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STRUCTURAL CHANGES IN LECITHIN-WATER SYSTEMS

THERMAL-TURBIDIMETRIC STUDIES

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SUMMARY

Turbidity measurements were made of dilute aqueous dispersions of 1,2-dipalmitoyl-L-lecithin as the temperature was varied. In the range from 24 to 33° a decrease in turbidity is associated with the penetration of water between the layers of lipid in the crystalline structure. At the transition to the liquid crystalline form (39–41°) a sharp decrease in turbidity occurs. In solutions of 0.06 M LiCl, 1 mM $CaCl_2$, $MgCl_2$ or 0.05 M PO_4^{3-} an increase in turbidity resulted at the transition temperature, whereas in 0.1 M NaCl, or quaternary ammonium salts the turbidity decreased. The presence of small amounts of dicetylphosphoric acid mixed with the lecithin decreased the turbidity, and at elevated pH levels there was no change at the transition temperature.

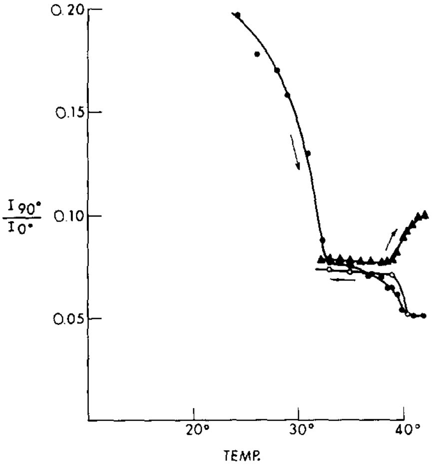
INTRODUCTION

Studies of aqueous systems of phospholipids using differential thermal analysis or differential scanning calorimetry[1,2] have shown that these dispersions when composed of a single molecular species undergo a sharp thermal transition from a gel-type structure to a liquid crystalline lamellar form. This transition involves the melting of the acyl chains to form a liquid hydrocarbon layer. In a report from this laboratory[3] we described changes in the ionic properties of lecithin that also accompany this structural transition. This paper describes a procedure using turbidimetric measurements for studying the structural changes in lecithin–water systems that result from changes in temperature, ionic environment, or the charges of the particles. In this way, it serves as a means for exploring some of the physico-chemical characteristics of a model membrane system.

Dispersions of lecithin were made by ultrasonic radiation. 1,2 Dipalmitoyl-L-lecithin from Mann Research Laboratories, New York, was purified by silicic acid chromatography. The dimyristoyl analog was from La Motte Chemical Co. Light scattering measurements were made of 10-ml systems containing 0.1–0.12 mg/ml in 0.025 M Tris–HCl at pH 7.2, unless otherwise indicated. A Brice–Phoenix universal light scattering photometer was equipped with a heating stage, a magnetic stirrer, and thermistor temperature probe. The intensity of 546-nm light was measured at angles of 0, 90, 45, and 135° at temperature intervals of 0.5 or 1.0°.

Fig. 1 shows the changes in light scattering, as indicated by the ratio I_{90°/I_0, for a system of dipalmitoyl lecithin. A large decrease in turbidity is observed as the system is heated from 22 to 32°. A second change occurs sharply in the narrow range from 39 to 41°. On cooling, this second change is reversed but the first is not. A second heating reverses the cooling curve very closely, showing a single major decrease in turbidity from 39 to 41°. Similar results were obtained with 1,2-dimyristoyl lecithin except that the sharp drop in turbidity occurred at 23°. The changes in turbidity observed on heating these lecithin–water systems may be related to the thermal transitions shown by differential thermal analysis or differential scanning calorimetry studies. The reversible change that occurs at 39° for dipalmitoyl lecithin and at 23° for the dimyristoyl analog results from the melting of the hydrocarbon chains.

To study the effects of added ionic solutes on these changes in turbidity, the following procedure was used. Equal 10-ml aliquots of a dispersion of dipalmitoyl lecithin in 0.025 M Tris–HCl were taken. In each experiment readings of light intensity were made as the system was heated from 20 to 43° and then cooled to 30°. This procedure of heating and cooling was followed to be assured that in the course of the thermal changes the lipid molecules go through the fluid state so that the polar portions of the molecules become oriented at the aqueous interface. The desired electrolyte was then added at 32–33° and after standing 20 min for equilibration the heating was continued to 45° with measurement of the turbidity at each 0.5°. The results observed on heating dipalmitoyl lecithin with different salts fell into two



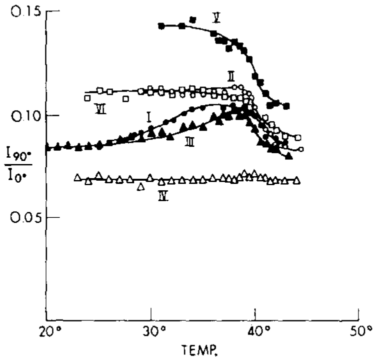


Fig. 1. Changes in light scattering with temperature. The system contained 1 mg of 1,2-dipalmitoyl L-lecithin in 10 ml 0.025 M Tris–HCl (pH 7.2). It was heated (●) and cooled (○). At 32°, LiCl was added to make the concentration of LiCl 0.06 M, and the system was reheated (▲). 546-nm light was used.

