Accelerated Publications

Quantitative Determination of Hydrocarbon Chain Conformational Order in Bilayers of Saturated Phosphatidylcholines of Various Chain Lengths by Fourier Transform Infrared Spectroscopy†

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ABSTRACT: The infrared spectra of aqueous dispersions of a homologous series of symmetric-chain, disaturated phosphatidylcholines, with fatty acyl chain lengths ranging from 12 to 19 carbons, have been measured at comparable reduced temperatures in their liquid-crystalline phases. The infrared spectra of these compounds contain bands that are dependent on the conformation of the fatty acyl chains. In particular, in the 1400–1300-cm^{-1} spectral region, there are bands due to CH_2 wagging which are specific for the different types of gauche conformers. Thus, gauche–trans–gauche′ sequences (or kinks) give a band at 1367 cm^{-1}, end-gauche conformers a band at 1341 cm^{-1}, and double-gauche conformers a band at 1355 cm^{-1}. The intensities of these bands were determined and normalized to the intensity of the conformation-insensitive band due to symmetric methyl bending at 1378 cm^{-1}. The intensities of the different "gauche" bands yield a "per chain" intensity, which is directly related to the concentration of the different types of conformational defects. We find that, within experimental error, the concentration of end-gauche and double-gauche conformers is relatively low and practically invariant with chain length when a series of homologous phosphatidylcholines are compared at the same reduced temperature. In contrast, the concentration of gauche–trans–gauche′ sequences (kink defects) is much higher and increases as the chain length increases. For dipalmitoylphosphatidylcholine we find that there are about 1.2 kink, 0.5–0.6 end-gauche, and 0.4 double-gauche conformers per hydrocarbon chain. Thus, for this phospholipid, the total number of gauche bonds per chain is about 3.7, in excellent agreement with that measured recently by Mendelsohn et al. [Mendelsohn, R., Davies, M. A., Brauner, J. W., Schuster, H. F., & Dluhy, R. A. (1989) *Biochemistry 28*, 8934–8939] and with earlier estimations derived from spectroscopic and thermodynamic measurements. The number of gauche bonds per hydrocarbon chain in liquid-crystalline bilayers is less than that in liquid *n*-alkanes or detergent micelles of comparable hydrocarbon chain length, due primarily to the lower concentration of double-gauche conformers present in the former.

The organization of phospholipid bilayers in water has been studied extensively by a wide variety of spectroscopic and other physical techniques over the past two decades [see Cevc and Marsh (1987) and Gennis (1989)]. Many of these spectroscopic studies have focused on the determination of the orientational order and dynamics of the phospholipid hydrocarbon chains, especially in their biologically relevant liquid-crystalline bilayer states. Although a fairly coherent qualitative picture of the general organization of the hydrocarbon core of the phospholipid bilayer has emerged from these studies, many questions remain to be answered. In particular, the number and types of nonplanar conformers that exist above the chain-melting phase transition temperature and their localization within the hydrocarbon chain have yet to be unambiguously determined, even for a relatively simple, chain-symmetric saturated phosphatidylcholine such as DPPC.[1] Moreover, the effect of variations in the structure and length of the phospholipid hydrocarbon chain on the nature and localization of these nonplanar conformers remains even more obscure.

The application of a variety of spectroscopic techniques to the problem of elucidating the orientational order and dynamics of the hydrocarbon chains of lipid bilayers, although necessary to furnish a complete picture of the organization of such structures, has engendered a certain amount of uncertainty and confusion in this field of research. This uncertainty arises largely from two sources, one having to do with the vastly different time scales sampled by each technique and the other with the necessity to utilize molecular probes in some cases. The technique of ^2H NMR spectroscopy, while relatively direct and nonperturbing, is sensitive to a variety of motions as well as to gauche–trans isomerization in the hydrocarbon chains. Thus, evaluating the relative contributions of trans–gauche isomerization, acyl chain librations, rigid body motions, etc. to the spectrum can be difficult and is model dependent [see Seelig and Seelig (1980) and Davis (1983)]. The techniques of ESR (Keith et al., 1973; Schreier et al., 1978) and fluorescence (Shinitzky & Barenholz, 1978; Sklar, 1984)

