LATERAL PHASE SEPARATIONS AND PERPENDICULAR TRANSPORT IN MEMBRANES

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Received October 1, 1973

SUMMARY

A valinomycin-mediated potassium conductivity has been studied using a glass U-tube in which two aqueous compartments are separated by a fritted glass filter impregnated with valinomycin and one or more pure phospholipids. This system can be used to detect the beginning and end of lateral phase separations in binary lipid mixtures, and also demonstrates a pronounced maximum in electrical conductivity of dipalmitoyl phosphatidylcholine at the transition temperature, 41°C.

INTRODUCTION

Two recent publications have described an interesting effect of lateral phase separations in membranes on transport through the membranes. In one study, Papahadjopolous et al.,[1] have shown that the passive leak rate of $^{22}Na^{+}$ from dipalmitoyl phosphatidylcholine liposomes decreases with decreasing temperature, except in the temperature region of 41°C which corresponds to the temperature of the gel-liquid crystal transition. In the vicinity of this transition temperature, there is a maximum in the leak rate. In a second study, the rate of active uptake of β-glucosides and β-galactosides by a fatty acid auxotroph of E. coli grown on elaidic acid was found by Linden et al.,[2] to show a marked, apparently discontinuous enhancement of uptake of these sugars with decreasing temperature, at the temperature corresponding to the onset of lateral phase separation of the phospholipids as detected by spin labels. The purposes of the present paper are (a) to report a third instance where there is enhanced transport in the vicinity of a transition temperature of a pure phospholipid, and (b) to show that the onset and completion of the lateral phase separations of two-component lipid-systems can

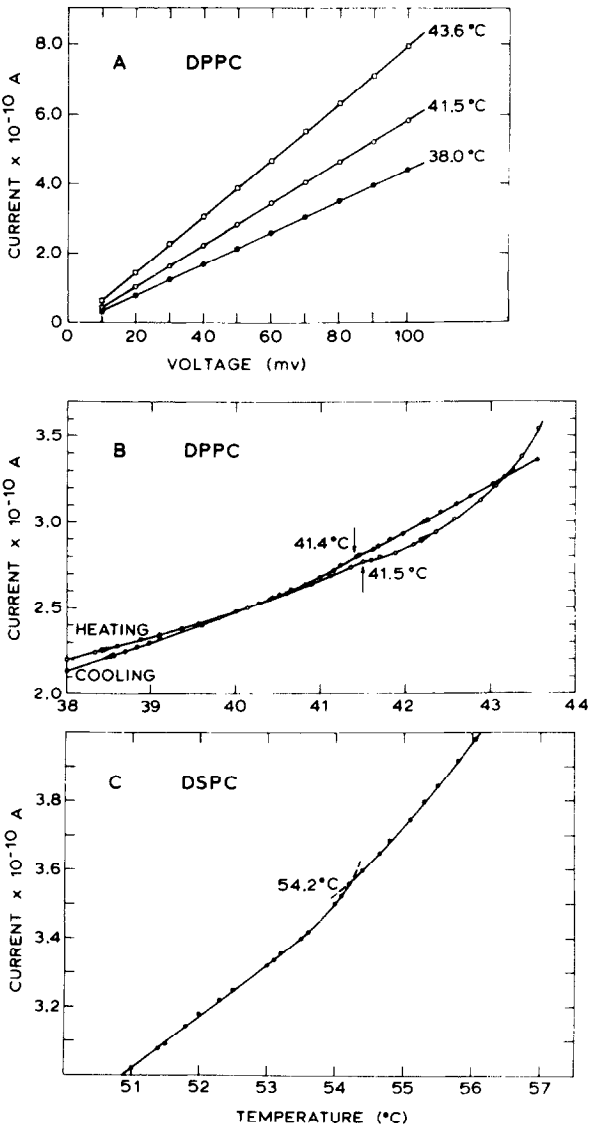

Figure 1(A). Current-voltage curves of DPPC membrane at three different temperatures. (B) Current vs. temperature curve for the phase transition of pure DPPC with applied voltage 50MV. (C) Current vs. temperature curve for the phase transition of pure DSPC with applied voltage 100MV. In (A), (B) and (C), the membranes contained 0.5mg lipid in aqueous medium of 0.1M KCl and 0.01M potassium phosphate at pH = 7.00.

