A NEW CRYSTALLINE PHASE OF L-α-DIPALMITOYL PHOSPHATIDYLCHOLINE MONOHYDRATE

URS PETER FRINGELI, *Laboratory for Physical Chemistry, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zurich, Switzerland*

ABSTRACT A new phase transition of L-α-dipalmitoyl phosphatidylcholine (DPPC) monohydrate from the "biaxial" phase to a crystalline phase (*C* phase) has been found at 71°C by means of infrared attenuated total reflection (IR-ATR) spectroscopy. The transition is characterized by drastic conformational changes in the glycerophosphorylcholine moiety, which led on the one hand to an alignment of the turn near the ester group in the hydrocarbon chain at glycerol C(2) position. On the other hand a uniform conformation of the glycerophosphorylcholine moiety is found to be typical for the *C* phase, in contrast to nonuniform head group conformations of DPPC in other regions of the DPPC/water phase diagram investigated so far.

INTRODUCTION

An important factor in the structure analysis of lipid-water systems is the characterization of the different phases as a function of temperature and water content. A lot of work has been done in the past years to construct such phase diagrams (Chapman, 1968; Luzzati, 1968; Tardieu et al., 1973; Luzzati and Tardieu, 1974; Lee, 1977). Detailed information is available now on phase diagrams of synthetic lecithins. Different experimental techniques or combinations of them have been applied, such as x-ray diffraction (Chapman et al., 1967; Tardieu et al., 1973; Gottlieb and Eanes, 1974; Janiak et al., 1976), differential thermal analysis, proton and deuterium magnetic resonance (Gally et al., 1975), fluorescence studies (Galla and Sackmann, 1974; Jacobson and Papahadjopoulos, 1975), Raman spectroscopy (Lippert and Peticolas, 1972), diffusion studies in lipid-water lamellar phases (Rigaud et al., 1972), and conoscopic studies by light microscopy (Powers and Pershan, 1977). In the latter investigation, monodomain samples of dipalmitoyl phosphatidylcholine (DPPC)[1] with varying concentrations of water and other ingredients have been used. The resulting phase diagram is reproduced in Fig. 1. It reveals the existence of inhomogenous regions at the phase boundaries, which probably consist of two phases. The phase diagram has been completed, however, by a new crystalline phase of DPPC monohydrate. Preliminary results concerning this phase transition have been reported earlier (Fringeli et al., 1977). In this paper, detailed results of an IR-ATR spectroscopic investigation of the phase transition of DPPC monohydrate (~ 2% wt H_2O) near 71°C are reported. This transition resulted in a new phase which has not been

---

[1] *Abbreviations used in this paper: C* phase, crystalline phase of DPPC monohydrate (phase transition at ~71°C); DPPC, L-α-dipalmitoyl phosphatidylcholine; DPPC-$N(^{13}CH_3)_3$, DPPC with C-13 isotope labeled *N*-methyl groups; IR ATR, infrared attenuated total reflection.

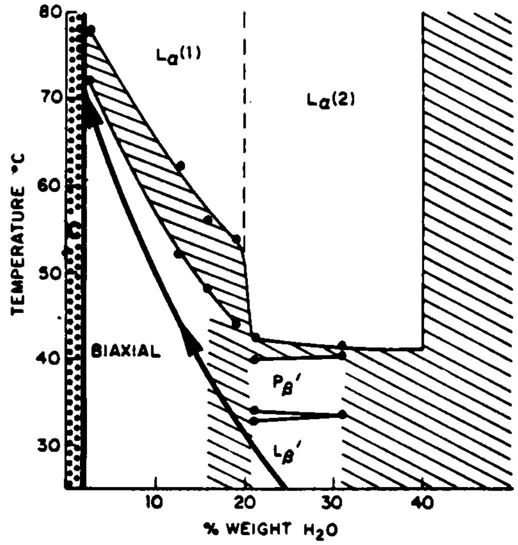

FIGURE 1 Modified phase diagram for oriented mixtures of DPPC and water (cf. Powers and Pershan, 1977). The slash marks indicate where the samples do not appear homogeneous. Phase conversion biaxial to C required heating of the sample along the path indicated in the diagram.

characterized so far. Both the hydrocarbon chain and the polar head group ordering of DPPC turned out to be considerably higher in the new phase than in the "biaxial" phase (Fig. 1). Because of the single crystalline character, the new phase is referred to as *C* phase throughout this paper. Independently, a corresponding phase transition has been found with DL-α-DPPC by means of infrared transmission spectroscopy.[2] Furthermore, new aspects concerning the molecular structure of the polar head group of DPPC have been derived from the *C* phase.

MATERIALS AND METHODS

DPPC, 1-palmitoyl lysophosphatidylcholine and DPPC-*N* ($^{13}CH_3)_3$ were synthesized by R. Berchtold, Biochemical Laboratory, Bern, Switzerland. Oriented multibilayers (mean thickness, 10–20 bilayers) have been prepared on one side of an ATR-plate from dilute (10^{-2}–10^{-3} M) chloroform solution by evaporation of the solvent. The plate served as one wall of a sealed cell, enabling the investigation of the lipid multilayer in a nitrogen gas atmosphere of controlled relative humidity (closed cycle). The second side of the plate was in close contact with the thermostated sample holder to establish a homogeneous temperature distribution in the sample. Spectra were scanned on a Perkin Elmer Model 225 infrared spectrometer (Perkin Elmer Corp., Instrument Div., Norwalk, Conn.) equipped with a KBr grid polarizer and an ATR attachment (Wilks Scientific Corp. Model 50). Single-pass trapezoid multiple internal reflection plates of germanium, zinc selenide (both 50 x 20 x 1 mm), and cadmium teluride (50 x 20x 2 mm) were obtained from Harrick Sci. Corp., Ossining, N. Y. The angle of incidence was 45°, resulting in 20 active internal reflections with germanium and zinc selenide plates and ten active reflections with cadmium teluride plates, respectively. For general information on ATR technique, the reader is referred to Harrick (1967), whereas more details concerning its application in membrane research are reported elsewhere (Fringeli, 1977; Fringeli and Fringeli, 1979; Fringeli, 1980).

The experiments were performed as follows. The DPPC multibilayer system was hydrated on the ATR-plate at 22°C in a nitrogen atmosphere containing 30,000-ppm H_2O, which corresponds to ~90%

---

[2] Jürgens et al. Manuscript submitted for publication.

relative humidity. The amount of bound water was ~25% by weight as determined from the O—H stretching absorption band at ~3,400 cm^{-1} using Lambert-Beer's law adapted to internal reflection spectroscopy (Fringeli, 1980). The corresponding normalized absorption coefficient was μ_0^2(3,400 cm^{-1}, 22°C) = (1.3 ± 0.1)· 10^{-18} reflection $^{-1}$ · molecule $^{-1}$ · cm $^{-2}$. Fig. 2 shows polarized IR-ATR spectra which are typical for this state. Now the temperature was enhanced stepwise, while the water content of the atmosphere remained constant at 30,000 ppm. This procedure led to a partial dehydration of the DPPC bilayers. The approximate pathway is indicated in the phase diagram in Fig. 1. It should be noted, however, that no attempt was made to investigate discontinuities in the dehydration process during the heating period. Measurements were performed at 22° (Fig. 2), 35°, 45°, 55°, 62° (Fig. 3), 71° (Figs. 4 and 5), 76°, 81°, and 90°C. The sample was equilibrated for at least 5 h at each temperature before the spectrum was scanned to see if slow conformational changes (such as the phase transition biaxial → *C* phase) were initiated under the corresponding conditions. The same sample was used for all temperatures.