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[1] Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DLPC, dilauroylphosphatidylcholine; DNPC, dinonadecanoylphosphatidylcholine; ESR, electron spin resonance; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

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spectroscopy, while very sensitive and responsive to a narrower range of more rapid chain motions, require the use of probe molecules whose structures differ appreciably from those of the fatty acyl chains which they monitor. The use of such probes raises the questions of how well these probe molecules reflect the true behavior of the phospholipid hydrocarbon chains and whether or not the presence of the probe molecules perturbs the orientation and dynamics of these chains in the bilayer. Also, with each of these latter techniques the results obtained are influenced by the structural and motional model used to analyze the experimentally derived spectra.

Infrared (Casal & Mantsch, 1984; Wong et al., 1988) and Raman (Lord & Mendelsohn, 1981; Carey, 1982; Wong et al., 1988) spectroscopy are nonperturbing techniques that monitor molecular vibrations and thus operate on a very short time scale. Moreover, the frequencies of certain CH_2 vibrational modes are sensitive exclusively to the conformation of the lipid hydrocarbon chains. For this reason a direct and quantitative determination of the number and types of nonplanar conformers present in the hydrocarbon core of a phospholipid bilayer is possible, at least in principle. Such a determination is not only important in its own right but would greatly assist in the interpretation of the data gathered by other spectroscopic techniques that are sensitive not only to hydrocarbon chain conformation but to other types of slower motions. However, until quite recently, vibrational spectroscopy has been used only in a qualitative or semiquantitative manner to monitor overall fatty acyl chain conformation in phospholipid bilayers (Casal & Mantsch, 1984; Wong et al., 1988). However, IR spectroscopy has been used for the quantitative determination of chain conformation in *n*-alkanes (Snyder, 1967; Maroncelli et al., 1982, 1983) and SDS micelles (Holler & Callis, 1989). In this paper we extend these studies to phospholipid bilayers.

Mendelsohn et al. (1989) have recently demonstrated that FTIR spectroscopy can be used to quantitatively determine the conformational order in the acyl chains of specifically deuterated derivatives of DPPC in both the gel and liquid-crystalline states. This determination is based on an analysis of the CD_2 rocking modes of the hydrocarbon chains, which differ in frequency if the CD_2 segment is surrounded by a trans C–C–C skeleton or by a skeleton with one or more gauche conformers (Snyder et al., 1983; Maroncelli et al., 1985). Although this method of analysis is experimentally demanding, requires the use of specifically deuterated hydrocarbon chains, and cannot differentiate between kink and single-gauche rotamers, it has the major advantage of providing information on the concentration of gauche rotamers at each position along the hydrocarbon chain. In contrast, the FTIR method described here, which is based on an analysis of the CH_2 wagging vibrations, is experimentally facile, does not require the deuteration of the hydrocarbon chain, and does differentiate between kinks and several other types of gauche rotamers. However, it does not provide information on the location along the chain of the kink and double-gauche rotamers. If both techniques are applied together, however, a complete description of the number, type, and location of the gauche rotamers of phospholipid hydrocarbon chains can be obtained.

