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# The Influence of Monovalent Anions on Dipalmitoylphosphatidylcholine Bilayer Phase Transitions: A Time Resolved X-Ray Diffraction Study

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# The Influence of Monovalent Anions on Dipalmitoylphosphatidylcholine Bilayer Phase Transitions: A Time Resolved X-Ray Diffraction Study

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The effect of monovalent anions on the packing structures and transitions of the lamellar phases of dipalmitoylphosphatidylcholine bilayers has been investigated at the Daresbury Synchrotron Laboratory (U.K.). Fully hydrated DPPC bilayers in the presence of 1M KBr swell past the usual repeat spacing observed in water by approximately 20Å and in the presence of 1M KSCN form an interdigitated gel phase. The transition temperatures, determined by changes in the bilayer parameters in the small and wide angle scattering profiles, occur at temperatures previously observed. Our observations also indicate that the changes in the bilayer unit cell produced by the presence of Br or $CNS^-$ do not change the nature of the phase transition.

## INTRODUCTION

There is increasing evidence that monovalent ions influence the structure and packing of mesophases made of dipalmitoylphosphatidylcholine and other zwitterionic lipids.[1–15] Recently, these effects have been investigated using a time resolved x-ray diffraction tech-

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nique.[16-19] Time resolved studies make use of the high intensity of x-rays from a synchrotron source to obtain x-ray diffraction patterns in 100 ms or longer while driving the sample through the bilayer phase transitions. Our studies on the effects of monovalent cations[19] on the DPPC bilayer transitions indicated that the rate of heating the sample influences the time course of the transition for DPPC bilayers in 1 M KCl.

In this report, the phase transition temperatures and dynamic structures are determined for DPPC bilayers hydrated in the presence of 1M KBr or 1M KSCN. We have previously shown by calorimetry and static x-ray diffraction[11] that these ions dramatically influence the bilayer repeat spacings and/or the acyl chain packing within the subcell. We have confirmed that DPPC bilayers in the presence of 1M KBr swell past the usual limiting d-spacing of 6.2 nm to well over 8.0 nm and that DPPC bilayers in 1M KSCN adopt a bilayer configuration with interdigitated acyl chains. In addition, a determination of the DPPC bilayer structural parameters as a function of temperature in the presence of these salts indicate that the character of the phase transitions, which are first order in a thermodynamic sense, is unchanged when compared to DPPC bilayers in water. Specifically, the transitions proceed by a process of phase coexistence.

## MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Birmingham, AL) and used without further purification. Salt solutions were made with reagent grade salts and distilled water. X-ray samples were prepared by mixing DPPC powder with a large excess (>90wt%) of salt solution. The samples were equilibrated by standing at room temperature for four days. The samples were then mounted between mica sheets 1 mm apart in an x-ray sample holder.

The x-ray experiments were carried out by using a monochromatic (0.15 nm) focussed X-ray beam at station 7.3 of the Daresbury Synchrotron Laboratory as previously described.[20] A cylindrically bend single crystal of Ge[21] and a long float glass mirror were used for monochromatization and horizontal focussing, providing $2 \times 10^9$ photon $\cdot$ $s^{-1}$ down a 0.2 mm colimator at 2.0 GeV and 100 to 200 mA of electron beam current. A Keele flat plate camera was used with a linear detector fabricated at Daresbury. The dead-time between frames was 50 $\mu$s, with the temporal resolution of each frame of 1.2s.

X-ray scattering has been plotted as a function of tan 2 θ using teflon (0.48 nm) as a calibration standard.[22] No corrections were applied to path distances from the sample to the linear detector consequently wide-angle spacings will be slightly longer than measured directly by the detector. Repeat spacings were determined by using Bragg's Law.[23]

The temperatures scans were produced by water baths connected internally to the sample mount of the X-ray camera. The rate of change of temperature was about $10° \cdot min^{-1}$ in both heating and cooling modes. The temperature of the sample was monitored internally using a thermocouple placed adjacent to the sample region of the X-ray sample holder.