Fig. 2. Changes in light scattering with temperature, effect of pH. Systems contained 1–1.2 mg of mixed lipids: 95.5 mole% 1,2-dipalmitoyl-L-lecithin and 7.5 mole% dicetylphosphoric acid. Each system was in 10 ml 0.025 M Tris–HCl brought to a specific pH level and heated. I, pH 5.82 (●); II, pH 7.20 (○); III, pH 7.60 (▲); IV, pH 8.27 (△); V, pH 7.60 in 0.060 M NaCl (■); VI, pH 8.44 in 0.060 M NaCl (□).

classes. In some the second heating produced the customary drop in turbidity in the range from 39 to 41°. Other systems, however, showed a sharp and dramatic increase in turbidity at 39°. This is shown in Fig. 1 for a system containing 0.06 M LiCl. Similar effects were observed with systems of lecithin in 0.025 M Tris which were then brought to $1 \cdot 10^{-3}$ M $CaCl_2$ or $1 \cdot 10^{-3}$ M $MgCl_2$. When these systems were heated, a sharp increase in turbidity resulted at 39°. Solutions of NaCl, KCl, tetramethylammonium chloride or choline chloride in concentrations to 0.10 M did not produce this increase in turbidity at 39°. A system heated in 0.01 M Na_2HPO_4 did not produce an increase. However, when the concentration was brought to 0.05 M and the system was reheated, a sharp increase in turbidity resulted beginning at 38.5°. Similar results were obtained in 0.05 M K_2HPO_4 or 0.03 M Tris–phosphate.

In another series of experiments the effect of adding an anionic lipid to the bi-ionic lecithin was studied. Dicetylphosphoric acid was mixed with dipalmitoyl lecithin in organic solvent and after evaporation of the solvent the mixture containing from 6.5 to 7.5 mole% of dicetylphosphoric acid was dispersed in water. These dispersions had lower turbidities than those containing lecithin alone. When heated from 20° they did not show the decrease in turbidity found consistently with the lecithin. As shown in Fig. 2, Curve II the major change observed on heating occurs at the mesomorphic transition which begins at 38.5° but is not complete until 42°. In the presence of salts, an increase in turbidity occurs in LiCl, $CaCl_2$ and $MgCl_2$ in a manner analogous to the lecithin systems described above. The curves in Fig. 2 show the effect of increasing pH. For these experiments the mixed lipid dispersion was heated in 0.025 M Tris at several pH levels. In Curve IV at pH 8.27 the system has an extremely low initial turbidity and does not show the transition at 38.5°.

The decreases in turbidity that are observed in these experiments are related to increased hydration of the polar regions of the dispersed molecules. CHAPMAN *et al.*[1] showed from X-ray diffraction that dipalmitoyl lecithin–water systems when heated above the transition temperature undergo an abrupt decrease in the long spacing, the distance between the lamellar layers. This is accompanied by a change in the small dimension from a sharp line at 4.27 Å to a diffuse one at 4.5–4.6 Å. This increase in the cross sectional dimension could lead to greater exposure of the polar groups to water. A changing dielectric constant in this region could result in disruptions of intramolecular and intermolecular linkages. In a related manner the decrease in turbidity that is observed from 22 to 32° may be the effect of water penetrating between the layers of polar groups. Similar penetration of water between the layers of polar groups is found for many amphiphilic compounds such as long-chain alkanols or fatty acids[4]. Differential thermal analysis diagrams of dipalmitoyl lecithin systems[5] show a broad endothermal change in this temperature range. Other studies of these systems at elevated pH levels show a drop in pH from 20 to 32°, with another sharp drop at the transition temperature[3]. The increase in turbidity that occurs in some of the salt solutions as lecithin undergoes the thermal transition to the liquid crystal form is related to the availability of the ionic groups for reactions with ionic solutes. The effectiveness of Ca^{2+}, Mg^{2+} or Li^{+} in producing these increases in turbidity may be related to the strong affinity of these ions for the phosphate of the lecithin or possibly their effectiveness in disrupting the water structures at the lipid–water interface[6]. When the pH of the systems containing dicetylphosphoric acid was raised the increased ionization led to increased repulsions between layers and also to greater hydration of the charged groups. The resulting turbidities are lower, and in the system in Curve IV no further change occurs on heating. In the presence of 0.060 M NaCl the electrostatic effects are reduced, and the turbidity values increased and the thermal transition was shown.

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