Mudd, S. H., & Cantoni, G. L. (1957) *Nature (London) 180*, 1052.
Ng, H., & Anderson, J. W. (1978) *Phytochemistry 17*, 2069–2074.
Ohshima, T., & Soda, K. (1979) *Eur. J. Biochem. 100*, 29–39.
Ohshima, T., Misono, H., & Soda, K. (1978) *J. Biol. Chem. 253*, 5719–5725.
Olson, O. E., Novacek, E. J., Whitehead, E. I., & Palmer, I. S. (1970) *Phytochemistry 9*, 1181–1188.
Painter, E. P. (1947) *J. Am. Chem. Soc. 69*, 229–234.
Pan, F., & Tarver, H. (1967) *Arch. Biochem. Biophys. 119*, 429–434.
Shrift, A. (1973) in *Organic Selenium Compounds: Their Chemistry and Biology* (Klayman, D. L., & Günther, W. H. H., Eds.) pp 763–814, Wiley, New York.
Snell, E. E., & DiMari, S. J. (1970) *Enzymes, 3rd Ed. 2*, 335–370.
Soda, K. (1968) *Anal. Biochem. 25*, 228–235.
Stadtman, T. C. (1980) *Annu. Rev. Biochem. 49*, 93–110.
Sunde, R. A., & Hoekstra, W. G. (1980) *Biochem. Biophys. Res. Commun. 93*, 1181–1188.
Walsh, C. (1979) in *Enzymatic Reaction Mechanism*, pp 816–827, W. H. Freeman, San Francisco, CA.
Zdanski, G. (1968) *Ark. Kemi 29*, 449–453.

Dependence of Acyl Chain Packing of Phospholipids on the Head Group and Acyl Chain Length†

David G. Cameron,* Eva F. Gudgin, and Henry H. Mantsch

ABSTRACT: The temperature dependencies of the factor group splitting of the infrared-active CH_2-scissoring bands of a series of fully hydrated gel phase diacyl phospholipids were determined. It is shown that, in all cases, the acyl chains are packed in an orthorhombic subcell and that the degree of rigidity of the subcell increases with increasing chain length. It is also demonstrated that the subcell in 3-*sn*-phosphatidylcholines differs from that found in 3-*sn*-phosphatidylethanolamines and 3-phosphatidic acids.

While the gel phases of model membranes such as 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC)[1] and 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine (DPPE) may be generally categorized as solidlike with the acyl chains in predominantly trans conformations, they in fact exhibit subtle but complex thermotropic behavior. The pretransition, T_{pre}, of 3-*sn*-phosphatidylcholines has recently been characterized as a particular solid–solid phase transition, similar to a transition found in *n*-alkanes (Cameron et al., 1980a; Janiak et al., 1976, 1979). Between T_{pre} and the chain melting phase transition, T_m, the acyl chain packing is hexagonal. Immediately below T_{pre}, the packing is orthorhombic (Figure 1) with a high degree of torsional and librational motion about the long axes of the acyl chains. As the temperature is reduced, the rates and amplitudes of motion about the long axes decrease (Davis, 1979; Marsh, 1980), and at low temperatures (−50 °C), the orthorhombic packing is quite rigid (Cameron et al., 1980b). Further, it has recently been reported (Chen et al., 1980) that incubation of phosphatidylcholines at 0 °C for long periods (1–3 days) results in a third exothermic transition near 15–20 °C.

Gel phase thermotropic behavior dependent on both the chain length and the head group has been previously reported. In a series of homologous phosphatidylcholines, the difference in temperature between T_{pre} and T_m decreases as the chain length is increased (Mabrey & Sturtevant, 1976). There is also a dependence of T_{pre} on whether the chain length is odd or even (Silvius et al., 1979), similar to differences observed in homologous series of fatty acids and other terminally substituted *n*-alkanes (Malkin, 1952).