SPECTROSCOPIC CONSIDERATIONS

Assignment of Significant Absorption Bands

Structural analysis was mainly based on the following group vibrations:

$\gamma_w(CH_2)$: Wagging progression of the methylene groups of a hydrocarbon chain in all-*trans* conformation, 1,180–1,350 cm^{-1}. The progression is erased by one or more *gauche* defects in

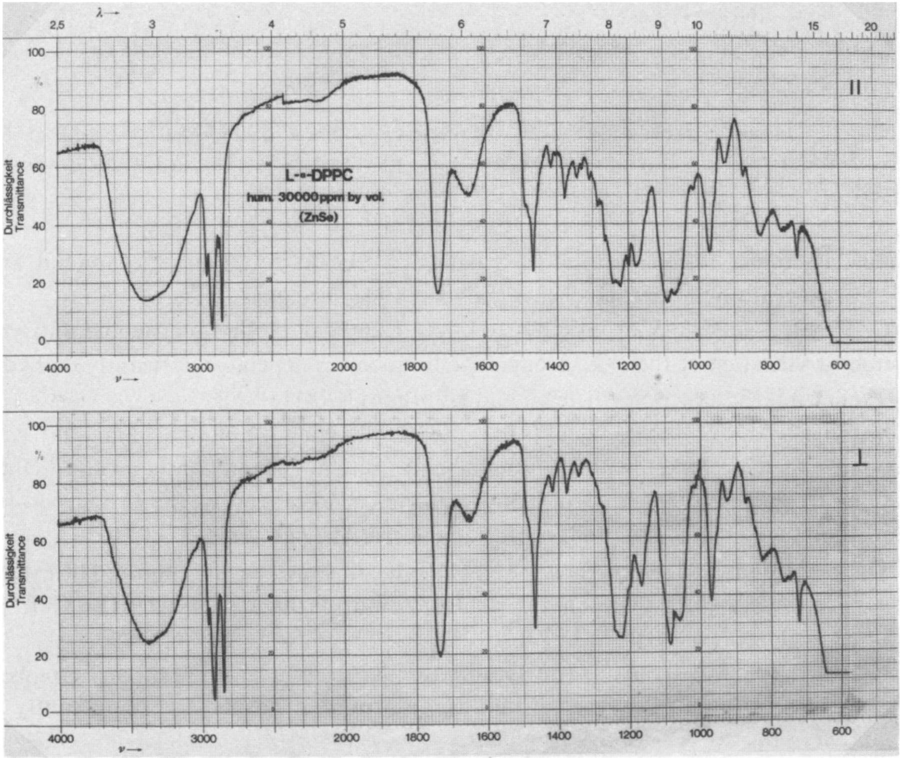

FIGURE 2 Oriented layers of DPPC at 22°C in nitrogen atmosphere containing 30,000 ppm H_2O (~90% relative humidity at 25°C). The spectra are typical for DPPC in the $L_{\beta'}$-phase (Fig. 1). (||) parallel and (⊥) perpendicular polarized incident light. ZnSe ATR-plate.

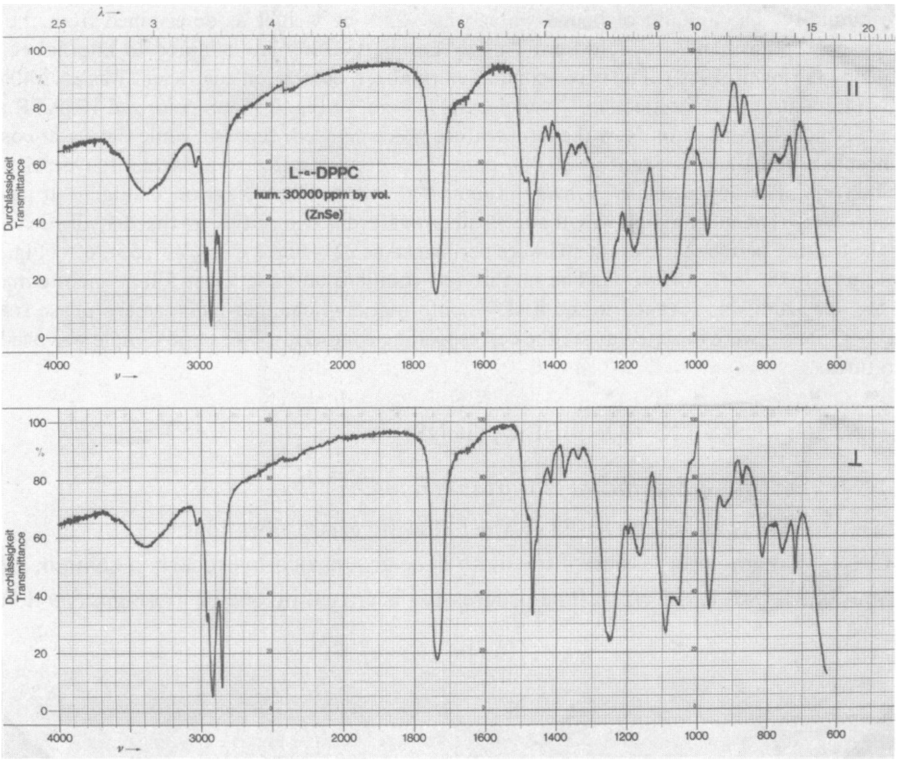

FIGURE 3 Oriented layers of DPPC at 62°C in nitrogen atmosphere containing 30,000 ppm H_2O. The spectra are typical for DPPC in the biaxial phase (~6% wt H_2O, 62°C, cf. Fig. 1). (∥) parallel and (⊥) perpendicular polarized incident light. ZnSe ATR-plate.

the chain. Therefore it can be used as a natural probe to determine the amount and the direction of all-*trans* hydrocarbon chains (Snyder, 1967; Fringeli, 1977).

$\nu_s(PO_2^-)$ (1,085–1,100 cm^{-1}), $\nu_{as}(PO_2^-)$ (1,200–1,260 cm^{-1}): Symmetric and antisymmetric stretching vibration of the PO_2^- group. The frequencies depend significantly on hydration and intermolecular interaction (Fringeli and Günthard, 1981).

$\nu_s(P[OR]_2)$ (755–765 cm^{-1}), $\nu_{as}(P[OR]_2)$ (810–825 cm^{-1}): Symmetric and antisymmetric PO single bond stretching with CO single bond stretching significantly involved (Fringeli, 1977). The frequencies depend significantly on the conformation of the C—O—P—O—C fragment (Shimanouchi et al., 1964; Forrest and Lord, 1977).

$\nu(CO[P])$ (1,040–1,080 cm^{-1}): C—O single bond stretching of phosphate ester. The frequency depends significantly on the conformation of the C—O—P—O—C fragment (Shimanouchi et al., 1964; Rihak, 1979).

$\nu_{as}(N[CH_3]_3)$ (950–970 cm^{-1}): Antisymmetric NC stretching of the methyl groups of the choline head group. The frequencies of the two absorption bands depend significantly on the conformation of the O—C—C—N fragment.