Materials and Methods

1,2-Didodecanoyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine, and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine were obtained from Sigma Chemical Co. (St. Louis, MO); 1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine, 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine, 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine, 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine, and 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine (DNPC) were purchased from Avanti Polar Lipids (Birmingham, AL). The purity of these lipids was checked by thin-layer chromatography. Hydrated dispersions were prepared following the same procedures for all samples: 6 mg of lipid was mixed with 50 μL of doubly distilled water (pH 6–7), vortexed, heated to a few degrees above their gel to liquid-crystal phase transition temperatures, and vortexed while warm; the vortexing–heating cycle was repeated three times. Translucent, homogeneous dispersions were obtained for all samples. For infrared measurement these dispersions were placed in cells of 50-μm path length fitted with CaF_2 windows. Infrared spectra at 2-cm^{-1} resolution were recorded with a Digilab FTS-60 Fourier transform spectrometer equipped with a DTGS detector. For all spectra 600 interferograms were coadded and apodized with a triangular function. For each sample, spectra were recorded at 5 ± 1 °C above the corresponding gel to liquid-crystal transition temperature. For each compound three independent samples were measured, and for each of these samples two replicate spectra were collected. The 1400–1300-cm^{-1} spectral region was analyzed by curve fitting each spectrum with bands due to the different vibrational modes (see Theory); a linear base line, extending from 1396 to 1330 cm^{-1}, was subtracted from each spectrum prior to curve fitting. The resulting spectra were fitted with Lorentzian curves at positions corresponding to those of the different bands by a least-squares algorithm (Jones & Young, 1969). The error in the band areas determined from the fitted spectra was about 10%, as estimated by comparison of the results of fitting spectra recorded from three different preparations of the same phospholipid.

Theory

The basis for the present conformational study is provided by the extensive investigations of Snyder and co-workers on the infrared spectra of *n*-alkanes (Snyder, 1967; Maroncelli et al., 1982). These studies have determined the frequencies of the bond vibrations in *n*-alkane chains, including the localized vibrations of nonplanar structures containing gauche bonds. Furthermore, Snyder's work also provides the basis for utilizing the intensities of the bands characteristic of nonplanar conformers in a quantitative manner to calculate the concentrations of these conformers. For this study we have chosen the bands in the 1400–1300-cm^{-1} region, which are due to localized wagging vibrations of CH_2 groups involved in nonplanar conformers. In this region there are four bands at 1367, 1354, 1341, and 1306 cm^{-1} that are useful for conformational analyses. The bands at 1367 and 1306 cm^{-1} are due to wagging of CH_2 groups in kink (gauche–trans–gauche′) sequences, the band at 1341 cm^{-1} is due to CH_2 wagging of end-gauche conformers, and the band at 1354 cm^{-1} is due to CH_2 wagging of the double-gauche conformers [for details of assignment and designation of the different conformers, see Table III in Maroncelli et al. (1982)].

In the infrared spectra of phosphatidylcholines studied here, there is a strong band at $\sim$1225 cm^{-1} due to antisymmetric stretching of the phosphate group. The high-frequency wing of this band overlaps heavily the 1306-cm^{-1} CH_2 wagging band due to kink conformers, making the determination of its intensity impractical. We have therefore not studied this band and relied solely on the intensity of the 1367-cm^{-1} band for determining the concentration of kink conformers.

In the spectral region of interest (1400–1300 cm^{-1}) there also appears a band at 1378 cm^{-1} due to the symmetric bending

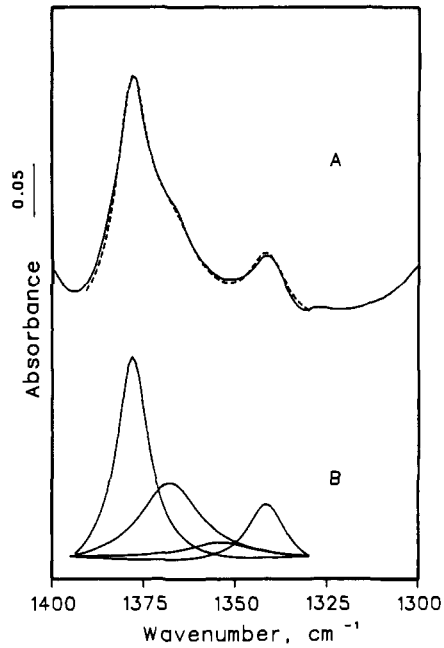

FIGURE 1: (A) Infrared spectrum (1400–1300 cm^{-1}) of a DLPC hydrated dispersion at 3.2 °C. The dotted trace superimposed on the spectrum is the result of curve fitting. (B) Individual curves used to fit the observed spectrum in (A).