Differences related to the head group are 2-fold. First, the pretransition has only been observed in phosphatidylcholines and in the closely related phosphatidylsulfocholines (Tremblay & Kates, 1981). Second, in phosphatidylethanolamines, T_m is generally observed some 10–30 °C higher than the T_m of the corresponding phosphatidylcholine. Indications of progressive changes in the acyl chain packing at reduced temperatures have also been observed in X-ray studies of DPPE bilayers (Harlos, 1978). In addition, X-ray studies of DPPE and DPPC (Janiak et al., 1976, 1979; Harlos & Eibl, 1980; McIntosh, 1980) have shown substantial differences in the bilayer thicknesses. This has been attributed to a tilting of the acyl chains of DPPC but not those of DPPE, the tilt resulting from the relatively large volume occupied by the phosphatidylcholine head group compared to that occupied by the phosphatidylethanolamine head group (McIntosh, 1980).

Infrared spectroscopy provides an extremely sensitive method for monitoring changes in the acyl chain packing of phospholipids. In the orthorhombic subcell, common to solid polyethylene, *n*-alkanes, and terminally substituted *n*-alkanes, the interchain interactions result in factor group splitting of the CH_2-scissoring band at 1468 cm^{-1} and the CH_2-rocking band at 720 cm^{-1} (Snyder, 1961, 1979). This factor group

† From the Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada. *Received January 16, 1981.* This paper is NRCC Publication No. 19131.

[1] Abbreviations used: DLPC, 1,2-dilauryl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DHPC, 1,2-diheptadecanoyl-3-*sn*-phosphatidylcholine; DSPC, 1,2-distearoyl-3-*sn*-phosphatidylcholine; DBPC, 1,2-dibehenoyl-3-*sn*-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine; DPPA, 1,2-dipalmitoyl-3-*sn*-phosphatidic acid; FT-IR, Fourier-transform–infrared spectroscopy.

0006-2960/81/0420-4496$01.25/0

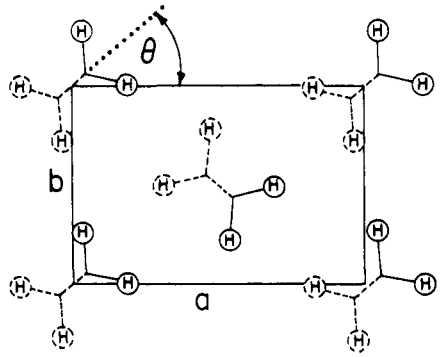

FIGURE 1: Two-dimensional orthorhombic subcell of *n*-diacyl phospholipids. The *a*–*b* plane corresponds to the plane of the bilayer surface; the long axes of the acyl chains are projecting from the page.

splitting is specific to orthorhombic packing and does not occur in the alternative hexagonal and triclinic subcells; hence, its observation permits one to characterize the packing of the lipid acyl chains. In addition, the magnitude of the band separation is indicative of the degree of interchain interaction (Decius & Hexter, 1977) and thus, indirectly, of the amplitudes and rates of motion about the long axes of the acyl chains.

In this study, we have determined the gel phase temperature dependencies of the CH_2-scissoring bands of a series of phosphatidylcholines, ranging from DLPC, with short acyl chains (C12:0), to DBPC, with very long acyl chains (C22:0). In addition, we have investigated the thermotropic behavior of the bands in a series of lipids with the same acyl chains but different head groups. The results demonstrate a dependence of the acyl chain packing on both the chain length and the nature of the head group.

Experimental Procedures

All lipids used in this study were obtained from Sigma (St. Louis, MO). Purities were checked by thin-layer chromatography; in all cases, only one spot was observed. Thin films were prepared by dissolving the lipids in $CHCl_3$/MeOH (4/1 v/v), depositing droplets on CaF_2 windows, and evaporating off the solvent at atmospheric pressure and then under vacuum for a period of 14 h. The samples were then hydrated (all at pH 7, DPPA and DPPE also at pH 11) and assembled into a 6-μm-thick cell following the procedures outlined in detail elsewhere (Cameron et al., 1979). No evidence of dichroism was observed in polarized spectra.

The cell was placed in an evacuable variable temperature chamber located in the sample compartment of a Perkin-Elmer 180 infrared spectrophotometer. Spectra were recorded at a resolution of 1.5 cm^{-1} at temperatures ranging from −100 to 30 °C; the temperature was monitored by a copper–constantan thermocouple located against the cell windows. The spectrum of DLPC at −190 °C was measured with a cold-finger liquid nitrogen cell.