## RESULTS AND DISCUSSION

Dipalmitoylphosphatidylcholine bilayers in excess water undergo two major phase transitions: a pre-transition which occurs at approx. 37°C and a main transition which occurs at approx. 41°C. The pre- transition, defined as the change in the phase from the $L_{\beta'}$ (gel) bilayer phase to the $P_{\beta}$, (rippled) bilayer phase, is characterized by changes in the relative intensities of several peaks in the small angle x-ray scattering (SAXS) region descriptive of the three-dimensional packing array.[24-26] The main transition, defined as the change in phase from the $P_{\beta'}$ phase to the $L_{\alpha}$ (liquid crystal) bilayer phase, is characterized by changes in the relative intensities of peaks in the SAXS region and by changes from 4.2 to 4.75Å in the wide angle x-ray scattering (WAXS) region which is descriptive of the two-dimensional packing between the acyl chains.[25,26] The change in acyl chain packing within a subcell is even observed in systems where the "ripple" phase does not occur as an intermediate in the transition from gel to liquid crystal bilayer states. Fully hydrated DPPC bilayers in water are observed to have a repeat spacing of approx. 64Å in the $L_{\beta'}$ phase and of approx. 62Å in the $L_{\alpha}$ phase.[19]

Samples of dipalmitoylphosphatidylcholine bilayers in excess solutions of 1M KBr and 1M KSCN were subjected to a temperature scan of 10°C/min. The initial structure of DPPC bilayers in 1M KSCN at approx. 22°C indicate that the bilayer state has a repeat spacing of approx. 40.8Å and an acyl chain subcell spacing of approx. 4.06Å. This decrease in the repeat spacing is the result of a decrease in the bilayer thickness caused by the foundation of an interdigitated bilayer phase which is characterized by an acyl chain packing of 4.09Å.[27-33]

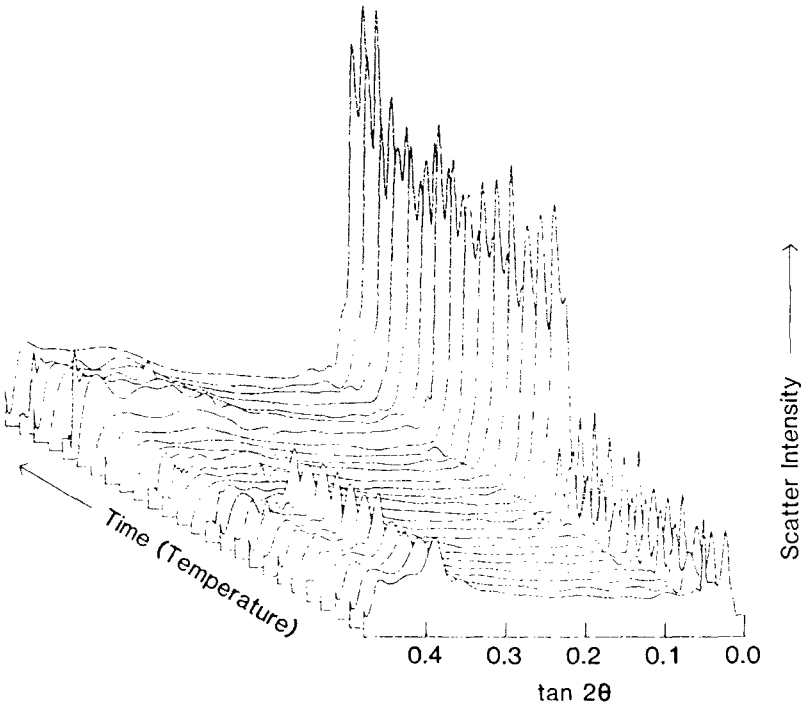


FIGURE 1 Three dimensional plot of scattered x-ray intensity, scattering angles and temperature for dipalmitoylphosphatidylcholine in 1M KSCN. Temperature was scanning at 10°C per minute between 22 and 73°C. A total of 255 frames of 1.2 S duration each were collected. Every tenth frame is presented in the figure.
Note: Peaks indexing at 6.38 nm in SAXS and 0.44 nm in WAXS are from unhydrated DPPC which was mixed with our sample.

We have previously observed this interdigitated phase for DPPC in 1M KSCN at 25°C using static x-ray diffraction.[11] The formation of an interdigitated bilayer phase of DPPC in 1M KSCN is most probably caused by a change in the solvent structure or water polarization adjacent to the bilayer surface. The final structure at 69°C is an interdigitated bilayer state having melted hydrocarbon chains with a bilayer repeat of approx. 57Å and an acyl chain subcell spacing of 4.4Å.

The diffraction patterns for DPPC bilayers in 1M KSCN collected as a function of time and therefore temperature indicate the presence of the main transition at approx. 46°C (Figure 1). This transition involves a change from the interdigitated bilayer phase to an $L_\alpha$ phase as observed by changes in the acyl chain packing from 4.06Å to 4.4Å in the WAXS region. The transition proceeds via the coexistence of

the interdigitated and $L_\alpha$ bilayer phases as determined by the coexistence of the diffraction patterns from the acyl chain subcells during the course of the transition. We do not observe a pre-transition due to the presence of an interdigitated bilayer phase. These results agree with previous determinations of the phase transitions by calorimetry.[11] The transit times for the main transition is approx. 1–2 sec which indicates that this transition is a first order phase transition. Several order phase transitions generally require intermediate phases with longer transit times.[34]

The initial structure of DPPC in 1M KBr at approx. 24°C was a gel state bilayer with repeat spacings of approx. 85Å and an acyl chain hexagonal subcell with a spacing of 4.2Å. The final structure at 55°C was a liquid crystal state bilayer with a repeat spacing of approx. 74Å and an acyl chain hexagonal subcell which does not produce a discernable diffraction peak but whose spacing is assumed to be approx. 4.7Å. We have previously shown that the increase in the repeat spacing of DPPC in 1M KBr is the result of an increase in the water separation between the bilayers. The increase in the repulsion between DPPC bilayers in the presence of 1M KBr is caused by increased ion (probably $K^+$) binding to the lipid headgroup, and a change in the polarization of the water between bilayers which causes an increase in the electrostatic and hydration forces, respectively. This increase in the interbilayer separation was also measured using static x-ray diffraction[11] and the limiting repeat spacing was found to be approx. 72Å at 70 wt % lipid for the bilayers in the gel phase. An increase in the bilayer repeat by ~10Å in the time resolved studies may be due to the large amount of salt solution (>80 wt %) present thus enabling more solvent to be imbibed between the bilayers.

Our time resolved collection of diffraction patterns as a function of time and therefore temperature (not shown) indicate that DPPC bilayers in the presence of 1M KBr underwent the pre-transition at approx. 36°C as determined by changes in intensities of peaks in the SAXS region and the main transition at approx. 44°C as determined by changes in the acyl chain packing in the WAXS region. Both the pre- and main transition temperatures occur at approximately the same temperatures as obtained by calorimetry.[11] Transit times for both transitions are 1–2 sec indicating that these transitions are first order phase transitions which is also observed for DPPC bilayers in water.[19] The transition proceeds by the coexistence of the $P_{\beta'}$ and $L_\alpha$ phases as again determined by the coexistence of the acyl chain subcell diffraction peaks during the course of the transition.

It can be concluded that both monovalent cations and anions in-

fluence the structure and packing of DPPC bilayers by binding to the lipid headgroup and/or changing the water structure in the double layer region induced by these ions. Time resolved x-ray diffraction measurements indicate that both ions do not affect the mode (i.e., coexistance of initial and final state) of these transitions.



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