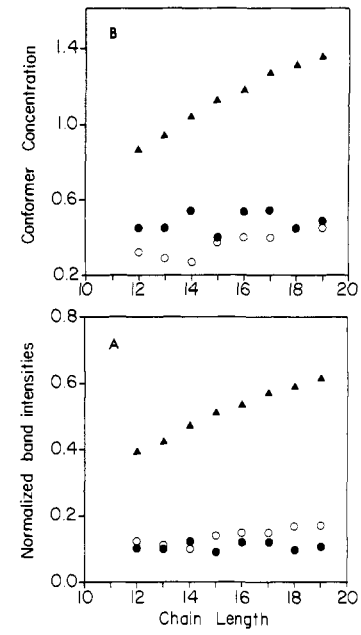

FIGURE 2: (A) Plot of the intensities, as integrated areas, of the bands due to the different nonplanar conformers [(▲) kinks; (○) double gauche; (●) end gauche] versus the total number of C atoms in the acyl chains of a homologous series of saturated phosphatidylcholines. These intensities were determined by curve fitting of the experimental spectra and have been normalized by dividing by the area of the CH_3 bending band at 1378 cm^{-1} in each case. (B) Plot of the concentration of different nonplanar conformers per acyl chain as a function of hydrocarbon chain length for a series of saturated phosphatidylcholines.

of the terminal CH_3 group in the fatty acyl chains. This band is insensitive to conformation and to chain length. Its intensity may be used as an internal standard with which to normalize the intensity of the conformation-sensitive CH_2 wagging bands. Such an approach was adopted successfully recently in a study of chain conformations in micelles of SDS (Holler & Callis, 1989).

It should be noted that single-gauche conformers in the interior of the hydrocarbon chain cannot be specifically monitored in this FTIR spectroscopic analysis. However, because such conformers result in a marked bend in the hydrocarbon chain, their concentration even in liquid *n*-alkanes is relatively low (Snyder, 1967). Since the overall conformational order of the hydrocarbon chains of phospholipid bilayers is greater than in liquid hydrocarbons of comparable chain length, it is unlikely that internal single-gauche conformers make a major contribution to the total conformational disorder in liquid-crystal bilayers. We cannot, however, rule out a minor contribution from this type of nonplanar conformer. We should also point out that this FTIR spectroscopic approach does not provide information on the location of the nonplanar conformers within the hydrocarbon chain.

The relationship between the intensities of the "gauche" CH_2 wagging bands and the actual concentrations of the different conformers has been established in studies of the determination of nonplanar conformations in the high-temperature solid phases of *n*-alkanes (Maroncelli et al., 1982) and in the recent work on SDS micelles (Holler & Callis, 1989). Therefore, a complete analysis of the bands observed in the infrared spectra of phospholipid bilayers between 1400 and 1330 cm^{-1} should yield a quantitative conformational analysis of the fatty acyl chains. In the following section we present the results of such a FTIR spectroscopic study for a homologous series of saturated phosphatidylcholines.

RESULTS

As representative of the whole series of spectra recorded, we show in Figure 1A the 1400–1300-cm^{-1} region of the FTIR spectrum of fully hydrated DLPC at 3.2 °C; the traces shown in Figure 1B are the result of curve fitting. The bands of interest are observed at 1380, 1367, 1355, and 1341 cm^{-1}. There is also a very weak band at ~1328 cm^{-1} that is probably due to vibrations of CH_2 or CH_3 groups in the polar headgroup of the molecule. Its intensity was not taken into consideration for the present conformational analysis.

