PRESSURE LOCKING OF THE SUBGEL PHASE OF HYDRATED DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYERS.

A Raman Spectroscopic Study

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ABSTRACT Raman spectra of aqueous dispersions of 1,2-dipalmitoyl-phosphatidylcholine (DPPC) have been measured as a function of pressure (up to 46 kbar) for samples incubated at 2°C and for nonincubated DPPC samples subjected to equally high pressure. The nature of the transition from the GII gel phase of the hydrated lipid into the subgel phase on incubation is entirely different from that of the transition from the GII gel phase into the GIII gel phase of the nonincubated lipid. The GIII gel phase has a monoclinic interchain packing, while the subgel phase exhibits a triclinic interchain structure. It is shown that pressure cannot induce the transition from the GII gel phase to the subgel phase; however, it does stabilize the subgel phase above the subtransition temperature. The mechanism for the formation of the subgel phase and the complex phase behavior of the gel phase of DPPC are rationalized in terms of the dynamic properties of the acyl chains of the lipid molecule.

INTRODUCTION

The existence of multiple endothermic and barotropic phase transitions in aqueous 1,2-dipalmitoyl-phosphatidylcholine (DPPC) bilayers is well documented (Wong 1984; Casal and Mantsch, 1984; Wong and Mantsch, 1985*b*, *c*, and references therein). At ambient temperature and pressure DPPC bilayers exist in the GII gel phase. On heating, the GII gel phase converts to the GI gel phase at ~35°C (T_p, pretransition) and the GI gel phase converts to the liquid crystalline phase at 41.5°C (T_m, main transition). Both transitions have been well characterized. Upon gradual cooling, the GII gel phase of DPPC converts to the GIII gel phase at −30°C (Wong and Mantsch, 1983). However, when kept for an extended period at a temperature between −8° and 6°C, DPPC converts from the GII gel phase to a so-called "subgel" phase. On heating, the subgel (SG) phase reconverts to the GII phase at ~15°C (T_s, subtransition) (Chen et al., 1980; Füldner, 1981; Cameron and Mantsch, 1982; Ruocco and Shipley, 1982; Nagle and Wilkinson, 1982; Ter-Minassian-Seraga and Madelmont, 1984; Wu et al., 1985; Wong et al., 1985). When pressure is applied to DPPC dispersions at ambient temperature the lipid converts from the GII gel phase to the GIII gel phase at 1.7 kbar (Wong and Mantsch, 1984, 1985*a*). On the other hand, pressure can retard, or even prevent the GII → SG transition from occurring (Wong et al., 1985; Wu et al., 1985). The nature of this complex phase behavior among the GII, GIII, and SG gel phases of DPPC is still unknown.

The interchain structure of the GII and GIII gel phases has been characterized as hexagonal and monoclinic, respectively (Wong 1984, and references therein); however, the interchain structure of the SG phase has been described as orthorhombic (Ruocco and Shipley, 1982) and also as triclinic (Magni and Sheridan, 1982; Cameron and Mantsch, 1982; Wong et al., 1985). Thus, the interchain structure of the SG phase is not definitively established, nor is the mechanism of the GII → SG phase transition well understood. Recent results from our laboratory indicate that in *n*-alkanes known to have triclinic interchain structure the triclinic phase is stable up to pressures as high as 60 kbar, whereas in *n*-alkanes with known hexagonal interchain structure pressure induces a number of phase transitions, first from hexagonal to monoclinic, then to orthorhombic packing (unpublished results from this laboratory). Accordingly, the subgel phase of DPPC, if it is indeed triclinic, should also be stabilized by pressure. Therefore, in the present Raman spectroscopic study, we have investigated the effect of pressure on incubated and nonincubated samples of DPPC in the GII gel phase with the aim to confirm the triclinic structure of the subgel phase of DPPC. Furthermore, it was hoped that the present study would also shed some light on the mechanism behind the formation of the subgel

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phase, and thus clarify the complex phase behavior among the GII, GIII, and SG phases of DPPC.

EXPERIMENTAL

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) obtained from Avanti Polar Lipids Inc., Birmingham, Alabama (purity ≥99%) was suspended in double distilled water (50 wt.% H_2O), heated to 60°C, immediately vortexed and cooled to ambient temperature. The heating, vortexing, and cooling cycle was repeated several times to reconstitute the DPPC multilamellar assemblies. The sample was then kept at 2°C to incubate for 5 wk. The annealed DPPC sample, together with a small amount of powdered ruby (30 μm maximum dimension) were loaded into a 0.34 mm drillhole on a 0.23 mm thick stainless steel gasket mounted on a diamond anvil cell that had been precooled to the sample temperature (i.e., 2°C). The pressure on the sample was raised immediately to 26 kbar, and the cell mounted on the Raman spectrometer; a back scattering geometry was used. Raman spectra were recorded at various pressures up to 46.3 kbar. Eventually the pressure was fully released and a Raman spectrum was recorded at atmospheric pressure (0.001 kbar). Then, a second set of Raman spectra were recorded while the pressure was raised again up to 55.7 kbar. The pressure on the sample was monitored by the ruby R-line method with an accuracy of 0.01 kbar (see Wong and Mantsch 1984); the temperature was 28°C (which included the laser heating).