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also be detected by their effect on transport. The present work utilizes a simple glass U-tube in which two aqueous compartments are separated by a fritted glass filter. In the present work, this filter was impregnated with a small amount of valinomycin and phospholipid. In general, the phos-

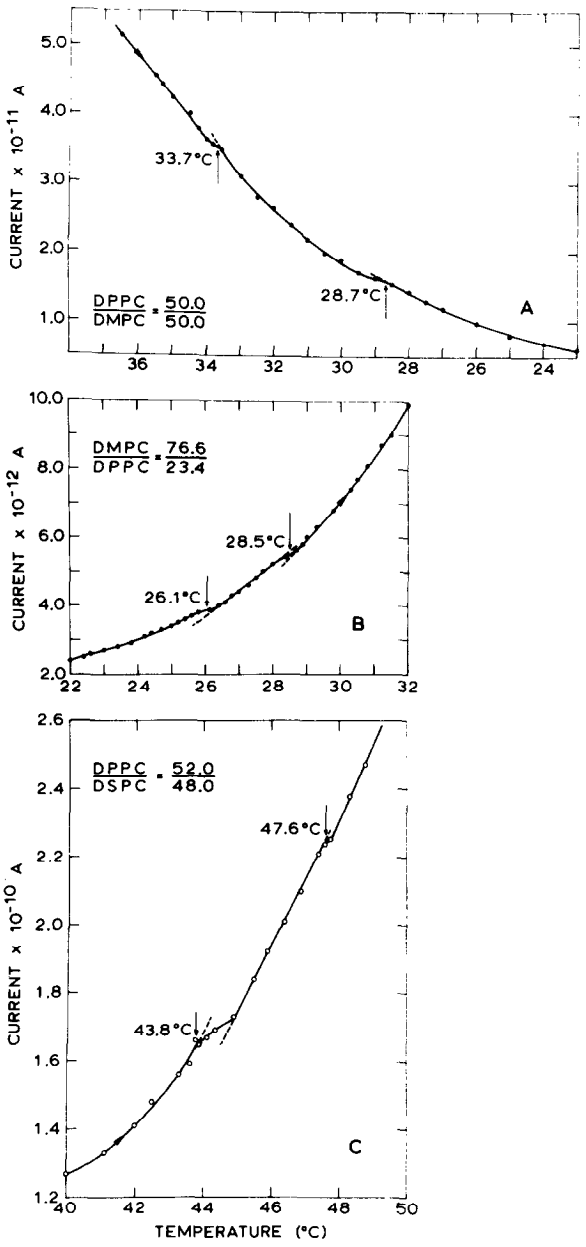

Figure 2(A). Current vs. temperature curve for the phase separation of binary mixtures of 50.0 mole% DPPC and 50.0 mole% DMPC with applied voltage 100MV. (B) Current vs. temperature curve for the phase separation of binary mixtures of 73.6 mole% DMPC and 26.4 mole% DPPC with valinomycin to lipid ratio of 1:2000 and applied voltage 50MV. (C) Current vs. temperature curve for the phase separation of binary mixtures of 52.0 mole% DPPC and 42.0 mole% DSPC with valinomycin to lipid ratio of 1:300 and applied voltage 50MV. In (A), (B) and (C), the membranes contained 0.5 mg lipid. The aqueous solution contained 0.1M KC1 in (A), (B) and 0.1M KC1 buffered with 0.01M potassium phosphate in (C).