The intensities as integrated areas of the four bands of interest were determined from curve-fitted spectra such as the one shown in Figure 1B; the intensity of the methyl symmetric bending mode at 1380 cm^{-1} was assigned a value of 1, and the intensities of the other three bands, which are sensitive to conformation, were normalized to this value. The combined results of all measurements are shown in Figure 2. The data of Figure 2 give the normalized areas of the bands due to kink (1367 cm^{-1}), end-gauche (1341 cm^{-1}), and double-gauche (1355 cm^{-1}) conformers as a function of chain length. The values shown in Figure 2A were all determined from spectra recorded at a reduced temperature of 5 ± 1 °C, i.e., at 5 ± 1 °C above the corresponding gel to liquid-crystal transition temperatures for each phosphatidylcholine. These transition temperatures range from −2.1 °C for DLPC to 61.8 °C for DNPC (Lewis et al., 1987). The normalized areas of the conformation-sensitive bands shown in Figure 2A give a "per chain" intensity, since there is only one terminal CH_3 group per chain and the intensity of the methyl band was used as the normalizing factor. It is clear from Figure 2 that even for the shortest chain PC the kink sequence is the dominant nonplanar conformer present. Moreover, the intensity of the band due to kink conformers increases linearly as the chain length of the phosphatidylcholine increases. Thus, the number of kink conformers in the liquid-crystalline phase at a given reduced temperature is larger for longer chains. On the other hand, the intensities of the band due to end-gauche conformers is, within experimental error, the same for all chain lengths studies, as expected. There also seems to be a small increase in the double-gauche intensity as the chain length increases. However, the error associated in determining the intensity of

this minor band is relatively large so no definitive statement can be made.

The concentrations of the various nonplanar chain conformers are given by the band intensities. Maroncelli et al. (1982) in their study of high-temperature solid phases of *n*-alkanes used the spectra of liquid *n*-alkanes and the results of statistical calculations of gauche bond concentrations in them to estimate the gauche bond concentration in the solid phases from the intensity of CH_2 wagging bands. A similar approach was used by Holler and Callis (1989) in their study of SDS micelles. Their results furnish conversion factors that relate band intensity to gauche bond concentration. These factors are $a_k = 2.2$ for the band due to kinks, $a_{gg} = 2.7$ for double gauche, and $a_{eg} = 4.5$ for end gauche. By use of these factors, the actual concentrations of the various conformers are obtained. The corresponding concentrations are plotted versus chain length in Figure 2B. The chain-length dependence of the actual conformer concentrations is obviously the same as that of the normalized intensities presented in Figure 2A.

Discussion

The results presented here represent a direct experimental determination of conformational order (defined as the type and number of gauche bonds) in the fatty acyl chains of a homologous series of phosphatidylcholines. We emphasize that in the general approach adopted for the present conformational analysis there is no need to invoke any structural or motional model. In this analysis the assumptions made are that the value of the energy difference between gauche and trans conformers is about 500 cal/mol and that Flory's (1969) rotational isomeric state approach for calculating the gauche content of a liquid *n*-alkane is valid. These two assumptions are very reasonable and have proven valid for many systems [see Cevc and Marsh (1987)]. These two assumptions permit the determination of the concentrations of the various nonplanar chain conformers from the FTIR CH_2 wagging band intensities of the phosphatidylcholine hydrocarbon chains.

The concentration of gauche bonds determined here may be compared with those derived by others. The only other experimental FTIR spectroscopic determination of gauche bond concentration in lipid bilayers is the recent work of Mendelsohn et al. (1989). In that study specifically deuterated derivatives of DPPC were examined. The gauche bond concentrations at different chain positions were established from the intensities of the conformationally sensitive CD_2 rocking bands. Our approach does not give information regarding the exact location of gauche bonds along the fatty acyl chain, and therefore, we cannot establish a direct comparison with this aspect of Mendelsohn's results. However, Mendelsohn et al. (1989) were also able to estimate that in the liquid-crystalline phase of DPPC there are *at least* 3.6 and *at most* 4.2 gauche bonds for chain. This yields corresponding values of 7.2 and 8.4 gauche conformers per DPPC molecule. Our results for DPPC (see Figure 2B) yield the following values per hydrocarbon chain: 1.19 ± 0.1 kink, 0.54 ± 0.1 end-gauche, and 0.4 ± 0.1 double gauche conformers. Since there are two gauche conformers in each kink and double-gauche sequence but only a single gauche conformer in the end-gauche sequence, this gives a total of 3.7 ± 0.2 gauche conformers per hydrocarbon chain or 7.4 ± 0.4 gauche bonds per molecule. Thus, our values for the total number of gauche conformers present are close to the lower value estimated from the study of the specifically deuterated DPPC.

Although the good agreement between the numbers of gauche conformers per DPPC hydrocarbon chain determined in the present study and in the FTIR spectroscopic study of Mendelsohn et al. (1989) is gratifying, we should note that in both cases the values obtained may be lower than the true values. This is because in our analysis we have ignored the potential contribution of single-gauche conformers in the interior of the hydrocarbon chain to the total conformational disorder. Similarly, Mendelsohn et al. (1989) neglected the contribution of the double-gauche conformers in their analysis. Although we find here that the double-gauche conformer is indeed present in relatively low concentrations in the liquid-crystalline DPPC bilayer, it nevertheless contributes an average of 0.8 gauche bonds per hydrocarbon chain. It is thus likely that the actual number of gauche conformers per chain may be somewhat greater than the range of 3.6–4.2 reported by Mendelsohn. Similarly, our value of 3.7 gauche conformers per DPPC chain is also likely to be low, perhaps by a similar amount. This is because although the concentration of single-gauche conformers would be expected to be greater than the concentration of double-gauche conformers on energetic grounds, each single-gauche conformer contributes only one gauche bond per chain to the total.

Table I: A Comparison of the Type and Number of Gauche Conformers per Hydrocarbon Chain for a Liquid *n*-Alkane, a Detergent Micelle, and a Phospholipid Bilayer

	no. of gauche bonds per chain		
type of conformer	tridecane[a]	SDS micelle[a]	DLPC bilayer
kink	0.77	0.68	0.88
end gauche	0.68	0.36	0.45
double gauche	0.64	0.77	0.32
total gauche conformers per chain	3.50	3.26	2.85

[a] Holler and Callis (1989).

The results of the present FTIR hydrocarbon conformational analysis of DPPC can be compared to the results obtained by other physical techniques. Nagle and Wilkinson (1978), on the basis of the volume change at the gel to liquid-crystalline phase transition and the energy difference accompanying this phase change, have calculated that 3.8 gauche conformers per chain of DPPC exist in the liquid-crystalline state just above the phase transition temperature. On the other hand, Schindler and Seelig (1975), using the ^2H NMR order parameter profile of the hydrocarbon chains of DPPC and the Marcelja (1974) molecular field model, calculated a vlaue of 4.3 gauche conformers per chain just above the phase transition temperature. These values are in good agreement with the value of 3.7 gauche conformers per DPPC hydrocarbon chain obtained in the present study, particularly as our value may slightly underestimate the true value.

We now compare the gauche bond concentrations found here for the series of disaturated PC's with those found in other related systems. Systematic, quantitative determinations of hydrocarbon chain conformation order exist only for *n*-alkanes (Maroncelli et al., 1982, 1985; Snyder, 1967) and SDS micelles (Holler & Callis, 1989). The numbers and type of gauche conformers present in an *n*-alkane, in micelles of SDS, and in a PC bilayer containing an equivalent number of methylene groups are compared in Table I. This comparison reveals that the total number of gauche conformers per hydrocarbon chain varies somewhat in the three systems. The liquid hydrocarbon overall has the largest number of total gauche bonds followed by the SDS micelles. The hydrocarbon chains of the phosphatidylcholine bilayer clearly contain a smaller number of gauche bonds, and thus a smaller degree of conformational disorder. This result is in agreement with findings from a variety of other studies on liquid alkanes, amphiphile micelles,

and phospholipid bilayers [see Cevc and Marsh (1987) and Gennis (1989)].

The distribution of gauche-conformer types between the three systems shows an even larger variation than does the total number of gauche bonds. In liquid tridecane, the concentration of kink, end-gauche, and double-gauche sequences are nearly comparable, although the kink conformer is present in slightly greater number. In contrast, although the number of kink conformers is roughly similar in the alkane, SDS micelle, and DLPC bilayer, the number of end-gauche conformers is significantly reduced in these latter two systems. The major difference between the phospholipid bilayer and the other two systems, however, is in the much-reduced number of double-gauche conformers present in the former. Since the formation of the double-gauche sequence results in a severe departure of the hydrocarbon chain alignment from the bilayer normal, the repression of the formation of this conformer in the more highly ordered and more tightly packed interior of the lipid bilayer is understandable. On the other hand, the relatively large content of kink conformers in the DLPC bilayer is also understandable, since the overall alignment of the hydrocarbon chain is minimally perturbed by this sequence. Considering that the tridecane molecule has two methyl termini per hydrocarbon chain whereas the hydrocarbon chains of the SDS micelle and DLPC bilayer each have one, the concentration of end-gauche conformers per chain terminus is roughly comparable in the three systems. It is interesting to note that the overall smaller total number of gauche bonds in the phospholipid bilayer system is due primarily to the marked decrease in the concentration of double-gauche conformers present, despite an apparent increase in the number of kink conformers present. Thus our results support the kink model of phospholipid hydrocarbon chain disorder in the liquid-crystalline state proposed by Trauble (1971).

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References

Carey, P. R. (1982) in *Biochemical Applications of Raman and Resonance Raman Spectroscopy*, pp 208–233, Academic Press, New York.

Casal, H. L., & Mantsch, H. H. (1984) *Biochim. Biophys. Acta 779*, 381–401.

Cevc, G., & Marsh, D. (1987) *Phospholipid Bilayers. Physical Principles and Models*, Wiley, New York.

Davis, J. R. (1983) *Biochim. Biophys. Acta 737*, 117–171.

Flory, P. J. (1969) *Statistical Mechanics of Chain Molecules*, Wiley-Interscience, New York.

Gennis, R. B. (1989) *Biomembranes. Molecular Structure and Function*, Springer-Verlag, New York.

Holler, F., & Callis, J. B. (1989) *J. Phys. Chem. 93*, 2053–2058.

Jones, R. N., & Young, R. P. (1969) Bulletin No. 13, National Research Council of Canada, Ottawa, ON.

Keith, A. D., Sharnoff, M., & Cohn, G. E. (1973) *Biochim. Biophys. Acta 300*, 379–419.

Lewis, R. N. A. H., Mak, N., & McElhaney, R. N. (1987) *Biochemistry 26*, 6118–6126.

Lord, R. C., & Mendelsohn, R. (1981) in *Membrane Spectroscopy* (Grell, E., Ed.) pp 377–436, Springer-Verlag, Berlin.

Marcelja, S. (1974) *Biochim. Biophys. Acta 367*, 165–176.

Maroncelli, M., Qi, S. P., Strauss, H. L., & Snyder, R. G. (1982) *J. Am. Chem. Soc. 104*, 6237–6247.

Maroncelli, M., Strauss, H. L., & Snyder, R. G. (1985) *J. Phys. Chem. 89*, 4390–4397.

Mendelsohn, R., Davies, M. A., Brauner, J. W., Schuster, H. F., & Dluhy, R. A. (1989) *Biochemistry 28*, 8934–8939.

Nagle, J. F., & Wilkinson, D. A. (1978) *Biophys. J. 23*, 159–175.

Schindler, H., & Seelig, J. (1975) *Biochemistry 14*, 2283–2287.

Schreier, S., Polnaszek, C. F., & Smith, I. C. P. (1978) *Biochim. Biophys. Acta 515*, 375–436.

Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19–61.

Shinitzky, M., & Barenholz, Y. (1978) *Biochim. Biophys. Acta 515*, 367–394.

Sklar, L. A. (1984) *Biomembranes 12*, 99–131.

Snyder, R. G. (1967) *J. Chem. Phys. 47*, 1316–1360.

Snyder, R. G., Maroncelli, M., Strauss, H. L., Elliger, C. A., Cameron, D. G., Casal, H. L., & Mantsch, H. H. (1983) *J. Am. Chem. Soc. 105*, 133–134.

Trauble, H. (1971) *J. Membr. Biol. 4*, 193–208.

Wong, P. T. T., Siminovitch, D. J., & Mantsch, H. H. (1988) *Biochim. