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Order-Disorder and the Pretransition in Dipalmitoyl Phosphatidylcholine Multilayers

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Summary

Using x-ray diffraction, the interaction of calcium ions with dipalmitoyl phosphatidylcholine (DPPC) multilayers in excess solution has been studied as a function of temperature over a range of concentrations. We have discovered order-disorder-order transformations as a function of both increasing temperature and calcium concentration. For pure DPPC, the pretransition is characterized by a large increase in lattice disorder. For low calcium concentrations, a similar increase in lattice distortion occurs for all temperatures. The high temperature phase is marked by a significant reduction in this disorder. Likewise ordering of the multilayer is regained at high calcium concentrations.

Introduction

Using x-ray diffraction, Inoko, et. al.[1] identified several structural states as a function of $CaCl_2$ concentration in dipalmitoyl phosphatidylcholine (DPPC) multilayers at 5°C. In this report, we use differential thermal analysis (DTA) and x-ray diffraction to study the phase behavior of these structural states as a function of temperature. Other investigators have reported structural changes in DPPC in pure water as a function of temperature.[2-4] We have varied both parameters simultaneously and found new results which give a more detailed view of the pretransition. However, if either parameter varies separately, our results correspond to those found by others.[1-4] In fully hydrated (DPPC) without ions, two transitions are known to occur in the region of biological interest: a large, first-order transition at 41.5°C, and a smaller transition (called the pre-transition) at 34.5°C. Recent evidence suggests that perturbations of these lipid thermal transitions using cations may be biologically significant.[5]

We have collected data which show that disorder may be induced in multilayers of pure DPPC by raising the temperature to the pretransition, and significantly increased by adding small amounts of calcium. Reordering is brought about either by high temperatures (above the main transition) or by sufficiently large amounts of calcium. Additional data indicate that the pretransition endotherm shifts monotonically toward the main transition for increasing calcium concentrations above about 50 mM.

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When the Ca^{2+} concentration is such that the pretransition endotherm is shifting, x-ray diffraction profiles reveal a gradual increase in lattice ordering as the concentration is increased.

Materials and Methods

Samples of DPPC were purchased from Sigma Chemical Co. and checked for purity by thin-layer chromatography and differential thermal analysis. Salts used were of reagent grade and baked at 200°C for 72 hours to remove any organic contaminants. Triply distilled, deionized water was used for all dispersions.

Phospholipid dispersions were prepared over N_2 gravimetrically. The dispersions were homogenized in a glass vessel using a low power, bath type sonicator. For x-ray measurements samples were centrifuged in 1.0 mm quartz capillaries. This produced a lipid-rich phase and a purely aqueous phase. The samples were placed in a dewar designed for x-ray measurements with a temperature stability of ±10 millidegrees. A Mettler TA2000 Differential Thermal Analyzer was used for DTA.

$CuK\alpha$ x-rays were generated with a bright rotating anode tube and focussed with a mirror monochromator. Diffracted x-rays passed through a vacuum path and were collected with a stable position-sensitive detector.[6]

Results

Results of DTA scans of DPPC dispersions for representative concentrations of Ca^{2+} are depicted in Figure 1. As the molar ratio of Ca^{2+} to lipid is increased, the pretransitional energy decreases in magnitude relative to the main transition for low concentrations of $CaCl_2$. The onset and midpoint temperatures do not significantly shift at these concentrations.

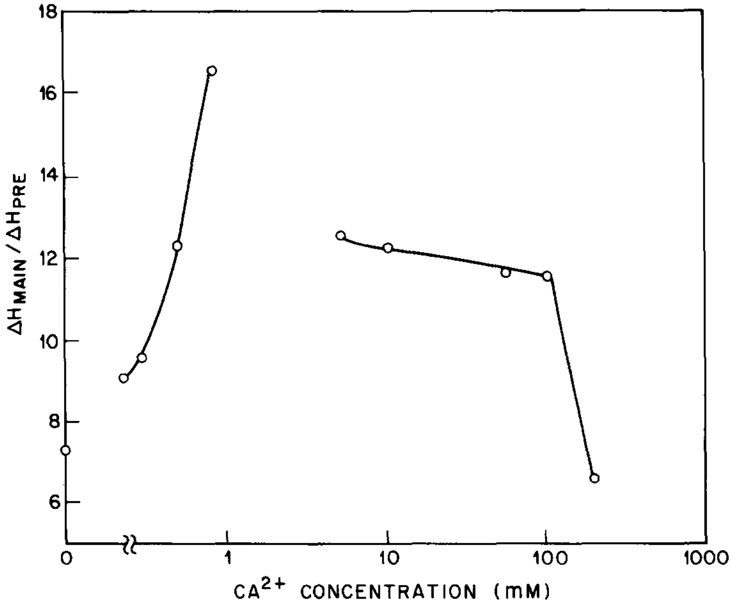

Fig. 1. The variation in the ratio of the enthalpic change at the main transition to that at the pretransition as a function of $CaCl_2$ concentration.

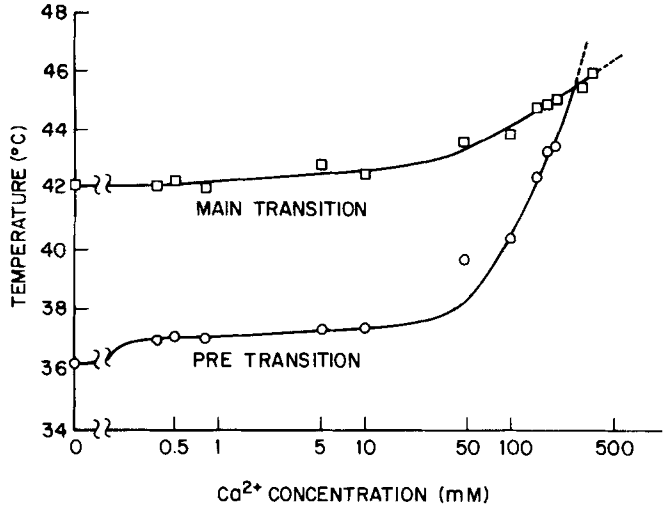

Fig. 2. The dependence of the midpoint temperatures of the pre- and main transitions on $CaCl_2$ concentration.

At Ca^{2+} concentrations above 50mM, the pretransition midpoint temperature is shifted (Figure 2). No significant broadening of the endotherm occurs, but the magnitude of the pretransition approaches that of the pure lipid. At 250 mM concentration, the temperature of the pretransition coincides with that of the main transition and the two cannot be distinguished by DTA.

By ultracentrifugation of samples containing low levels of Ca^{2+}, we were able to visually observe a macroscopic phase separation between more dense and less dense regions. This behavior is similar to that observed with tobacco mosaic particles[7] and with polystyrene latex systems.[8] Starting at $\approx 0.1\,mM$ Ca^{2+} concentration, the lower density region increased proportionally with increasing calcium until the concentration reached 1*mM*, where none of the higher density phase could be observed. From $\approx 5\,mM$ to 250*mM*, the fraction of the less dense phase decreased. Finally, at 250*mM*, only the phase of greater density remained. The less dense phase in all samples showed a higher ratio of main transition to pretransition endotherms. Since we were unable to extract the separated phases for x-ray analysis, the mixture was examined. An increase in lattice disorder could, however, be correlated with an increase in the proportion of the less dense phase.

For ordered multilayered systems, x-rays will diffract coherently and the pattern will consist of sharp Bragg peaks at regularly spaced intervals which correspond to the interlayer repeat distance. However, if the system is disordered, incoherent scattering will be observed which corresponds to a lack of long