The thermal history of the lipid samples used in the experiment was as follows. Samples were heated to above the temperature of the corresponding acyl chain melting phase transition and then within 30 min cooled to −100 °C. The spectra were then recorded at increasing temperatures during the course of 4–6 h. As the experiment was completed within 7 h, we observe no evidence of the third phase transition recently reported by Chen et al. (1980) which requires a long incubation (1–3 days) at temperatures near 0 °C.

Results

Figure 2 shows the CH_2-scissoring bands of the infrared spectra of fully hydrated multibilayers of DLPC, DMPC,

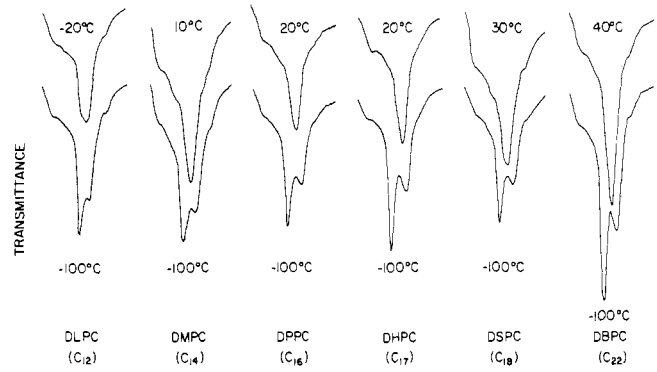

FIGURE 2: CH_2-scissoring region of the infrared spectra of the diacyl phosphatidylcholines at −100 °C (bottom row) and 20–30 °C below the T_m (top row). In each case, spectra are shown from 1500 (left) to 1440 (right) cm^{-1}; i.e., frequencies decrease from left to right.

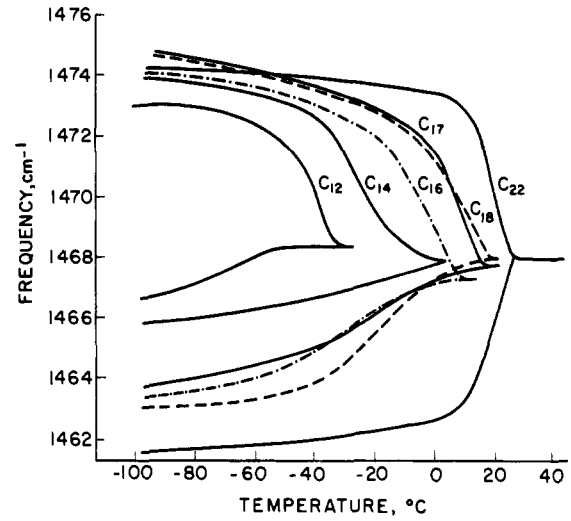

FIGURE 3: Temperature-dependent factor group splitting of the acyl chain CH_2-scissoring mode of the diacyl phosphatidylcholines. In order to contain the data on one plot and permit a visualization of differences resulting from variations in the chain length, continuous curves have been plotted through the experimental data, and error bars have been omitted. For an example of the uncertainties, see Cameron et al. (1980b).

DPPC, DHPC, DSPC, and DBPC at −100 °C and at a temperature about 20–30 °C below the T_m of the respective lipid. The intense narrow absorptions which dominate this region result from the out-of-phase CH_2-scissoring mode of the fully extended acyl chains, while the bending modes of the glycero and choline methylene groups, and those of the methyl groups, result in the underlying weak lines.

In all cases at temperatures near the T_m, a single CH_2-scissoring band contour is observed at 1468 cm^{-1}. At −100 °C, the band is split due to interchain coupling typical of that resulting from packing of the acyl chains in an orthorhombic subcell. As we have demonstrated in the case of DPPC (Cameron et al., 1980a), the splitting commences at T_{pre}, and the separation between the component bands increases progressively as the temperature is reduced below T_{pre}. Initially, the splitting is so small that it can only be monitored by means of FT–IR difference spectra and by changes in bandwidth. As the temperature is further reduced, the splitting increases, and two peak maxima are resolved; the temperature dependence of the splitting can then be monitored via the peak positions (Cameron et al., 1980b).

