped with first derivative, timer, wavelength programmer, cell programmer, and temperature read-out accessories, as well as the digital interface port and printer. The temperature was controlled by water circulated through jacketed cuvettes from a Lauda K2/RD refrigerated bath, and the temperature was monitored by the built-in thermistor which was immersed in a jacketed cuvette hooked in series with the sample cuvettes; the temperature data was printed out along with the absorption data on the Cary Smart Printer. The rate of heating could be varied, and the rate used was between 0.5 and 1.5 K/min, except in the transition region where it was slowed to 0.2 K/min. In general two samples were followed simultaneously; this improved the temperature resolution by permitting comparison of two samples at the same temperature. The reproducibility of the absolute temperature measurement was ± 0.1°C.

Results

Fig. 1 shows a plot of the absorbance at 400 nm of DPPC against temperature, with several concentrations of sucrose and glycerol in the solvents. Several effects are observed in this graph. The absolute absorbance is seen to decrease as the sucrose concentration increases. This effect is even more apparent with the glycerol solution. What is more interesting, however, is the observation that the change in absorbance associated with the main transition increases with increasing sucrose concentration, while the contribution of change associated with the pretransition decreases. Again, this effect is enhanced in the presence of 36% glycerol.

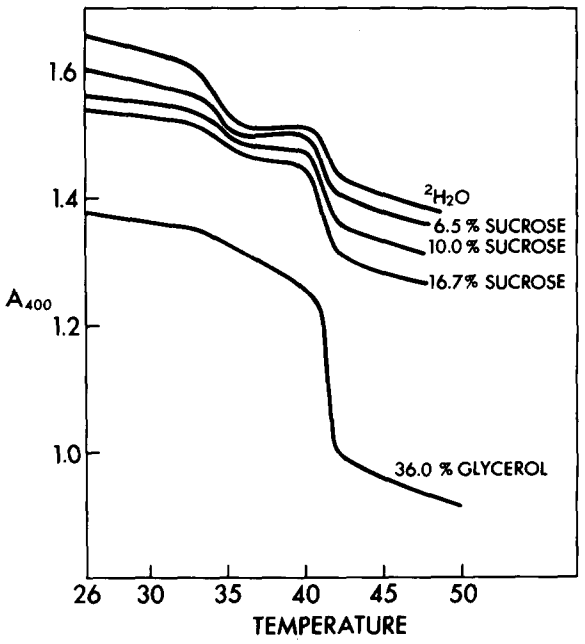

Fig. 1. Effect of solvent additives on the phase transition of DPPC observed by absorbance at 400 nm. 0.55 mg/ml lipid. The H_2O control was identical to the ^2H_2O curve.

Several experiments were done to test possible causes for these effects. To distinguish whether the pretransition was being thermodynamically eliminated, shifted to lower temperatures, or just becoming optically transparent, the kinetics of the sucrose effects were investigated. It was found that the change in absorbance of the suspensions at 25°C was instantaneous with the addition of sucrose. This eliminated the possibility that the pretransition had been shifted to temperatures below our observation temperature, because the kinetics of the pretransition are slow. On the other hand, the kinetics of the change in absorbance in going from 25 to 37°C, through the pretransition, were slow as expected, regardless of the sucrose concentration; only the magnitude of the optical change was affected. These experiments indicate that the effect of sucrose on the pretransition is optical rather than thermodynamic. In addition, it has been reported [4] that sucrose has no effect on the calorimetric measurement of phase transitions

of DPPC. We have observed no effect of sucrose on the main transition midpoint of either DMPC or DPPC in the presence and absence of ethanol [5,6]. Thus, it appears that the effect of the solvent additives sucrose and glycerol is an optical effect rather than a thermodynamic one.

The possible causes of the optical effects include solvent density and solvent index of refraction. The lack of any effect of ^2H_2O on the optical properties rules out the density factor. In addition, an experiment was done in which the effect of two glycerol concentrations were compared with 16.7% sucrose, one matched in index of refraction, and one matched in density. The samples which were matched in index of refraction gave similar results, whereas the glycerol sample which had the greater index of refraction gave results correlated with the index of refraction.

Several correlations were investigated to explore this optical effect. Fig. 2 shows a plot of absorbance against solvent index of refraction at 25°C. There is a clear correlation of absorbance and solvent index of refraction. This is as would be expected, since the light scattering which gives rise to the absorbance depends upon the index of refraction difference between the solute and solvent.

More interesting than the decrease in absolute absorbance with solvent index of refraction is the shift in relative proportions of change in absorbance for the two transitions. Fig. 3 shows the effect of solvent index of refraction on the contribution of the change in absorbance of the main transition as a fraction of the total absorbance at 25°C. It is seen that there is a steep linear dependence of this parameter on solvent index of refraction, regardless of whether sucrose or glycerol is used to increase the solvent index of refraction.

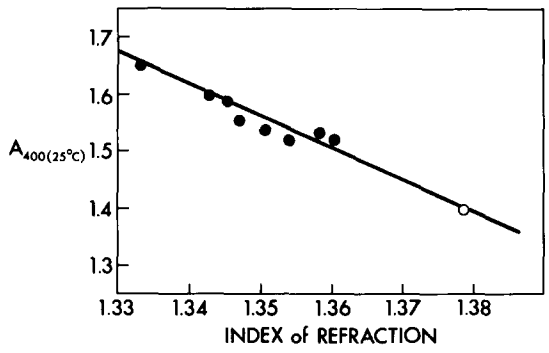

Fig. 2. Effect of solvent index of refraction on the absorbance of liposome suspensions at 25°C. ●, sucrose added; ○, glycerol added.

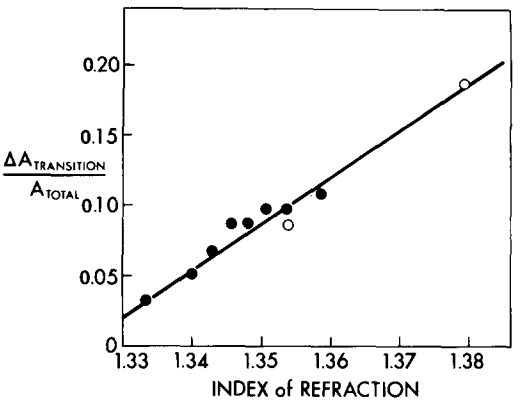

Fig. 3. Relative contribution of change in absorbance during main transition as a fraction of total absorbance at 25°C. ●, sucrose added; ○, glycerol added.

Discussion

The absorbance or light scattering changes associated with the main phase transition of synthetic phosphatidylcholines have been used by several investigators to follow phase transitions [1,5–13]. By theoretical analysis of index of refraction increments, light scattering, and turbidity measurements, Yi and MacDonald [1] showed that the major portion of the turbidity change in the main transition is accounted for by the change in refractive index of the lipid, which is due to the lipid density change. The remainder of the turbidity change was attributed to the change in optical anisotropy of the lipid bilayer during the transition. We have found that the absolute value of the absorbance at 400 nm decreased with increasing solvent index of refraction. This is as expected since the light scattering depends upon the index of refraction increment, i.e. the difference in index of refraction between the solvent and the solute.

The effect of solvent index of refraction on the relative changes in absorbance during the pretransition and the main transition may arise from several sources. As has been observed by others as

well [1,13], in the absence of sucrose, the pretransition gives a large change in turbidity which is difficult to reconcile with its small calorimetric contribution [14]. There is no change in index of refraction associated with the pretransition [1]. The physical change which occurs during the pretransition is a change in surface topography, and in tilt of the acyl chains [15], and it appears likely that these changes give rise to a change in optical anisotropy of the bilayer and bilayer surface which can change the light scattering [1]. The increase in solvent index of refraction apparently neutralizes the optical effect of this phenomenon, so that the light scattering properties of the intermediate state between the two transitions are more similar to those of the low temperature state than at lower solvent refractive index.

The change in relative proportions of the contributions of the two transitions does not completely account for the effect of solvent index of refraction, however. It is evident by inspection of Fig. 1 that the total change between 25 and 45°C is also increasing with index of refraction. A possible explanation for this effect is that the multiple scattering effects are greater for the low temperature high turbidity state than for the high temperature lower turbidity state. This explanation is consistent with the observation that Beer's law does not apply for these suspensions at the concentrations used here. It would be expected that at lower total concentrations this effect would be decreased.

The results reported here provide an improvement in the use of turbidity measurements for studying the main phase transition of phosphatidylcholines. As shown here, using a solvent with a relatively large index of refraction greatly improves the precision possible in following the transition by this method. Since the change in absorbance of the lipid suspension results from changes in the physical state of the lipid itself, this method has distinct advantages over the optical methods which involve the use of probe molecules. Any small deviation from linearity in the transition region due to differential multiple scattering effects of the high and low temperature states would be negligible compared to the experimental error, which is limited by the precision of the temperature control.

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