$\nu_s(N[CH_3]_3)$ (875 and ~910, ~930 cm^{-1}): Symmetric NC stretching of $N-(CH_3)_3^+$. There is a significant dependence on the conformation of the O—C—C—N fragment of choline. Two bands were found for *gauche* conformation at 875 and ~910 cm^{-1}, and one band

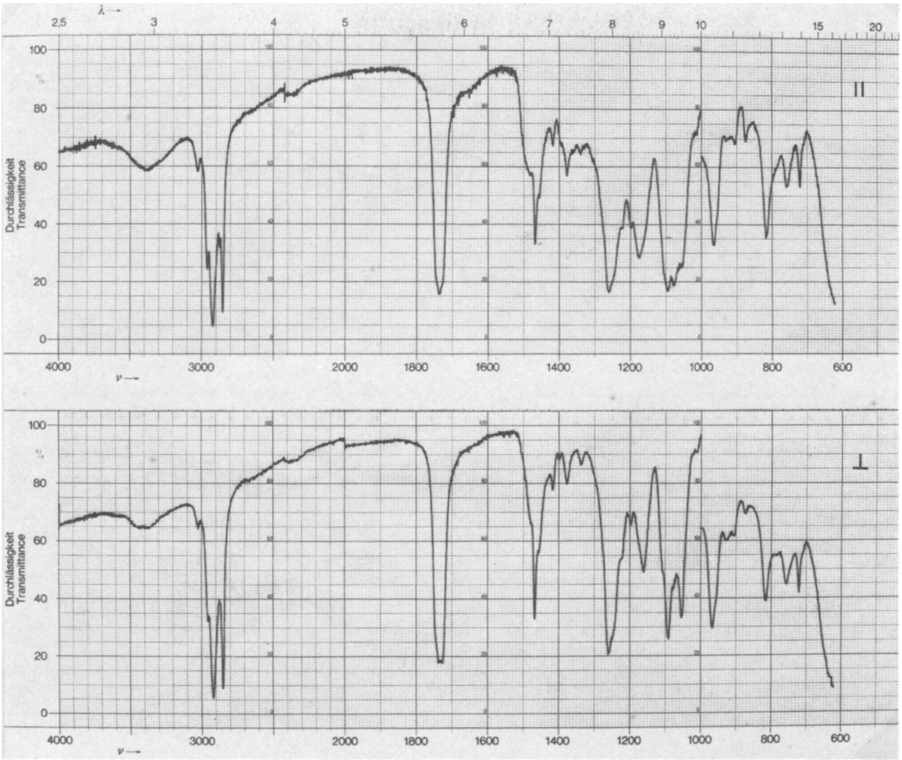

FIGURE 4 Oriented layers of DPPC at 71°C in nitrogen atmosphere containing 30,000 ppm H_2O. The spectra are scanned during the biaxial → C-phase transition of DPPC monohydrate. (∥) parallel and (⊥) perpendicular polarized incident light. ZnSe ATR-plate.

for *trans* conformation at ~930 cm^{-1} (Rihak et al., 1977; Fringeli, 1977; Rihak, 1979; Fringeli and Günthard, 1980). These bands may be used to estimate the *gauche* and *trans* fractions of the O—C—C—N fragment in DPPC. Potential energy distrubutions were calculated by means of normal coordinates analysis of choline and related compounds. It was found that the amount of ν_s ($N[CH_3]_3$) is >90% for *trans* conformation of O—C—C—N, i.e., the 930-cm^{-1} mode may be considered as an "isolated" group vibration. However, strong coupling of ν_s ($N[CH_3]_3$) with other vibrations occurs in the case of *gauche* conformation. The corresponding potential energy distributions were found to be ν_s ($N[CH_3]_3$) (67%) and ν_s (NC_4) (13%) for the 875-cm^{-1} band, and ν_s ($N[CH_3]_3$) (21%), ν (CO) (33%), γ_r (α-CH_2) (20%), and γ_w (β-CH_2) (14%) for the 910-cm^{-1} band, respectively. Furthermore, it has turned out that both the intensity and the frequency of the latter vibration are influenced by bound water and the crystallinity of the sample. One should expect that these effects originate from the large amount of (P) C—O single bond stretching, i.e., this band should also be sensitive to conformational and hydrational changes in the phosphate group region. This is indeed the case, as discussed below.

ν_s (NC_4) (710–720, ~760 cm^{-1}): Totally symmetric NC stretching vibration of C—N $(CH_3)_3^+$. The frequency depends strongly on the conformation of the O—C—C—N fragment. It was found to be at 710–720 cm^{-1} for *gauche*, and at ~760 cm^{-1} for *trans*

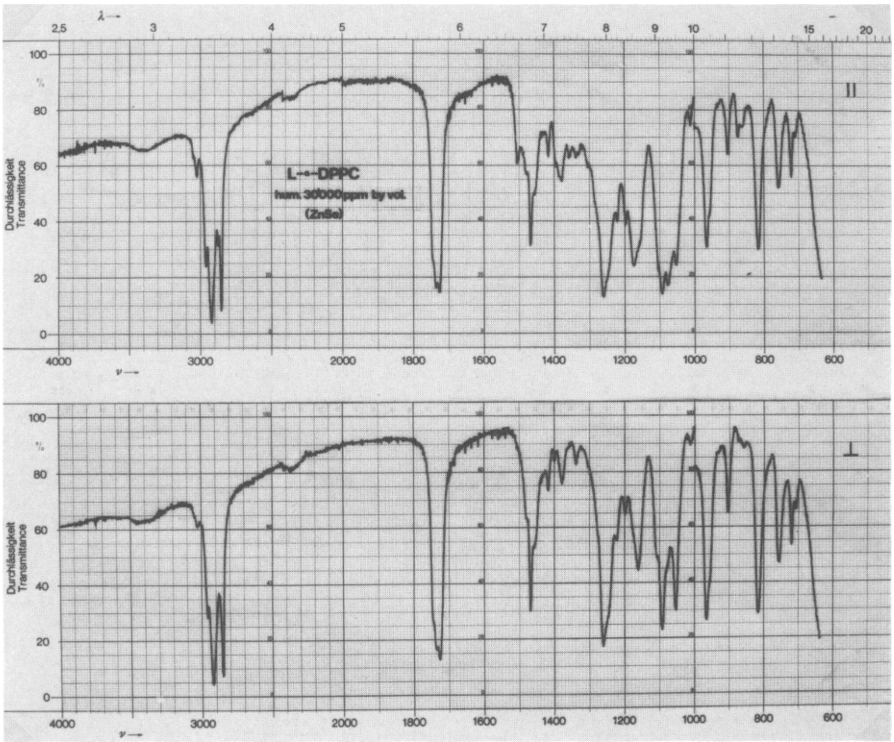

FIGURE 5 Oriented layers of DPPC at 71°C in nitrogen atmosphere containing 30,000 ppm H_2O. The spectra are typical for DPPC monohydrate in the crystalline phase (*C* phase, cf. Fig. 1). (‖) parallel and (⊥) perpendicular polarized incident light. ZnSe ATR-plate.

conformation, respectively. The corresponding potential energy distributions were calculated to be $\nu_s(NC_4)$ (88%) and $\nu_s(N[CH_3]_3)$ (14%) for *gauche*, and $\nu_s(NC_4)$ (92%) for *trans* conformation. This couple of bands is most valuable for conformational analysis of the O—C—C—N frame in choline groups by means of Raman spectroscopy. In infrared spectroscopy, however, these bands are mostly of small intensity because of the approximate T_d symmetry of the group. Nevertheless, strong totally symmetric NC stretching bands may occur, especially by $\nu(NC_4)$ coupling with a second vibration, which itself has an intense vibrating dipole moment. An example is given in Fig. 6, which shows infrared absorption spectra ($\alpha = -\ln T$) of chlorocholine. The potential energy distributions for the 768- and 715-cm^{-1} bands, respectively, were found to be $\nu_s(NC_4)$ (64%), $\nu(CCl)$ (23%) and $\nu(CC)$ (16%) (*trans* conformation of Cl—C—C—N), and $\nu_s(NC_4)$ (82%), $\nu_s(N[CH_3]_3)$ (14%) (*gauche* conformation of Cl—C—C—N). The considerable intensity of the *trans* band at 768 cm^{-1} (Fig. 6 *a*) thus results from coupling with the C—Cl stretching vibration $\nu(CCl)$.