Figure 3 shows plots of temperature versus peak position of the CH_2-scissoring bands of the diacyl phosphatidylcholines. From this figure, it can be seen that the infrared spectra of

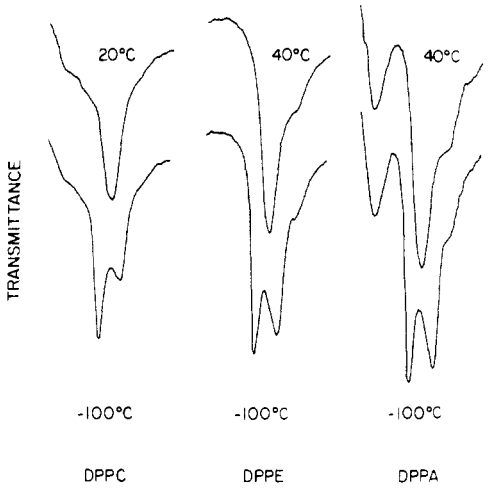

FIGURE 4: CH_2-scissoring region of the infrared spectra of dipalmitoylphospholipids at −100 °C (bottom) and 20–30 °C below the T_m (top). In each case, spectra are shown from 1500 (left) to 1440 (right) cm^{-1}; i.e., frequencies decrease from left to right.

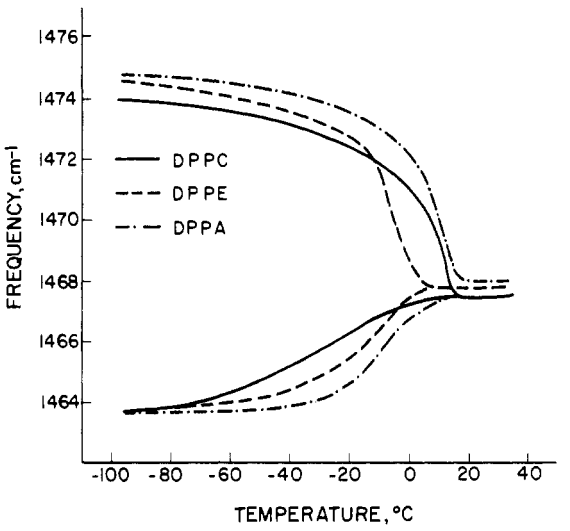

FIGURE 5: Temperature dependence of the factor group splitting of the CH_2-scissoring modes of dipalmitoylphospholipids. DPPE and DPPA were measured at pH 7 and 11; the curves were identical. See Figure 3 legend for additional details.

all the phosphatidylcholines exhibit a similar thermotropic behavior but that there are specific differences related to the acyl chain length. Although the low-temperature band contours in Figure 2 are similar, the data in Figure 3 shows that there are differences in the magnitudes of the splitting. The greatest band separation is observed in the DBPC spectra. As the acyl chain length decreases, so does the splitting, the rate of reduction being particularly large in the series DPPC, DMPC, and DLPC. This dependence of the magnitude on the chain length is emphasized by the results of a measurement of the spectrum of DLPC at −190 °C. The CH_2-scissoring bands were observed at 1473.5 and 1465 cm^{-1}, splitting which is still substantially less than that in the spectrum of DPPC at −100 °C (1474 and 1463.5 cm^{-1}).

It is of further interest to consider the way in which the band separation changes as the temperature is increased. In all cases, a rapid reduction of the splitting commences about 50–70 °C below the T_m. The splitting then changes over a 20–40 °C temperature range until only a single band contour at 1468 cm^{-1} is observed. Differences in the rate of reduction of the splitting related to the chain length are not large. However, there is evidently a tendency for the reduction to occur over a smaller temperature range as the chain length is increased.

The results of varying the head group are given in Figure 4, which shows the CH_2-scissoring region of the infrared spectra of the dipalmitoylphospholipids DPPC, DPPE, and DPPA at −100 °C and ~20 °C below the T_m. Figure 5 shows the positions of the CH_2-scissoring bands in the spectra of these compounds as a function of temperature; in all three cases, the two components of the scissoring mode separate at similar rates, and the same separation is achieved at low temperature.

Although, at −100 °C, the magnitude of the splitting does not depend on the nature of the head group, there is a dependence of the rate of the splitting on the head group, particularly when we consider that the T_m of DPPC is about 20 °C lower than those of DPPE and DPPA. More clearly dependent on the nature of the head group are the changes of the band contours and the way in which they change as a function of temperature, as shown in Figure 6. The low-temperature band shapes in the spectra of DPPA and DPPE are typical of those found in the spectra of most terminally substituted alkanes, and in the spectrum of polyethylene (Krimm et al., 1956; Snyder et al., 1978; Tasumi et al., 1964). The two components have similar peak heights, in contrast to

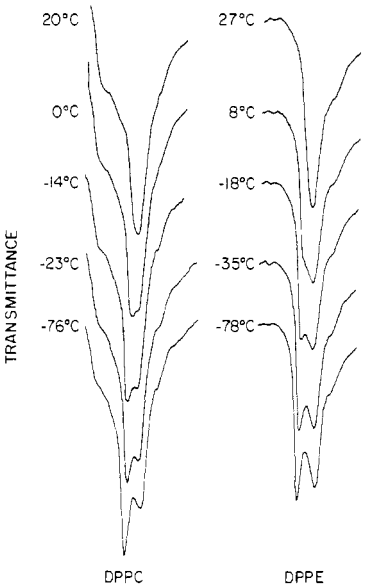

FIGURE 6: Dependence of the temperature-included changes in the CH_2-scissoring band contours of the dipalmitoylphospholipids on the nature of the head group. In each case, spectra are shown from 1500 (left) to 1440 (right) cm^{-1}; i.e., frequencies decrease from left to right.

the band contours of the phosphatidylcholines, in which the peak height of the 1474-cm^{-1} component is much greater than that of the 1464-cm^{-1} component. As expected on the basis of earlier studies, similar relative peak heights and bandwidths are evident in the CH_2-rocking band of DPPC at low temperatures (Mantsch et al., 1980), demonstrating that the effect in the CH_2-scissoring mode does not result from overlap with one of the underlying choline or terminal methyl bending bands. Figure 6 also illustrates that there are substantial differences in the temperature dependencies of the shapes of the CH_2-scissoring band contours of DPPC and DPPA. In the spectrum of DPPC (and all other phosphocholines), the peak height of the higher frequency component is greater than that of the lower frequency component at all temperatures. However, in the DPPE spectra (and those of DPPA), the lower frequency component has the greater peak height at higher temperatures, but as the temperature is reduced, the relative height of the high-frequency component increases until at −80 °C it is the stronger band.

Finally, we emphasize that the observation of a single maximum at 1468 cm^{-1} does not mark the cessation of splitting, only the point at which the bands overlap to the degree that they cannot be resolved in the absorption spectrum. Other FT-IR studies (this laboratory, unpublished experiments) demonstrate that in all cases factor group splitting continues up to T_{pre} (phosphatidylcholines) or T_m (DPPA, DPPE). We also note that in a X-ray study of DPPE (Harlos, 1978), it has been shown that there is a large change in the acyl chain packing at about 20 °C, and recent calorimetric data (Chen et al., 1980) demonstrate endothermic transitions of DPPC, DHPC, and DSPC multibilayers around 18–20 °C after annealing for several days at 0 °C. The temperature range roughly corresponds to the most rapid rate of change of the factor group splitting. However, recent studies (this laboratory, unpublished experiments) demonstrate that the correspondence is only fortuitous and that the results obtained by Chen et al. are specific to samples incubated for long periods at temperatures less than 6 °C and greater than −20 °C.

Discussion

Effects of Chain Length. We shall consider three aspects of the data in terms of the effects resulting from variations in the acyl chain length: (i) the low-temperature band contours, (ii) the magnitudes of the splittings at low temperatures, and (iii) the decrease in the factor group splitting with increasing temperature.

The band contour reflects the summation of the bands resulting from the two components of the CH_2-scissoring mode. The intensities, I_a and I_b, of the two bands are related to each other (Snyder, 1961) by

$$I_a/I_b = \tan^2 \theta$$

where θ is the angle between the carbon skeletal plane and the a axis of the subcell (Figure 1). Thus, the observation that the relative intensities of the two components are similar in all the phosphatidylcholines indicates that the overall shape of the subcell is the same, regardless of the chain length.

Since factor group splitting depends on the relative orientation of neighboring chains, the magnitude of the splitting at any given temperature results from two factors. First, there is a dynamic factor reflecting the degree of torsional and librational motion about the long axis of the acyl chains. The greater the amplitudes of such motions, the smaller the vibrational coupling between the chains (Decius & Hexter, 1977). This effect results in the reduction of the splitting with increasing temperature. It implies a loosening of the packing in the orthorhombic subcell, without a concomitant loss of the shape of the subcell. The other factor reflects the interchain distance and the degree to which the acyl chains can pack in the orthorhombic subcell. In an ideal case, that is, compounds being essentially comprised of methylene groups such as long alkanes and polyethylene, extremely uniform packing with strong van der Waals' interactions is obtained. The introduction of progressively larger terminal groups and the reduction of the chain length reduces the interchain interactions. This is essentially a steric effect, most evident near the terminal substituents (Hitchcock et al., 1974; Pearson & Pascher, 1979). Thus, in phospholipids, the interchain interactions will be greatest in the central regions of the acyl chains, the region which most closely resembles the *n*-alkanes and polyethylene. Such an effect was clearly evident when we studied a series of dipalmitoylphosphatidylcholines with specifically deuterated acyl chains. The pretransition, reflecting the loss of interchain interactions, is only observed in the central region of the acyl chains (Cameron et al., 1981).

Consequently, we consider that the relatively small splitting in the DLPC spectra reflects the perturbations to the packing introduced by the terminal methyl and head groups. The effects of the latter will be particularly large, due to the complex conformation in the region of the ester linkages (Hitchcock et al., 1974; Pearson & Pascher, 1979) and, in the case of the phosphatidylcholines, the relatively large cross-sectional area of the head group (McIntosh, 1980), which impose a large interchain distance near the bilayer surface.

As the acyl chains are lengthened, the potential for van der Waals' interactions is increased, while the steric perturbations to the packing are kept constant. The consequence is that the factor group splitting, reflecting the degree of interaction over the total length of the acyl chains, increases. The results of this are most evident at low temperatures, where the dynamic effects are minimal. However, the larger interchain interaction in the longer acyl chains also results in the relatively large splitting at higher temperatures and, indeed, the increase in T_m with increasing chain length.

Effects of the Head Group. As discussed earlier, the shape of the unit cell determines the relative intensities of the two components of the CH_2-scissoring and CH_2-rocking bands. From the large differences in the band shapes in the spectra of DPPE and DPPA compared to those in the spectra of the phosphatidylcholines, it is apparent that the orthorhombic subcells of these two classes of phospholipids differ. The most plausible explantation for the differences lies in the angles of tilt of the acyl chains with respect to the plane of the bilayer surface. Phosphatidylcholines exhibit a tilt angle of about 30° while the acyl chains in phosphatidylethanolamines are normal to the bilayer surface (McIntosh, 1980). As a consequence of this, there will be differences in the three-dimensional packing of the acyl chains which will result in differences in the vibrational coupling within the two-dimensional subcell.

It has been postulated that the tilt results from differences in the area occupied by the head group, the PC group being larger than the PE group (McIntosh, 1980). Indirect support for this comes from the fact that the band shapes observed in the CH_2-rocking and -scissoring modes of DPPE and DPPA are similar to those in the spectra of *n*-alkanes, while those of DPPC are quite different. In fact, we are unable to find a report of spectra of alkanes or terminally substituted alkanes exhibiting similar band shapes.

Conclusions

As demonstrated by the factor group splitting of the infrared-active CH_2-scissoring modes, the acyl chains of all gel phase phospholipids investigated in this study are packed in a two-dimensional orthorhombic subcell. With hindsight, this is to be expected, as the orthorhombic subcell is common to terminally substituted *n*-alkanes while the alternate hexagonal and triclinic subcells are rarely observed.

The acyl chains of all investigated 3-*sn*-phosphatidylcholines pack in the same way, which results in the anomalous CH_2-scissoring and -rocking band contours as compared to those found in the spectra of other acyl compounds. This anomalous band contour may be related to the large angle of tilt of the acyl chains in the phosphatidylcholines. On the other hand, the band contours in the spectra of DPPE and DPPA are similar to those found in the spectra of acyl compounds.