The Raman spectra of DPPC dispersions and of the ruby R-lines were recorded at a spectral resolution of ~2 cm^{-1} with a double monochromator (model 14018; Spex Industries Inc., Edison, NJ) equipped with 1800/mm holographic gratings and a cooled photomultiplier (model C31034; RCA, Lancaster, PA). A CRL Model 12 argon ion laser projected, at the sample, ~300 mW of excitation power at 514.5 nm. When measuring the ruby R-lines the 514.5 nm laser line was unfocused with 10 mW of power. Raman spectral data were acquired by a Datamate processor (model DM1; Spex Industries Inc.). Data was then transferred to a PDP 11 computer for data reduction.

RESULTS AND DISCUSSION

Representative Raman spectra of the methylene scissoring and the C-H stretching modes of incubated and nonincubated DPPC samples at several pressures are shown in Figs. 1 and 2, respectively. The main features in these spectral regions have been assigned and discussed in the literature (Boerio and Koenig, 1969; Snyder et al., 1978; Abbate et al., 1984; Wong, 1984; Levin, 1984).

Interchain Structure

It is immediately evident from Fig. 1 that the A_{1g} correlation field band near 1,410 cm^{-1} is present in the spectra of nonincubated DPPC at pressures above 1.7 kbar (part *B*), but is absent from the spectra of incubated DPPC (part *A*). The correlation field band of the CH_2 scissoring mode has been observed in the vibrational spectra of *n*-alkanes with either monoclinic or orthorhombic interchain structure, however, this characteristic band is absent in *n*-alkanes known to have hexagonal or triclinic interchain structure (Boerio and Koenig, 1982; unpublished results from this laboratory). Therefore, the absence of this correlation field band in the spectra of the subgel phase (Fig. 1 *A*) indicates that the possible interchain structures for this phase are either hexagonal or triclinic. Since the acyl chains are fully extended and orientationally highly ordered in the subgel phase (as indicated by all the Raman spectral parameters of this phase), the possibility of a less ordered hexagonal structure (Snyder et al., 1978; Levin, 1984) can be ruled out in this phase. Thus, the interchain structure in the SG phase is, in all likelihood, triclinic.

On the other hand, earlier Raman (Wong and Mantsch, 1984, 1985*a*) and infrared studies (Wong and Mantsch, 1985*c*) have shown that when a hydrostatic pressure is applied to the GII gel phase of DPPC it converts to the GIII, GIV, and GV gel phases at 1.7, 4.8, and 15 kbar, respectively. These phase transitions are associated with a change in correlation field splitting. There is no correlation field band in the distorted hexagonal GII phase (see

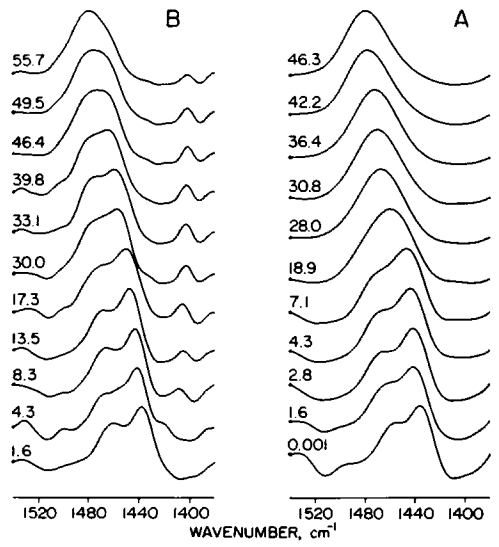

FIGURE 1 Raman spectra of fully hydrated DPPC bilayers in the methylene scissoring region at several pressures. (*A*) spectra for incubated and (*B*) spectra for nonincubated DPPC samples.