Mole Fractions of Conformers

If a reliable assignment of corresponding conformation dependent vibrations has been achieved, the mole fraction of a conformer can be determined from the integrated intensities of corresponding bands of the coexisting conformations. It should be noted, however, that, generally, corresponding absorption coefficients related to different conformations of a fragment are different, i.e., mole fractions may not be determined by simple comparison of absorption band areas. Corresponding absorption coefficients must be known, which furthermore may depend on the degree of hydration, i.e., on intermolecular interaction. This complicating fact is demonstrated by Fig. 6. Chlorocholine assumes predominantly *trans* conformation in the crystal and *gauche* conformation in aqueous solution. *Trans-gauche* equilibrium can be controlled in the cyrstalline state by relative humidity of the gas phase (Rihak, 1979). Chlorocholine turned out to be of particular interest for conformational studies of Cl—C—C—N (corresponding to O—C—C—N in choline or lecithin), because in addition to vibrations of the quaternary ammonium group (see above) there is an additional conformation sensitive vibration, the C—Cl stretching mode (ν[CCl]: Bellamy, 1975, Herzberg, 1945, Huber-Wälchli and Günthard, 1975). It is located near 735 cm^{-1} for *trans* and near 670 cm^{-1} for *gauche* conformation. The potential energy distributions of these vibrations are ν (CCl) (80%), (ν_s[NC_4], ν[CC], γ_w[α—CH_2]) (~20%) for *trans*, and ν (CCl) (100%) for *gauche* conformation (Rihak, 1979). Fig. 6 *a–c* demonstrates clearly the appearance of the *gauche* conformer (*G*) upon hydration of the crystal, ν_s (N[CH_3]$_3$): 860 and ~910 cm^{-1}, ν (CCl): 666 cm^{-1}, ν_s (NC_4): 715 cm^{-1}. The corresponding *trans* bands (*T*) are at 920 cm^{-1} (ν_s[N(CH_3)$_3$]), 735 cm^{-1} (ν[CCl]), and 768 cm^{-1} (ν_s[NC_4]), respectively.

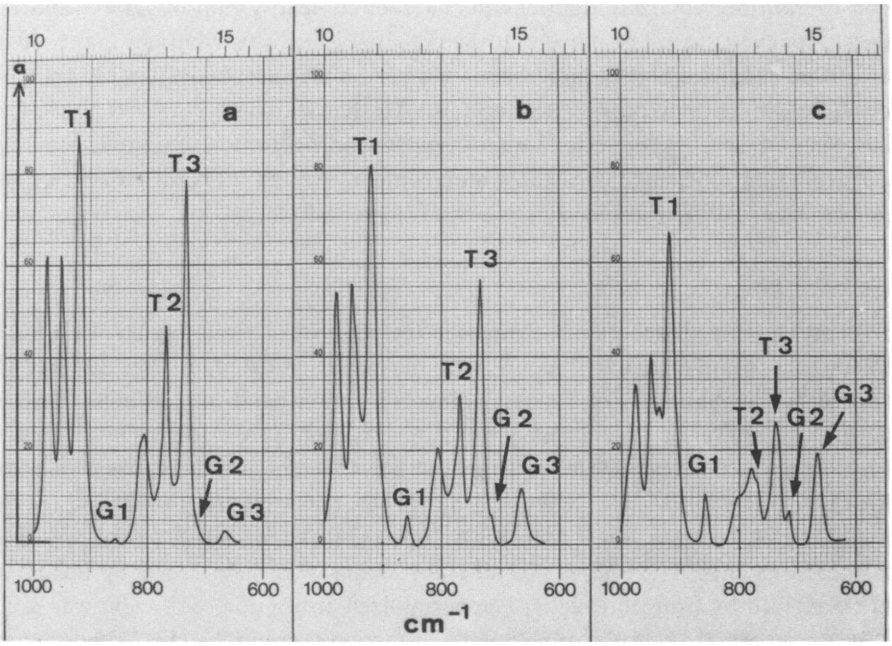

FIGURE 6 Conformation of the Cl—C—C—N fragment of equal amounts of cholorcholine as a function of the relative humidity of the gas phase (*a*) 1% relative humidity, (*b*) ~20% relative humidity D_2O, (*c*) 90% relative humidity D_2O. Typical conformation sensitive absorption bands are: ν_s(N[CH_3]$_3$): 920 cm^{-1} (*trans*,T1); ~910 and 860 cm^{-1} (*gauche*,G1): ν(NC_4): 769 cm^{-1} (*trans*,T2); 715 cm^{-1} (*gauche*,G2): (totally symmetric): ν(C—Cl): 735 cm^{-1} (*trans*,T3); 666 cm^{-1} (*gauche*,G3). ZnSe ATR-plate, parallel polarized incident light, T = 25°C.

Intensities of corresponding modes in Fig. 6 *a–c* reflect once more that the absorption coefficients critically depend on the conformation, and, in the case of the totally symmetric C—N stretching vibration ($\nu_s[NC_4]$, 768 cm^{-1}), also on the degree of hydration. Nevertheless, Fig. 6 *a–c* can be used to estimate the ratio of the absorption coefficients $\alpha_{trans}(\nu_s[N(CH_3)_3]$, 920 $cm^{-1})/\alpha_{gauche}(\nu_s[N(CH_3)_3]$, 860 cm^{-1}). Considering the potential energy distribution of ν (CCl) in *trans* and *gauche* conformation, as indicated above, one is led to the conclusion that the intensities of both bands should be determined predominantly by the oscillating dipole moment of the C—Cl stretching vibration. Therefore the ratio of *gauche*- and *trans*-band areas approximately equals the ratio of corresponding molar fractions, i.e., at 90% relative humidity (Fig. 6 *c*) chlorocholine exhibits about equal amounts of *gauche* and *trans* conformers. This conclusion is consistent with the 920-cm^{-1} band area in Fig. 6 *c* which is about one half the corresponding area in Fig. 6 *a* (overlapping by new *gauche* bands at 935 and ~910 cm^{-1} must be taken into account when estimating the area of this band in Fig. 6 *c*), indicating furthermore that $\nu_s(N[CH_3]_3)$ is not significantly affected by hydration. A comparison of the area of the 860-cm^{-1} band (Fig. 6 *c*) with that of the 920-cm^{-1} band (Fig. 6 *a*) reveals that the absorption coefficient of $\nu_s(N[CH_3]_3)$ has to be about seven times larger for *trans* conformation of Cl—C—C—N (920 cm^{-1}) than for *gauche* conformation (860 cm^{-1}). Moreover, since the corresponding potential energy distributions are found to be very similar in both choline and chlorocholine, the conclusion should also be valid for the O—C—C—N fragment of choline. This, however, enables an estimation of *gauche* and *trans* fractions in the choline head group of DPPC and related compounds. Finally, it should be noted that the potential energy distributions indicated in this paper have been calculated for isolated, noninteracting molecules: hydration effects have not been considered in normal coordinate analysis. As shown above, however, $\nu_s(N[CH_3]_3)$, which has been used for the determination of *gauche* and *trans* mole fractions, is an equally good approximation independent of the water content. An extensive discussion of further absorption bands of DPPC was given elsewhere (Fringeli, 1977).