Within a series of phosphatidylcholines, the magnitude of the factor group splitting is dependent on the acyl chain length and on the temperature. The different factor group splitting is attributed to the increased potential for strong van der Waals' interactions with increasing chain length, while the temperature dependence reflects the introduction of torsional

and librational motions into the acyl chains as the temperature is increased.

For a given chain length, variations of the head group have no major effect on the value of the factor group splitting at low temperatures. However, subtle differences are evident in the temperature range from T_m to T_m - 70 °C. We are continuing to study this by means of Fourier-transform–infrared spectroscopy.

The results of this study, together with the fact that the pretransition represents the final loss of the orthorhombic subcell, suggest that the forces governing the structure of the bilayer are comprised of two types, those associated with the head group and those resulting from the interchain interactions. The temperature and chain length dependencies of the factor group splitting indicate that the energetically most favored conformations of the two lipid components, the acyl chains and the head group, are incompatible and that the bilayer structure, at any given temperature and chain length, represents a compromise between the two. In the phosphatidylcholines, the pretransition represents an abrupt change in this balance, hence its sensitivity to perturbants, and variations in the chain length. The delicacy of the balance is indicated by the fact that the pretransition temperatures show the same odd–even alteration as do the melting point series of terminally substituted *n*-alkanes (Silvius et al., 1979).

Acknowledgments

We thank Hector L. Casal for helpful discussions and a critical reading of the manuscript.

References

Cameron, D. G., Casal, H. L., & Mantsch, H. H. (1979) *J. Biochem. Biophys. Methods 1*, 21–36.

Cameron, D. G., Casal, H. L., & Mantsch, H. H. (1980a) *Biochemistry 19*, 3665–3672.

Cameron, D. G., Gudgin, E. F., & Mantsch, H. H. (1980b) *Biochim. Biophys. Acta 596*, 463–467.

Cameron, D. G., Casal, H. L., Mantsch, H. H., Boulanger, Y., & Smith, I. C. P. (1981) *Biophys. J. 35*, 1–16.

Chen, S. C., Sturtevant, J. M., & Gaffney, B. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5060–5063.

Davis, J. H. (1979) *Biophys. J. 27*, 339–358.

Decius & Hexter (1977) *Molecular Vibrations in Crystals*, McGraw-Hill, New York.

Harlos, K. (1978) *Biochim. Biophys. Acta 511*, 348–355.

Harlos, K., & Eibl, H. (1980) *Biochim. Biophys. Acta 601*, 113–122.

Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3036–3040.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575–4580.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1979) *J. Biol. Chem. 254*, 6068–6078.

Krimm, S., Liang, C. Y., & Sutherland, G. B. B. M. (1956) *J. Chem. Phys. 25*, 549–562.

Mabrey, S., & Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3862–3866.

Malkin, T. (1952) *Prog. Chem. Fats Other Lipids 1*, 1–17.

Mantsch, H. H., Cameron, D. G., Umemura, J., & Casal, H. L. (1980) *J. Mol. Struct. 60*, 263–268.

Marsh, D. (1980) *Biochemistry 19*, 1632–1637.

McIntosh, T. J. (1980) *Biophys. J. 29*, 237–245.

Pearson, R. A., & Pascher, I. (1979) *Nature (London) 281*, 499–501.

Silvius, J. R., Read, B. D., & McElhaney, R. N. (1979) *Biochim. Biophys. Acta 555*, 175–178.

Snyder, R. G. (1961) *J. Mol. Spectrosc. 7*, 116–144.

Snyder, R. G. (1979) *J. Chem. Phys. 71*, 3229–3235.

Snyder, R. G., Hsu, S. L., & Krimm, S. (1978) *Spectrochim. Acta, Part A 34A*, 395–406.

Tasumi, M., Shimanouchi, T., Watanabe, A., & Goto, R. (1964) *Spectrochim. Acta 20*, 629–666.

Tremblay, P. A., & Kates, M. (1981) *Chem. Phys. Lipids* (in press).