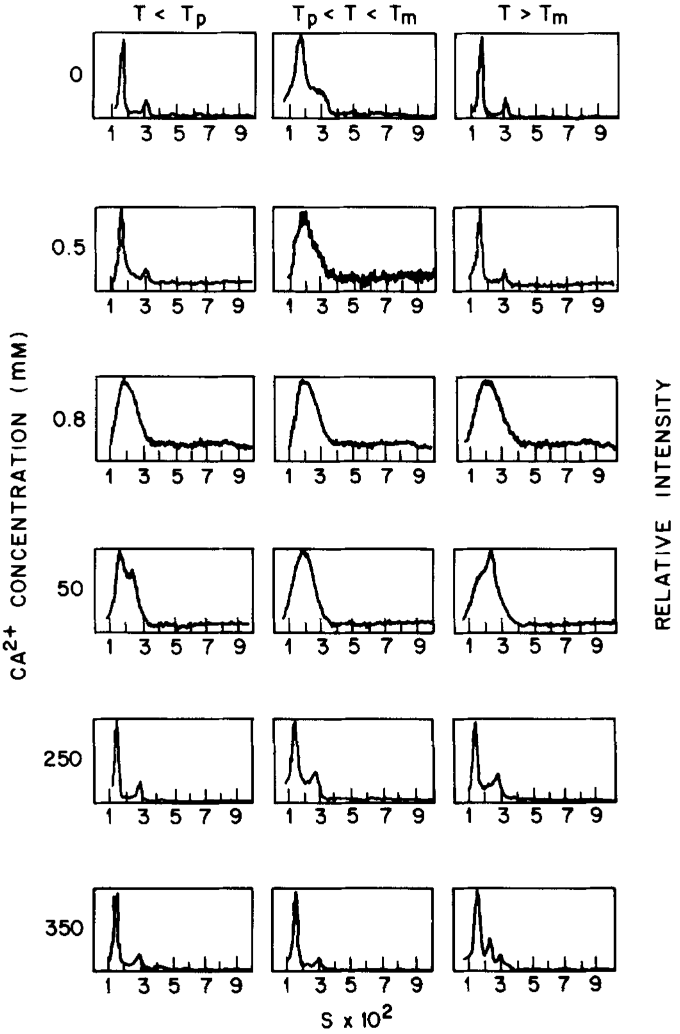

Fig. 3. X-ray diffraction patterns of dispersions of DPPC multilayers as a function of temperature and $CaCl_2$ concentration. Each column corresponds to the indicated temperature range. There are six rows corresponding to six concentrations of Ca^{2+}. The concentration range is from 0mM $CaCl_2$ (pure DPPC) to 350mM $CaCl_2$. For each plot, the vertical axis denotes relative intensity and the horizontal axis $S = 2\sin\theta/\lambda$ in units of $\mathring{A}^{-1}$.

range order.[9] In dispersions of single bilayers, for example, broad peaks are present which are attributed to electron density variation within the bilayer[10] modulated by very weak interbilayer correlations.

X-ray diffraction patterns (figure 3) for a number of calcium concentrations were taken at various temperatures. Beginning with pure DPPC at room temperature, and increasing the calcium content, we observe a gradual increase in the lattice disorder as evidenced by the increased incoherent scattering. At higher concentrations, the ordering gradually returns.

Between the pretransition and main transition (as determined by DTA), disordered profiles persist with increasing calcium until ≈ 100 *mM* concentrations are reached. Above this threshold, the scattering is coherent, and the repeat distance remains constant at ≈ 72Å. Here, the patterns show coherent Bragg scattering as in the dispersions with no calcium.

Above the main transition temperature, the lattice is reordered for both very low and very high calcium concentrations. However, intermediate concentrations show varying degrees of disorder with a maximum lattice distortion occurring between 0.8*mM* and 5*mM* as inferred from the linewidths. The observed lamellar spacing at high temperatures is diminished by the melting of the hydrocarbon chains. Evidence of a separate phase with a repeat distance of 46Å possibly due to membrane dehydration, can be seen at 350 mM calcium.

Discussion

Using DTA, we observe a halving in the magnitude of the pretransition in the presence of ~ 1 *mM* Ca^{2+}. For pure DPPC the x-ray diffraction data show an increase in lattice spacing and disorder at the pretransition as determined by linewidth measurements. We find that lattice disorder increases even below the pretransition temperature when $\sim$ *1 mM* Ca^{2+} is added. This disordering may be analyzed using the DLVO theory for colloidal dispersions in ionic media.[11] These results suggest that approximately one half of the pretransitional energy is associated with disordering the system of multilayers.

The remaining pretransitional energy seems to be associated with an intrinsic bilayer property. This intrinsic process may be incoherent rippling of single bilayers within the multilayer. Coherent rippling of the entire multilayer with no excess water present has been observed in this laboratory and by others.[3] The difference between excess water systems and constrained water is therefore largely manifested in incoherent and coherent scattering at the pretransition. The intrinsic process probably includes straightening of tilted chains[4] which would account for the increased lamellar spacing between the pre- and main transitions.

Without Ca^{2+}, the intrinsic process must initiate lattice disorder. With Ca^{2+}, lattice disorder occurs at a lower temperature than the pretransition but disordering continues at the pretransition. Further analysis of the x-ray intensity data should determine the structural changes associated with the disordering process.

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