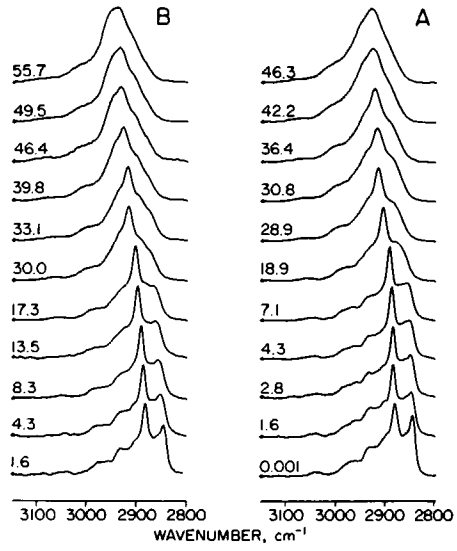

FIGURE 2 Raman spectra of fully hydrated DPPC bilayers in the region of the C-H stretching modes at several pressures. (*A*) incubated DPPC samples and (*B*) nonincubated DPPC samples.

spectrum at 1.6 kbar in Fig. 1 *B*). The correlation field band of the CH_2 scissoring mode appears above 1.7 kbar in the monoclinic GIII phase as a shoulder and becomes a well-defined band above 4.8 kbar in the orthorhombic GIV and GV phases. The correlation field band shifts to lower frequencies with increasing pressure, and an additional, weaker correlation field band appears above 15 kbar. The fact that the CH_2 correlation field band does not appear in the Raman spectra of the SG phase under pressure up to 46 kbar (see Fig. 1 *A*) confirms our earlier conclusion that the subgel phase of DPPC is triclinic, while the GIII phase, which is obtained at temperatures below −30°C, is monoclinic (see Wong et al., 1985).

Pressure Locking of the Subgel Phase

As discussed above, on cooling the distorted hexagonal GII gel phase of DPPC can convert either to the monoclinic GIII gel phase (at −30°C on continuous cooling), or to the subgel phase when equilibrated for an extended period at a temperature between −8° and 6°C. The subgel phase is stable at temperatures below the subtransition temperature T_s, but above T_s it immediately converts to the GII phase. On the other hand, it was shown that upon applying pressure up to 0.3 kbar the GII → SG phase transition could be retarded (Wu et al., 1985) and even prohibited at a higher pressure (Wong et al., 1985). From studies of the pressure effect on *n*-hexadecane, an alkane known to have triclinic interchain packing, we predicted that if the SG phase has the triclinic interchain structure, this phase should be stabilized above T_s by pressure. This prediction was indeed confirmed by the present study. The Raman spectra in Fig. 1 *A* show that when an incubated fully hydrated DPPC sample was kept under pressure greater than 1 kbar for over 1 wk at a temperature well above T_s, it remained in the SG phase. Thus, once the SG phase is formed, it can be stabilized by pressure. Upon releasing the pressure to 0.2 kbar, the SG phase reconverted immediately to the distorted hexagonal GII phase. Resubjecting this GII gel phase to increasing pressure it converted to the monoclinic GIII gel phase and then to the orthorhombic GIV and GV phases, in agreement with our earlier findings (Wong and Mantsch, 1984). These results demonstrate that although pressure cannot induce the GII → SG phase transition, it can stabilize the subgel phase. Thus, the triclinic interchain structure of the subgel phase is locked in by external pressure at temperatures above T_s.

Interchain Interactions

The Raman spectra in the C-H stretching region of both incubated and nonincubated DPPC (Fig. 2) show the presence of a band (at 2,858 cm^{-1} at atmospheric pressure) on the high frequency side of the $\nu_s CH_2$ mode. The emergence and increase in intensity of this component band becomes clearer when resolution enhancement (Kauppinen et al., 1981) is applied to this spectral region (see Fig. 3). A band at 2,858 cm^{-1} of intensity comparable

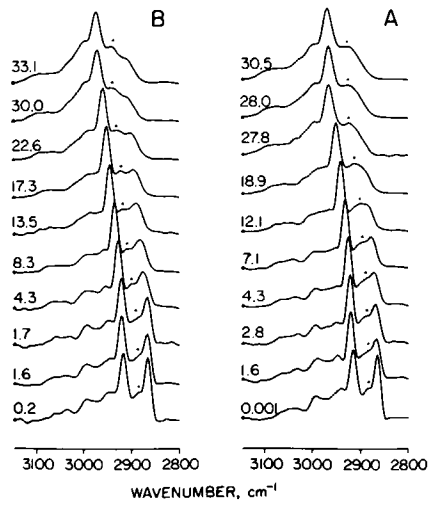

FIGURE 3 Representative Raman spectra of the C-H stretching modes of DPPC after band-narrowing by deconvolution with a 14 cm^{-1} wide Lorentzian line that reduces the intrinsic width of the bands by 10%.

