OPTICAL STUDIES OF THE PHASE BEHAVIOR OF MONODOMAIN SAMPLES OF DIPALMITOYL PHOSPHATIDYLCHOLINE CONTAINING CALCIUM CHLORIDE

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ABSTRACT Optical birefringence of the phases exhibited by monodomain samples of dipalmitoyl phosphatidylcholine containing 0.1 M–6 mM calcium chloride is measured over a range of temperature and water content. Little change was observed in the birefringence for this calcium chloride content range, and a phase diagram is constructed from these data and compared with that of monodomain samples of dipalmitoyl phosphatidylcholine and water. Effects of the presence of calcium chloride are most pronounced at low temperature and water content but the interaction with phosphatidylcholine cannot account for the effects observed with calcium on intermembrane interactions.

INTRODUCTION

There has been much interest in the phases (1–3) and transitions (4) exhibited by lipid-water mixtures because the lipid bilayer is the matrix of biological membranes. The various functions, however, are affected by the ionic character of the adjacent medium. One ion pair common to the surrounding medium of most biological membranes is Ca^{++} which has recently been shown to play a role in intermembrane interactions (e.g., cell fusion) (5–8) and can affect the molecular packing within a bilayer (9–11). This work addresses the effect of Ca^{++} and Cl^{-} ($10^{-3}-10^{-1}$M) on the phase behavior of multilamellar systems of dipalmitoyl phosphatidylcholine (DPPC).

MATERIALS AND METHODS

L-α-Phosphatidylcholine (β-γ-dipalmitoyl), DPPC, was obtained from Calbiochem-Behning Corp.(San Diego, Calif.) or Sigma Chemical Co. (St. Louis, Mo.). The purity of lipids used for sample preparation was determined by thin-layer chromatography to be >99.5% and the gel transition was 41.8°C for ≃30% wt water content. The width of this transition was determined to be ≃0.5°C using a light microscope with heating stage, and differential scanning calorimetry showed the width at half maximum of the sharp excess-specific heat peak to be $\lesssim$1°C at this water content (12). These results are consistent with those reported by Hinz and Sturtevant (13) for this purity. Solutions were prepared from triple-distilled water and analytic grade CaCl$_2$.

Samples were prepared from dispersions of lipid and 3–10% wt CaCl$_2$ solution (0.1 M–6 mM) between silane surfactant-coated slides with removable spacers, and annealed until they were monodomain according to the method of Powers and Clark (14). Annealing occurred at 90–95°C for these samples, temperatures ≃30°C lower than those of pure DPPC and water (14), and this process was

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monitored by conoscopic illumination between crossed polarizers. The maximum amount of Ca^{++}/lipid (mole ratio) that could be incorporated in this manner was ≃0.06, and higher Ca^{++} contents produced multiphase samples or ones that annealing did not render monodomain. Additional solution was added to the oriented samples by submersion of the sample assembly in the respective solutions at 90°C with removal of the spacer sides (1). The amount of solution uptake was controlled by the time the sample was exposed to the solution (varied up to ≃3 h) before replacement of the spacer sides. Samples were then removed from the solution and allowed to equilibrate for ≃2.5 h at 90°C before birefringence measurements were made.

Sample content of calcium and chlorine relative to phosphorous was measured at 25°C with x-ray fluorescence by scanning electron microscopy (Cambridge Stereo Scan 2A; Cambridge Instrument Co., Inc., Mansey, N.Y.) The detection limit for Ca^{++} is ≃400 ppm, and the results of peak integration were corrected for respective collecting efficiencies and background. After removal from the glass slides and spacer, the sample water content was determined to ± 1% at 25°C by the weight difference of the lipid mixture before and after heating for ≃24 h in a vacuum of ≃1 μm (1).

Birefringence was measured as a function of temperature (high to low) to ± 0.2°C for a particular water and $CaCl_2$ content by conoscopy (1) ($\lambda = 6{,}328$ Å). The uniaxial birefringence, $n_e - n_0$ ($= n_z - n_x$, where the z axis is normal to the bilayer planes [x, y]), and the biaxial birefringence, $n_y - n_x$, was calculated from the conoscopic interference figures, and the average refractive index derived from the results of Yi and McDonald (15) for pure DPPC-water mixtures. A detailed description of this method and the calculations (16) are given by Powers and Pershan (1). The refractive index is directly related to the molecular orientation or configuration (e.g., phase transformations).

RESULTS

Solutions used for swelling the monodomain samples varied in $CaCl_2$ concentration over roughly two orders of magnitude (0.1 M-6 mM), and the calcium content relative to phosphorous, $[Ca^{++}/P]$, changed by roughly an order of magnitude (0.06-0.008) for the water contents studied. Because the calcium content between the bilayers, $[Ca^{++}/P]$, is small compared with that of the swelling solution, the calcium bound to lipid phosphoryl groups in a 1:1 complex can be approximated by $[Ca^{++}/P]$, and calculation of the association constant using the law of mass action gives a value $K_{Ca^{++}} \simeq 0.8\ M^{-1}$. Although this calculation is crude, it is in reasonable agreement with that determined by Gottlieb (17) for dimyristoyl phosphatidylcholine and McLaughlin and McLaughlin (18) for egg phosphatidylcholine. Other quantities that were measured for these samples, such as alignment temperature, time required for swelling to a particular water content, and birefringence, changed little over this range of $CaCl_2$ concentration. Therefore, in discussion of these results, no particular concentration will be designated.

Birefringence measurements were made on samples containing 4, 9, 15, 20, 22, 25, and 30% wt water. Fig. 1 shows data calculated for the 4, 15, 20, and 30% samples as a function of decreasing temperature. For samples containing up to ≃22% wt water, the uniaxial birefringence observed after annealing increases monotonically with decreasing temperature. This increase is probably the result of elongation in the hydrocarbon chains which increases the bilayer thickness (3). The abrupt increase observed is accompanied by an increase in the amount of scattered light (decrease in transmitted light) and designated by an arrow. This turbid region in which the birefringence is nearly constant with decreasing temperature is interpreted as a two-phase region. The next change in birefringence is the appearance of biaxiality which increases monotonically with decreasing temperature in samples containing up to ≃20% wt water and is due to an orientation of the polar groups (1,14,19). The arrow at the onset of biaxiality indicates the samples now scattered less light because of a decrease in

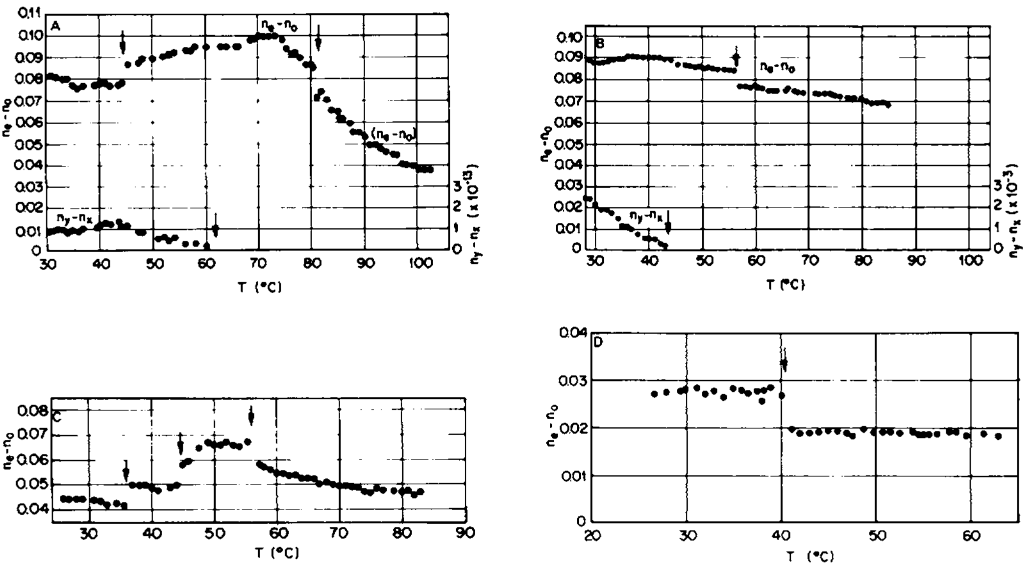

FIGURE 1 Representative optical birefringence data for various phase regions as a function of temperature. Arrows indicate change in transmitted intensity. $CaCl_2$ concentration range is 0.1 M-6 mM. (A) $\simeq$ 4% wt water; (B) $\simeq$ 15% wt water; (C) $\simeq$ 20% wt water; (D) $\simeq$ 30% wt water

turbidity. For water contents < $\simeq$10% wt, another transition occurs but the biaxial birefringence remains nearly constant for decreasing temperature. Comparison of these data with that reported by Powers et al. (1,14) for pure DPPC-water samples (Fig. 1) indicates that the presence of $CaCl_2$ at these water contents raises the temperature at which the onset of the two-phase region is observed, produces larger values of uniaxial birefringence, and produces a biaxial region at low temperature for which the biaxiality is nearly constant. An abrupt decrease in uniaxial birefringence is also observed between 9 and $\simeq$13% wt water for temperatures above the two-phase region that is not found in the pure DPPC-water samples (1) with both phases appearing homogeneous. Likewise, another decrease is observed between 20 and 22% wt water but is similar to one observed in pure DPPC-water samples (1) and that observed by Gary-Bobo et al. (20,21).

Higher water contents produce uniaxial birefringence below the annealing temperature that is nearly constant with decreasing temperature. A second phase is observed having higher turbidity and a "fuzzy" appearance, with a third, at low temperature, that is clear. The second phase could not be observed at 30% wt water content, whereas samples having larger water contents (water content determined at 25°C) could not be obtained by the swelling technique described above. Again, in comparision of this water content region with that observed for pure DPPC-water samples (1), the temperature corresponding to the gel transition is raised, as is that corresponding to the pretransition. At $\simeq$30% wt water content, however, the gel transition is the same but the pretransition is not observed.

DISCUSSION

A phase diagram deduced from the optical birefringence is shown in Fig. 2. Phases are labeled according to their birefringence and the designations $P\beta'$ and $L\beta'$ cannot be determined from

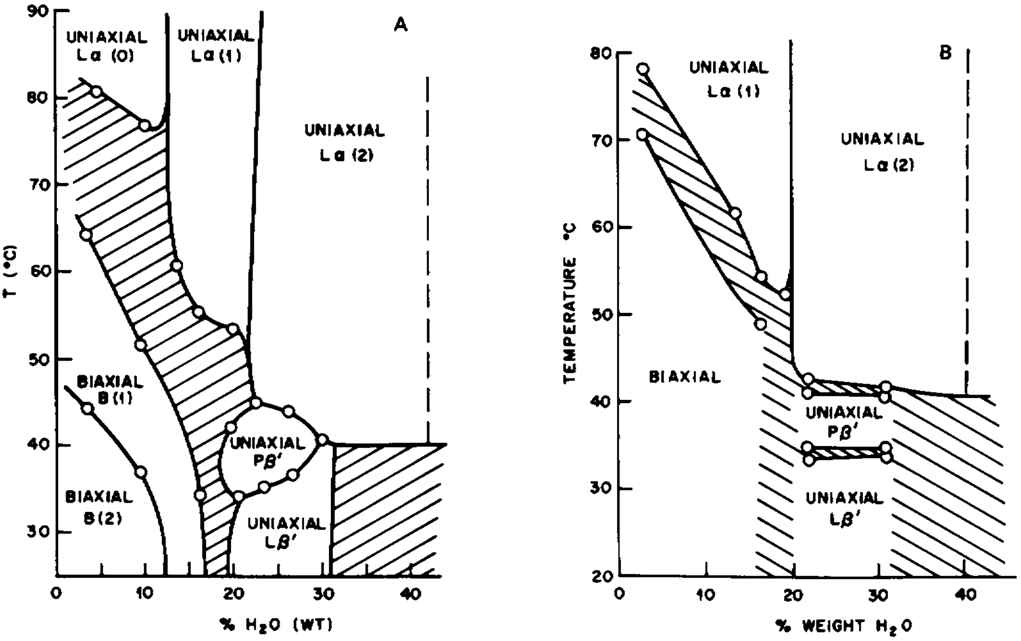

FIGURE 2 Phase diagrams for monodomain dipalmitoyl phosphatidylcholine water systems determined from optical birefringence measurements. Slashed regions indicate two-phase regions and circled points mean the phase boundary is accompanied by a change in transmitted intensity: (A) containing $CaCl_2$ in the concentration range 0.1 M-6 mM; (B) containing no other components (1).

our measurements (1) but are assigned from x-ray data in the literature (2,3). They are similar in temperature behavior of the birefringence and general appearance (turbidity, water content region, transition temperatures) to those observed in pure DPPC-water systems (1). In general, the effect of $CaCl_2$ in the concentration range of 0.1 M-6 mM on the phase behavior of DPPC-water systems is largest at low water content. A second biaxial phase, B(2), is observed whose biaxiality remains constant with decreasing temperature, unlike that of the biaxial phase observed in pure DPPC-water systems or the similar phase, B(1). In fact, the latter two phases are so similar that we suggest they have the same structure (19,22), whereas that of the B(2) phase remains unclear.

In the high temperature region an additional $L\alpha$ phase is observed and designated by (0). The $L\alpha(1)$ and $L\alpha(2)$ phases appear quite similar to those observed in pure DPPC-water systems where a change in polar group conformation proposed by Gary-Bobo et al. (20,21) for egg phosphatidylcholine was suggested as the distinguishing feature (1). A similar argument could be made that Ca^{++} and Cl^- ions in the presence of <5 or 6 water molecules/lipid [$L\alpha(0)$ region] form a rigid complex with the lipid polar groups in which the cation (anion) is strongly associated. When more water molecules are available, some of the polar group ligands are replaced by water, and the polar groups are able to interact as suggested by Gary-Bobo et al. (20,21). This argument, however, is one of many having the facts that (*a*) the annealing temperature is lowered by $\simeq$30°C and (*b*) the temperature for onset of the two-phase region is raised by 5-8°C for the $L\alpha(0)$ region compared with 0.5–2°C for the other $L\alpha$ regions as the only supporting evidence.

The onset of the two uniaxial regions (labeled $P\beta'$ and $L\beta'$) occurs at water contents similar to those for the pure DPPC-water systems, but the transition temperatures are raised by 0.5–2°C. The $P\beta'$ phase decreases in temperature range as water content is increased and is not observed at $\simeq$30% wt. This is consistent with observations in excess water, i.e., the two-phase region shown for water content > $\simeq$30% wt whose structure consists of the $L\beta'$ phase observed at the $\simeq$30% wt boundary in excess water (23). Finally, the boundary shown at $\simeq$42% wt water could not be determined accurately by our methods (1,14).

In summary, the phase diagram for incorporation of $CaCl_2$ in 0.1 M-6 mM concentrations between DPPC lipid bilayers presents more questions than it answers. However, the effects are most pronounced at low water content but the interaction with phosphatidylcholine is much less than that observed for charged phospholipids. No crystalline behavior is indicated by these studies at high water contents for temperatures at which this behavior is observed in phosphatidic acid (6), and there is no indication of aggregation or fusion caused by a Ca^{++}-induced isothermal phase change as in the case phosphatidylserine (7). It appears, then, that the effects observed with calcium on intermembrane interactions at high water content are the result primarily of interaction with charged lipids, and it is not until the water content is lowered ($\leq$10% wt) that similar effects are observed with phosphatidylcholine.

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