RESULTS

Evidently, only minor conformational changes in the polar head group region of DPPC occur upon heating the sample to 62°C along with the path indicated in Fig. 1. This fact is demonstrated by the similarity of corresponding absorption bands of the polar head group (Figs. 2 and 3). However, enhancement of temperature was paralleled by a continuous reduction of hydrocarbon chain ordering. Fig. 7 *a* shows the amount of all-*trans* hydrocarbon chains relative to the initial sample as determined via the wagging progression ($\gamma_w[CH_2]$: 1,200, 1,222, 1,244, 1,266, 1,288, 1,310, 1,332 cm^{-1}). The band at 1,200 cm^{-1} was used for intensity measurements. The amount of all-*trans* chains was determined by means of the procedure described by Fringeli (1980). The normalized absorption coefficient was μ_0^2 (1,200 cm^{-1}, 22°C) = $(1.8 \pm 0.1) \cdot 10^{-19}$ $reflection^{-1} \cdot$ functional $group^{-1} \cdot cm^{-2}$. It decreased by 0.2%/°C as the temperature was increased. Concerning the amount of hydrocarbon chains assuming all-*trans* conformation there is an approximately linear decrease of ~ (1.3 ± 0.2)%/°C. However, when the sample was kept for several hours at 71°C, recrystallization of DPPC monohydrate (Fig. 1) occurred. The most surprising features of this process were on the one hand the doubling of the number of hydrocarbon chains with all-*trans* conformation. About

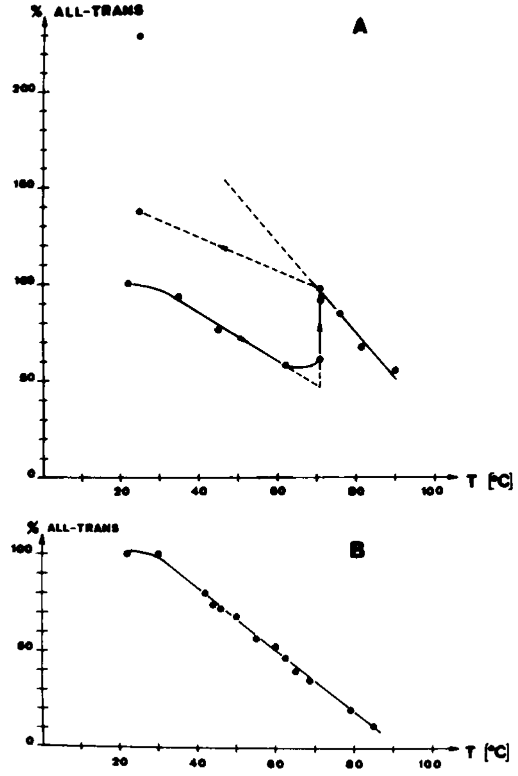

FIGURE 7 Effect of temperature on the structure of the hydrocarbon chains of oriented layers of DPPC. The percentage of all-*trans* chains was determined relative to the initial sample by means of the CH_2-wagging ($\gamma_w[CH_2]$) absorption band at 1,200 cm^{-1}. (*a*) Nitrogen atmosphere, 30,000 ppm H_2O. (The point at 228%/25°C was determined from *C*-phase DPPC in dry nitrogen, 200 ppm H_2O) (*b*) Nitrogen atmosphere, 200 ppm H_2O (~1% relative humidity at 25°C).

96% all-*trans* chains were found now with respect to the initial sample and, on the other hand, chain alignment was paralleled by a conformational change in the glycerophosphorylcholine part of DPPC, assuming now almost perfect orientation and uniform conformation (Figs. 4 and 5). The duration of the recrystallization process depended on the material of the ATR-plate, e.g., completion was achieved after 12 h on zinc selenide plates, but only after ~1 wk on germanium or cadmium teluride internal reflection plates. However, the structure of the *C* phase was found to be independent of the ATR-plate used, i.e., contamination by the plate material could not play a significant role. Further enhancement of the temperature up to 90°C resulted in only minor changes of the polar head group conformation. However, it led to a continuous reduction of hydrocarbon chain ordering. The reduction of all-*trans* hydrocarbon chains in the *C* phase was now determined from Fig. 7 *a* to be (2.6 ± 0.2)%/°C, i.e., twice the slope observed with DPPC in the biaxial phase under the same conditions.

Reduction of the temperature below 71°C reconverted DPPC into the original conformation. It should be noted, however, that hydrocarbon chain ordering was found now to be considerably better than in the initial sample (beginning of the cycle). On the other hand, if crystalline DPPC (*C* phase) is cooled down to 25°C in a dry nitrogen atmosphere (~200 ppm

H_2O) the molecular structure typical for the *C* phase remained stable over longer periods (the period of observation was 1 wk). Reconversion into the original structure occurred with a time constant of 1.2 min when *C*-phase DPPC was exposed at 25°C to a nitrogen atmosphere with 30,000 ppm H_2O (~90% relative humidity).

Finally, it should be noted that the transition from the biaxial phase (Fig. 1) to the *C* phase could not be accomplished by heating the sample in a dry nitrogen atmosphere. As demonstrated by Fig. 7 *b*, hydrocarbon chain ordering decreased linearly with increasing temperature. The slope was $(1.54 \pm 0.1)\%/°C$, i.e., approximately the same as already observed in humid atmosphere before the phase transition (cf. Figs. 1 and 7 *a*).

Drastic conformational and reorientational changes occured in the glycerophosphorylcholine region during the biaxial → *C* transition. A summary of the main features is given below: (*a*) Fatty acid ester groups: Typical absorption bands of this group result from C═O double-bond stretching (ν[C═O]: ~1,740 cm^{-1}) and C—O single-bond stretching (ν[C—,O]: ~1,180 cm^{-1}) (Fringeli, 1977). Splitting of ν(C═O) on the one hand and a more pronounced polarization of ν(C—O) at 1,175 cm^{-1} upon crystallization on the other hand (Figs. 3, 4, and 5) point to a more uniform conformation of the two fatty acid ester groups. The same information is obtained from the 1,090-cm^{-1} band, which is tentatively assigned to

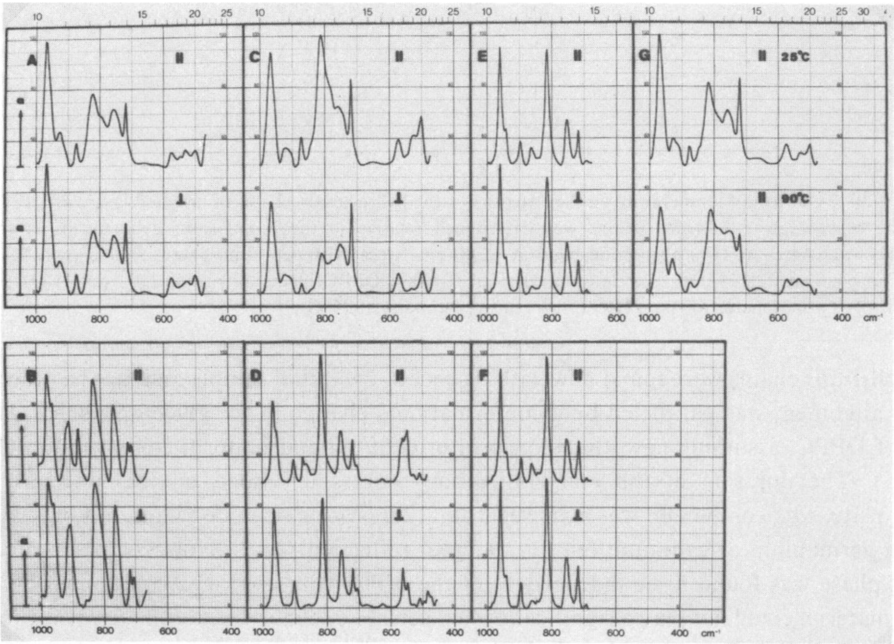

FIGURE 8 IR-ATR spectra of oriented layers of: (*a*) 1-palmitoyl lysophosphatidylcholine, N_2 atmosphere (200 ppm H_2O), "biaxial" phase, 22°C, KRS-5 ATR-plate; (*b*) 1-palmitoyl lysophosphatidylcholine, N_2 atmosphere (30,000 ppm H_2O), *C* phase, 71°C, ZnSe ATR-plate; (*c*) DPPC, N_2 atmosphere (200 ppm H_2O), "biaxial" phase, 25°C, CdTe ATR-plate (annealed sample); (*d*) DPPC, N_2 atmosphere (30,000 ppm H_2O), *C* phase, 71°C, CdTe ATR-plate; (*e*) DPPC-$N(^{13}CH_3)_3$, N_2 atmosphere (30,000 ppm H_2O), *C* phase, 67°C, ZnSe ATR-plate; (*f*) DPPC-$N(^{13}CH_3)_3$, N_2 atmosphere (200 ppm H_2O), *C* phase, 25°C, ZnSe ATR-plate; (*g*) DPPC, N_2 atmosphere (200 ppm H_2O), biaxial phase, 25° and 90°C, respectively, ZnSe ATR-plate. (∥) parallel and (⊥) perpendicular polarized incident light.

a stretching vibration of the O(1)—C(1)—C(2)—O(2) fragment of glycerol. (*b*) Phosphate group: Comparison of corresponding absorption bands (e.g., $\nu_{as}(PO_2^-)$(~1,250 cm^{-1}), $\nu_s(PO_2^-)$(~1,100 cm^{-1}), ν(CO[P])(1050 and 1080 cm^{-1}), ν_{as}(PO[C])(818 cm^{-1}) and ν_s(PO[C]) (759 cm^{-1}) as well as the phosphorylcholine deformation vibrations in the 400- to 600-cm^{-1} region (Fig. 8 *c*, *d*, and *g*) before (Fig. 3), during (Fig. 4) and after (Fig. 5) the phase transition biaxial → *C* reveals well resolved, distinct bands in the *C* phase. In contrast, broader and only weakly resolved bands are found in the biaxial and the $L_{\beta'}$-phase, respectively. The most reasonable explanation of this is that a uniform conformation of the phosphate group region can only be expected in the *C* phase. Nonuniform headgroup conformation had already been proposed earlier (Fringeli, 1977). More recent nuclear magnetic resonance (NMR) data of lysolecithin (Hauser et al., 1978) revealed the existence of three different conformations in the glycerol region, too.

Information about the orientation of the PO_2^- group was obtained from the polarizations of ν_{as} (PO_2^-) and ν_s (PO_2^-), respectively (Fringeli, 1977). In the *C* phase it was found that the connective line between the two nonesterified oxygen atoms of $>PO_2^-$ formed an angle of 60° ± 5° with the normal to the plane of bilayers. The corresponding angle of the bisector of O—P—O was found to be 33° ± 10°, i.e., the spacial orientation of the PO_2^- group remained approximately the same as in the biaxial and L-phases (Fringeli, 1977; Seelig, 1978; Griffin et al., 1978). Concerning the conformation of C(3)—O(3)—P—O(4)—C(4) the observed frequencies of ν_{as}(PO[C])(818 cm^{-1}) and ν_s(PO[C])(759 cm^{-1}) are in good agreement with the calculated frequencies (825 and 755 cm^{-1}, respectively) of g^+g^+ or g^-g^- conformation of this fragment (Shimanouchi, 1964). It should be noted, however, that comparison of Figs. 3 and 5 or Fig. 8 *c* and *d* reveal a drastic change in the polarization of ν_{as}(PO[C]) at 818 cm^{-1}, which probably results from internal rotations about the P—O(3) and O(3)—C(3) bonds. This conformational change orients the P—O(3)—C(3)—C(2) fragment in an upright position, probably assuming *trans* conformation. This interpretation is consistent with the strong ||-polarization of ν(CO[P]) at 1,078 cm^{-1}. On the other hand, the corresponding ν(CO[P]) vibration of the phosphocholine ester group at 1,052 cm^{-1} as well as the ν_s $(N[CH_3]_3)$, (*gauche*) vibration at 904 cm^{-1} (cf. Materials and Methods) exhibit a polarization typical for transition dipole moments oriented randomly in a plane parallel to the ATR-plate, i.e., parallel to the plane of the bilayers (Fringeli, 1977). Because normal coordinates analysis revealed that the directions of the transition dipole moments of both vibrations were significantly influenced by C—O single bond stretching, one should assume the C(4)—O(4) bond to deviate by <20° from the plane of the bilayers. Finally, some remarks should be made concerning the conformation of the choline head group. A number of typical vibrations have already been discussed in Materials and Methods. DPPC with ^{13}C-labeled *N*-methyl groups was used to check the assignments. This isotopic substitution led to frequency shifts of only those absorption band involving vibrations of the quaternary ammonium group. As demonstrated by Fig. 8 *d* and *e*, shifts of some wave numbers could indeed be observed with $\nu_{as}(N[CH_3]_3)$, $\nu_s(N[CH_3]_3)$ and $\nu_s(NC_4)$.

The absence of the 930-cm^{-1} component of $\nu_s(N[CH_3]_3)$ in the *C*-phase spectra (Figs. 5 and 8 *b*, *d*, *e*, and *f*) points to the absence of a *trans* conformer of 0(4)—C(4)—C(5)—N. Furthermore, the conformational changes in the glycerophosphorylcholine region had reoriented the C(5)—N bond, leading to distinct polarizations of $\nu_s(N[CH_3]_3)$ (*gauche*) at

875 cm^{-1} and of two *A*-type antisymmetric CH_3-bending vibrations of the *N*-methyl groups ($\delta_{as}[(N)CH_3]$, *A*-type) at 1,506 and 1,400 cm^{-1}, respectively. A more detailed discussion of the latter vibrations has been given earlier (Fringeli, 1977). The transition dipole moments of all three vibrations are directed approximately parallel to the C(5)—N bond, as concluded from normal coordinates analysis. Therefore, straightforward dichroic analysis (Fringeli, 1977; Fringeli and Günthard, 1980) resulted in 15° ± 5° for the angle between the C(5)—N bond and the normal to the plane of the bilayers. Moreover, the distinct polarization of these bands gives further support to the conclusion of uniform *gauche* conformation of O(4)—C(4)—C(5)—N in the *C* phase.

Furthermore, it should be mentioned that the conformation of the choline head group of DPPC in the biaxial and L-phases is not expected to be completely uniform, because the existence of the $\nu_s(N[CH_3]_3)$ band at ~930 cm^{-1} points to a certain amount of *trans* conformation. This amount could be enhanced upon heating DPPC in dry nitrogen atmosphere from 25° to 90°C. Enhancement of the *trans* band at ~930 cm^{-1} is paralleled by a decrease of the intensities of the two corresponding *gauche* bands at ~920 and 875 cm^{-1}, respectively (cf. Fig. 8 *g*). Accurate determination of *trans* and *gauche* fractions, however, is complicated by two facts, namely overlapping of $\nu_s(N[CH_3]_3)$(*trans*, ~930 cm^{-1}) and $\nu_s(N[CH_3]_3)$(*gauche*, ~920 cm^{-1}), as well as by considerable differences in the absorption coefficients of *gauche* and *trans* bands (cf. Materials and Methods). Nevertheless, one may estimate that at 25°C the amount of *trans* conformation of DPPC in the biaxial and L_β-phase is ~10%.

Finally, it should be noted that the phase transition biaxial → *C* reported for oriented layers of DPPC could also be observed with oriented layers of 1-palmitoyllysophosphatidylcholine under the same experimental conditions (Figs. 8 *a* and *b*).

DISCUSSION

The IR-ATR data reported in the preceding sections enable a detailed understanding of the phase transition biaxial → *C* (Fig. 1) on a molecular level. Evidently, the transition originates in a significant conformational change of the polar head group. This fact is documented on the one hand by the existence of the same phase transitions in DPPC and in 1-palmitoyllysophosphatidylcholine, and on the other hand by the apparent realignment of already melted hydrocarbon chains (cf. Fig. 7 *a*). The real reason for the enhancement of the amount of all-*trans* hydrocarbon chains, however, was a conformational change in the glycerol moiety, which led to an alignment of the turn near the fatty acid ester group attached to glycerol C(2) position. This turn has found to be typical for a variety of phospholipids (Hitchcock et al., 1974; Seelig and Seelig, 1975; Fringeli, 1977). Because of this turn, chain 2 could never contribute to the wagging progression (see above) as long as DPPC assumed the conformations typical for the biaxial phase, and $P_{\beta'}$-phase, or one of the L-phases (Fig. 1). Only in the *C* phase, all-*trans* conformation of chain 2 is established. This is documented by a doubling of the intensity of the CH_2-wagging progression in the course of the transition at 71°C, as well as by doubling of the slope of the percent of all-*trans* vs. temperature curve (Fig. 7 *a*). It should be noted, however, that alignment of chain 2 could also be induced by the peptide antibiotic alamthicin when incorporated into dry DPPC double layers (Fringeli and Fringeli, 1979).

Taking the molecular structure of dipalmitoylphosphatidylethanolmanine as determined by x-ray analysis into account (Hitchcock et al., 1974), one may conclude from a molecular framework model that at least four internal rotations are required to obtain a molecular structure of DPPC, consistent with the IR-ATR data reported here. Rotation about $C_O(2)$—$C_\alpha(2)$ ($C_O[2]$, $C_\alpha[2]$):C atoms of carboxyl group, and α-methlene group of chain 2, respectively), C(2)—O(2) and C(1)—O(1) would enable alignment of the hydrocarbon chain 2 at glycerol C(2) position, whereas rotation about the P—O(3) bond would bring the O(3)—C(3)—C(2) fragment in upright position as required from polarization measurements. The following tentative structure of DPPC in the *C* phase is consistent with the IR-ATR data reported in this paper: Chain 1: $C_\alpha(1)$—$C_O(1)$—O(1)—C(1), *trans;* $C_O(1)$—O(1)—C(1)—C(2), *gauche* (+); O(1)—C(1)—C(2)—C(3), *trans*. Chain 2: $C_\alpha(2)$—$C_O(2)$—O(2)—C(2); *trans;* $C_O(2)$—O(2)—C(2)—C(3), *trans;* O(2)—C(2)—C(3)—O(3), *gauche* (+). Phophorylcholine: C(2)—C(3)—O(3)—P, *trans;* C(3)—O(3)—P—O(4), *gauche* ($\mp$); O(3)—P—O(4)—C(4), *gauche* ($\mp$); P—O(4)—C(4)—C(5), *trans;* O(4)—C(4)—C(5)—N, *gauche* (±).

Evidently, the *C* phase of DPPC offers a chance for an accurate determination of the structure of glycerophosphorylcholine by x-ray diffraction. However, one should be aware that significant differences exist between the structure of DPPC in the *C* phase and under biologically relevant conditions.

Concerning the molecular structure of DPPC in the latter case it should be noted that the IR-ATR results reported above are generally in good qualitative agreement with recent NMR data, e.g., nonuniform conformation of the glycerol moiety of lysophosphatidylcholine was concluded from ^{1}H-NMR data (Hauser et al., 1978) and identical spacial orientations of the phosphate group were found by both ^{31}P-NMR experiments (Griffin et al. 1978; Seelig, 1978), and IR-ATR spectroscopy. The different interpretations of the phosphorylcholine structure, which are based on the one hand on ^{31}P- and ^{2}H-NMR data (Seelig, 1978) and on the other hand on IR-ATR data (Fringeli, 1977; this paper) are probably only apparently contradictory. IR-ATR spectroscopy reveals at least two different conformations for both the C(3)—O(3)—P—O(4)—C(4) and the O(4)—C(4)—C(5)—N fragments. In the latter case, IR-ATR data showed that *gauche* conformation was dominant. However, a significant amount (~10%) of the fragment was found to assume *trans* conformation. On the other hand, the structure of the polar head group of DPPC as concluded from NMR data is either all-*gauche* (+) or all-*gauche* (−), featuring rapid conformational transitions from one form into the other. It should be noted that "rapid" has to be considered in the time scale of NMR spectroscopy, i.e., in a microsecond scale. Obviously, intermediate conformations of the phosphorylcholine group must occur during an all-gauche (+) to all-gauche (−) transition and vice versa. However, the intermediates could be too short lived to be resolved by NMR spectroscopy. Since IR spectroscopy has a time resolution on the order of 10^{-13} transient conformations are always detected, unless their concentrations are too small. This fact could be a reasonable explanation for the detection of a small amount of *trans* conformation in the choline head group of DPPC by IR-ATR spectroscopy.

Finally, it should be mentioned that hydrocarbon chain ordering of DPPC in the $L_{\beta'}$ and biaxial phases was considerably enhanced by the annealing process $L_{\beta'} \longrightarrow$ biaxial $\longrightarrow C \longrightarrow$ biaxial $\longrightarrow L_{\beta'}$, as documented by Fig. 7 *a*. The mean tilt angle of the hydrocarbon chains is now found to be 20° ± 5° in the biaxial phase at 22°C and 2–4% water content by weight. A tilt angle of 29°–35° has been reported earlier (Fringeli, 1977) for nonannealed oriented layers of DPPC under the same experimental conditions. Recently, Stamatoff et al. (1979) have studied the hydrocarbon chain tilt angle of DPPC in the biaxial phase containing 2% and 10% water by weight. The x-ray analysis revealed a decrease of the tilt angle with increasing water content, paralleled by an increase of the mosaic spread from 4° to 10°. The tilt angle was reported to be 12.5° at 10% water content. This finding is in agreement with our infrared ATR data obtained from annealed DPPC multilayers under corresponding conditions.

The author thanks Mrs. M. Fringeli, Dr. A. M. Tatone, and Miss S. Wiedemann for skillful technical assistance, Dr. P. Rihak for valuable discussions, and Prof. W. Stoffel, Institute of Physiological Chemistry, University of Cologne, West Germany, for providing ^{13}C-labeled lecithins and sphingomyelins, which have been useful for spectroscopic assignment.

Supported by the Swiss National Foundation, Project No. 3.192.0.77 and by the Emil Barell Foundation (F. Hoffmann-La Roche, Basel, Switzerland).

Received for publication 29 April 1980 and in revised form 18 November 1980.

REFERENCES

Bellamy, L. J. 1975. The Infrared Spectra of Complex Molecules. Methuen & Co., London.

Chapman, D., R. M. Williams, and B. D. Ladbrooke, 1967. Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1,2-diacyl-phosphatidylcholines (lecithins). *Chem. Phys. Lipids.* 1:445–475.

Chapman, D., and D. F. H. Wallach. 1968. Recent physical studies of phospholipids and natural membranes. *In* Biological Membranes. D. Chapman, editor, Academic Press, Inc., London. 1:125–199.

Forrest, G., and R. C. Lord. 1977. Laser raman spectroscopy of biomolecules. X-frequency and intensity of the phosphodiester stretching vibrations of cyclic nucleotides. *J. Raman Spec.* 6:32–37.

Fringeli, U. P., P. Rihak, and Hs. H. Günthard. 1977. Temperature and hydration dependent conformational changes of the polar headgroup of L-α-dipalmitoyl-lecithin. *Experientia.* (*Basel*). 33:818.

Fringeli, U. P. 1977. The structure of lipids and proteins studied by attenuated total reflection (ATR) infrared spectroscopy. *Z. Naturforsch.* 32c:20–45.

Fringeli, U. P., and M. Fringeli. 1979. Pore formation in lipid membranes by alamethicin. *Proc. Natl. Acad. Sci. U.S.A.* 76:3852–3856.

Fringeli, U. P. 1980. Distribution and diffusion of alamethicin in a lecithin/water model membrane system. *J. Membr. Biol.* 54:203–212.

Fringeli, U. P., and Hs. H. Günthard. 1981. Infrared membrane spectroscopy. *In* Molecular Biology, Biochemistry and Biophysics. E. Grell, editor. Springer Verlag, Berlin. 31:270–332.

Galla, H. J., and E. Sackmann. 1974. Lateral diffusion in the hydrophobic region of membranes: use of pyrene excimers as optical probes. *Biochim. Biophys. Acta.* 339:103–115.

Gally, H., W. Niederberger, and J. Seelig. 1975. Conformation and motion of the choline head group in bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. *Biochemistry.* 14:3647–3652.

Gottlieb, M. H., and E. D. Eanes. 1974. Coexistence of rigid cristalline and liquid crystalline phases in lecithin-water mixtures. *Biophys. J.* 14:335–342.

Griffin, R. G., L. Powers, and P. S. Pershan. 1978. Head-group conformation in phospholipids: a phosphorus-31 nuclear magnetic resonance study of oriented monodomain dipalmitoylphosphatidylcholine bilayers. *Biochemistry.* 17:2718–2722.

Harrick, N. J. 1967. Internal Reflection Spectroscopy. Interscience Publishers, New York.

Hauser, H., W. Guyer, B. Levine, P. Skrabal, and R. J. P. Williams. 1978. The conformation of the polar group of lysophosphatidylcholine in H_2O; conformational changes induced by polyvalent cations. *Biochim. Biophys. Acta* 508:450–463.

Herzberg, G. 1945. Infrared and raman spectra of polyatomic molecules. Van Nostrand. Reinhold Corp., New York.

Hitchcock, P. B., R. Mason, K. M. Thomas, and G. G. Shipley. 1974. Structural chemistry of 1,2 dilauroyl-

DL-phosphatidylethanolamine: molecular conformation and intermolecular packing of phospholipids. *Proc. Natl. Acad. Sci. U.S.A.* 71:3036–3040.

Huber-Wälchli, P., and Hs. H. Günthard. 1975. Trapping of unstable molecular conformations in argon matrices: *gauche-* and *trans*-1,2-Difluoroethane. *Chem. Phys. Lett.* 30:347–351.

Jacobsen, K., and D. Papahadjopoulos. 1975. Phase transition and phase separations in phospholipid membranes induced by changes in temperature, pH and concentration of bivalent cations. *Biochemistry.* 14:152–161.

Janiak, M. J., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyllecithin. *Biochemistry.* 15:4575–4580.

Kjellander, R. 1978. Water—a structural element in model membrane system: an approach to some structural changes in the lecithin-water system. *J. Colloid Interface. Sci.* 66:303–312.

Lee, A. G. 1977. Lipid phase transitions and phase diagrams. I. Lipid phase transitions. *Biochim. Biophys. Acta.* 472:237–281.

Lippert, J. L., and W. L. Peticolas. 1972. Raman active vibrations in long-chain fatty acids and phospholipid sonicates. *Biochim. Biophys. Acta.* 282:8–17.

Luzzati, V. 1968. X-ray diffraction studies of lipid-water systems. *In* Biological Membranes. D. Chapman, editor. Academic Press, Inc., London, 1:71–121.

Luzzati, V., and A. Tardieu. 1974. Lipid phases: structure and structural transition. *Ann. Rev. Phys. Chem.* 25:79–94.

Powers, L., and P. S. Pershan. 1977. Monodomain samples of dipalmitoylphosphadidylcholine with varying concentrations of water and other ingredients. *Biophys. J.* 20:137–152.

Rigaud, J. L., C. M. Gary-Bobo, and Y. Lange. 1972. Diffusion processes in lipid-water lamellar phases. *Biochim. Biophys. Acta.* 266:72–84.

Rihak, P., U. P. Fringeli. and Hs. H. Günthard. 1977. Temperature and hydration dependent conformational changes of choline and glycerophosphorylcholine. *Experientia.* (*Basel*). 33:828.

Rihak, P. 1979. Infrarot-Spektroskopie von Cholin and Lecithin. Thesis ETH-Zürich *6393.*

Seelig, A., and J. Seelig. 1975. Bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. Conformational differences between the fatty acyl chains. *Biochim. Biophys. Acta.* 406:1–5.

Seelig, J. 1978. ^{31}P-nuclear magnetic resonance and the head group structure of phospholipids in membranes. *Biochim. Biophys. Acta.* 515:105–141.

Shimanouchi, R., M. Tsuboi, and Y. Kyogoku. 1964. Infrared spectra of nucleic acids and related compounds. *Adv. Chem. Phys.* 7:435–496.

Snyder, R. G. 1967. Vibrational study of the chain conformation of the liquid *n*-paraffins and molten polyethylene. *J. Chem. Phys.* 47:1316–1350.

Stamatoff, J. B., W. F. Graddick, L. Powers, and D. E. Moncton. 1979. Direct observation of the hydrcarbon chain tilt angle in phospholipid bilayers. *Biophys. J.* 25:253–261.

Tardieu, A., V. Luzzati, and F. C. Reman. 1973. Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.