with that of the $\nu_s CH_2$ band at 2,850 cm^{-1} was observed in the Raman spectrum of solid *n*-$C_{20}H_{42}$ an *n*-alkane with known triclinic interchain structure. This band had been suggested as a characteristic feature for the triclinic packing of methylene chains in *n*-alkanes (Gaber et al., 1978; Snyder et al., 1978). However, a similar, though less intense band, exists in the Raman spectra of solid *n*-alkanes with hexagonal, monoclinic, and orthorhombic structures (Larsson, 1973; Synder et al., 1978); this band disappears completely when the methylene chains are isotopically isolated (Snyder et al., 1978). Therefore, the 2,858 cm^{-1} band is attributed to interchain interactions and is often referred to as the interchain interaction band. This band is marked by an asterisk in Fig. 3. In the spectra of both incubated and nonincubated DPPC the interchain interaction band is much weaker than the $\nu_s CH_2$ band at ambient temperature and pressure. However, it shifts to higher frequencies and increases in intensity with increasing pressure; at very high pressures this band becomes even more intense than the $\nu_s CH_2$ band. We have observed the same phenomenon with solid *n*-alkanes under pressure, regardless of their interchain structure (unpublished results from this laboratory). Since the 2,858 cm^{-1} band is present in both incubated and nonincubated DPPC and because its intensity is pressure dependent we conclude that the height of the interchain interaction band is mainly determined by the magnitude of the interchain interactions and, that despite its considerable intensity at elevated pressures, it is not a Raman spectral marker for the triclinic interchain structure.

Mechanism for the Formation of the Subgel Phase

The complex phase behavior of the gel phase of DPPC can be rationalized in terms of the triclinic interchain packing

of the subgel phase in which the zig-zag planes of the acyl chains are parallel to each other.

The transition from the hexagonal GII gel phase to the monoclinic GIII gel phase involves the damping of the intermolecular rotational reorientations so that the orientations among DPPC molecules become highly ordered in the GIII gel phase (Wong, 1984). However, the transition from the GII gel phase to the subgel phase not only involves the damping of the reorientational fluctuations among the DPPC molecules, but also involves the reorientation of the two acyl chains within each DPPC molecule from nearly perpendicular to parallel to each other in the triclinic structure. This reorientation of the acyl chains is a slow process and only takes place over a limited temperature range (i.e., $-8°$ and $+6°C$). At temperatures above this range the mobility of the chains, especially the torsion or twisting motion of the acyl chains of each DPPC molecule, is too large to form an orientationally ordered phase such as the SG phase. On the other hand, at temperatures below this range the orientational mobility of the acyl chains, as well as that of the DPPC molecules, is highly restricted. In fact, at $-30°C$ the reorientational fluctuation among the DPPC molecules is almost completely damped and the GII gel phase can now convert to the highly ordered GIII gel phase in which the acyl chains of each DPPC molecule remain nearly perpendicular to each other due to the restricted chain reorientation at this low temperature. It is well documented (Wong, 1984) that pressure slows down the mobility of the chains. At 1.7 kbar and room temperature the rotational reorientations of the DPPC molecules are almost completely damped (basically the same effect as the decrease in temperature to $-30°C$), and the GII gel phase transforms to the highly ordered GIII gel phase. Therefore, due to the pressure restriction of the reorientation of the acyl chains of each DPPC molecule from nearly perpendicular to parallel, pressure does prohibit the transformation of the GII gel phase to the subgel phase. On the other hand, once the subgel phase is formed, pressure also restricts the reorientation of the acyl chains from parallel back to perpendicular to each other, and thus keeps the reorientational fluctuations highly damped. Consequently, the parallel orientation of the acyl chains in the triclinic subgel phase is locked in and stabilized by external pressure even when the temperature is raised well above the subtransition temperature.

As far as the role of water is concerned, it is clear that its presence is essential for the formation of the subgel phase (as it is for all the other gel phases). The pressure induced formation of ice VI at ~9 kbar and of ice VII of bulk water at ~20 kbar does not affect the structural changes at the GII → SG and GII → GIII phase transitions.

CONCLUSIONS

The present Raman study has helped to clarify the relationship among the GII, GIII, and SG gel phases of DPPC. On reducing the temperature, the distorted hexagonal GII gel phase of DPPC converts to the monoclinic GIII phase at $-30°C$, which is a fast process. Upon incubating the GII phase at ~2°C it transforms to the triclinic subgel phase, which is a slow process. Once the SG phase is formed, it is stable at temperatures below T_s and even below the temperature of the GII → GIII transition ($-30°C$). Above T_s the subgel phase immediately reconverts to the GII phase. However, under pressure, the subgel phase becomes stable above T_s; at 28°C, the SG phase is stable under pressures of up to 46 kbar. Pressure can only stabilize the SG phase but cannot convert the GII phase into the SG phase. Instead, pressure induces a transition from the GII gel phase to the GIII phase at ambient temperature. The mechanism of the GII → SG and GII → GIII transitions is governed by the intra and intermolecular reorientational mobility of the acyl chains.

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