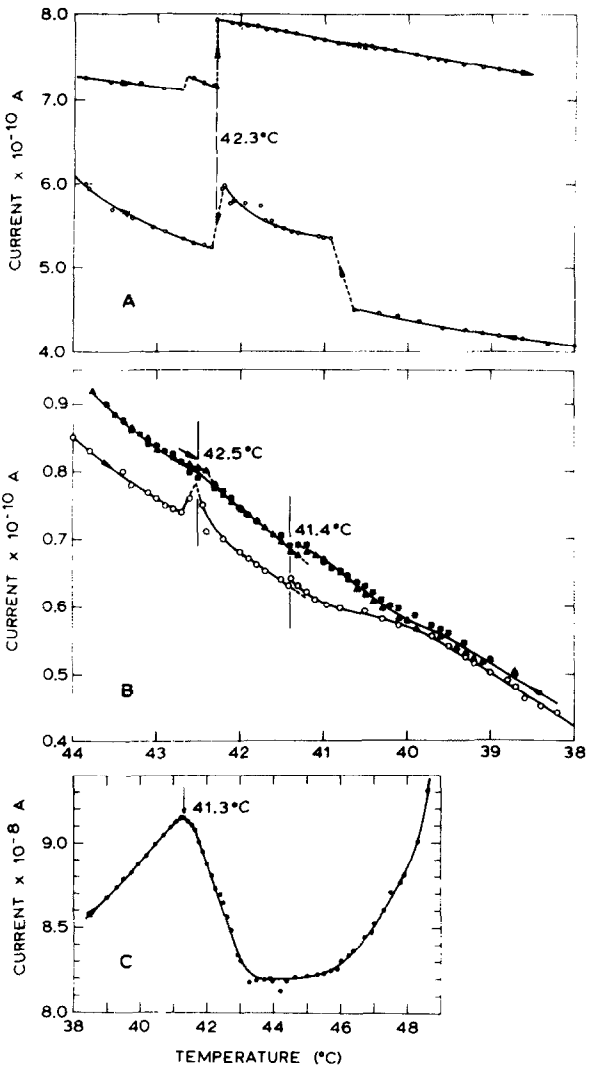

Figure 3. Current vs. temperature curves for the phase transition of DPPC membrane under different experimental conditions. (A) The membrane containing 0.5 mg lipid in a medium of 0.1 M KCl and 0.01 M potassium phosphate buffer at pH = 7.00 with applied voltage 50 MV. 5μg valinomycin was added to the positive side of aqueous solution. (B) The membrane contained 0.4 mg DPPC with valinomycin to lipid molar ratio of 1:150. The aqueous solution contained 0.024M KCl, 0.076M NaCl, and 0.01M sodium phosphate buffer at pH = 7.43, the applied voltage was 100MV. ▲ and O, cooling curves ■ , heating curve. (C) The membrane contained 0.5mg DPPC and 0.5μg valinomycin to start with initially. The aqueous solution contained 0.1 M KCl, 0.01 M potassium phosphate at pH = 7.00 and 0.5μg/ml valinomycin. The applied voltage was 50MV.

pholipid would have formed 10^3 bilayers if uniformly spread across the fritted glass surface. In spite of the obvious drawbacks of this set-up, it does have the advantages of stability over very long periods of time, precisely

defined lipid composition and the complete absence of lipid film "stabilizers". In earlier experiments performed with black lipid membranes[3,4,5] there has been some uncertainty in the precise composition of the lipid film.

MATERIALS AND METHODS

Dipalmitoyl phosphatidylcholine (DPPC) was purchased from General Biochemicals and contained less than 1% impurity as judged by thin layer chromatography. Dimyristoyl phosphatidylcholine (DMPC), distearoyl phosphatidylcholine (DSPC) and valinomycin were purchased from Calbiochem and were used without further purification. The salts used were AR grade reagents from Mallinckrodt.

U-tubes with fritted glass filters (Kontes) in the middle, which were very similar to polarographic cells, were used for membrane formation. Each side arm of the U-tube contained 4-5 ml salt solutions.

Multibilayer membranes[6] were formed by evaporation of 100$\mu\ell$ of a chloroform solution of 5 mg/ml lipid with or without valinomycin into the fritted glass filters. The chloroform solution of valinomycin (1.12 mg/ml in stock) was added directly to the lipid solution to reach the desired molar ratio of valinomycin to lipid. The valinomycin can also be added to the aqueous medium through ethanolic solution (1 mg/ml in stock). After evaporation of the organic solvent, and pumping under vacuum to remove all traces of solvent for at least 30 min, the lipids were hydrated by filling the cell with 0.1M salt solutions buffered with 0.01M potassium or sodium phosphate at pH = 7.00. Complete hydration can be achieved by keeping the sample cells at roo temperature for 2-4 days or by keeping the cells above the transition temperatures of the lipid for a shorter period of time, such as 10 hr. Both procedures gave similar results except that prolonged equilibration at room temperature seems to give more stable membranes and more reproducible results.

The electric current through the membrane was measured with a variable DC voltage source and a Keithly 615 digital electrometer with a sensitivity of 10^{-15}A full scale. Polarizing potentials were applied across the membrane through a pair of calomel electrodes (Beckman) with agar-saturated KCl bridges.

The sample cells were immersed in the bath of a Haake constant tem-

perature circulator (Model FT) with control accuracy of ± 0.002°C. A Beckman thermometer and a calibrated thermometer (accuracy ± 0.05°C) were used to measure the temperature of the bath. In the data reported here, a heating rate of 6-8°C hour and natural cooling rate of about 5° per hour were employed.

RESULTS AND DISCUSSION

In the presence of excess water, the lipid bilayers formed from DMPC, DPPC and DSPC, undergo transitions from the "gel phase" to the lamellar smectic liquid crystal phase. Transition temperatures determined using calorimetry are 23.70, 41.75 and 54.24, respectively.[7,8] Figure 1A shows typical current-voltage curves of a DPPC membrane at three different temperatures. Figures 1B and 1C show the temperature dependencies of our U-tube impregnated with DPPC and DSPC. There are distinct breaks in slope at the transition temperature of these lipids. Transition temperatures determined in this fashion for DMPC, DPPC and DSPC are 23.6, 41.5 and 54.2, respectively. The observed conductivities of the U-tube set-up are consistent with the idea that all, or much of the current passes through the bilayers. However, accurate determination of transition temperatures could nevertheless be possible even if much of the current did not pass through the bilayers, since the geometry of the lipid-glass matrix might change at the transition temperature.

Figure 2A shows that there are two discernable breaks in the temperature dependence of the electrical conductivity of a 50:50 mixture of DMPC and DPPC. The two temperatures 33.7 and 28.7 correspond closely to temperatures 33.9 and 28.8 known from spin-label studies to represent the onset and completion of lateral phase separations in this mixture of lipids.[9]

Figure 2B and 2C show the conductivities of DMPC-DPPC and DPPC-DSPC mixtures, this time in the presence of valinomycin. Again, breaks in slope of conductivity vs. temperature are seen at temperatures corresponding to the beginning and end of lateral phase separations, but there is no marked effect of valinomycin on the size of these rather small changes.

Figure 3 gives the results of three experiments involving pure DPPC in the presence of valinomycin. Here there are marked enhancements in

the vicinity of the gel-liquid crystal transition of DPPC. Although these curves are not quantitatively reproducible or reversible, the qualtitative observation of enhanced conductivity at the transition temperature 41-42° is reproducible.

The present work does not answer the question as to whether the co-existence of two phases in a two-component system leads to enhanced conductivity. The uncertainty here is experimental, and involves the question as to what fraction of the observed electrical current goes through the bilayers. Our work does show a marked enhancement of transport through our U-tube system when there is only valinomycin and DPPC in the fritted glass. This result is then analogous to that observed by Papahadjopolous et al.[1] These investigators suggested that the enhanced $^{22}Na^{+}$ permeation in DPPC at approximately 41°C may be due to a high permeability through a disordered region between the fluid and solid phase.[1] In studies of transport through bacterial membranes at the onset of a phase separation process (which is almost sharp enough to be a phase transition) we have suggested that the enhanced transport is due to the enhanced lipid lateral density fluctuations, related to the enhanced lateral compressibility when solid and liquid phases co-exist.[2] The two explanations are not necessarily different, since the density fluctuations may be enhanced in the regions of domain boundaries. However, it is not certain that the phase boundaries in pure DPPC are sharp at the melting temperature (whereas they are in mixed lipid systems, as judged by freeze-fracture electron microscopy[10]), since the transition temperature of this type of lipid appears to be equal to, or close to the critical temperature as judged by pressure-area monolayer studies.[11,12] Also, theoretical and experimental evidence has been presented that the gel-smectic liquid crystal transition is not a simple first-order transition.[13] Thus, Nagle has shown from dilatometry studies that the transition appears sharp at 41.36° but has a high temperature tail that goes to ~42°.[14] There may be large density fluctuation throughout this temperature range, which may account for the complex conductivity vs. temperature curves seen in Figure 3.

ACKNOWLEDGMENTS

We are indebted to Drs. E.J. Shimshick and Mark McNamee for

helpful discussions. This work was sponsored by the National Science Foundation under grant No. NSF GB 33501X1 and has benefited from facilities made available to Stanford University by the Advanced Research Projects Agency through the Center for Materials Research.

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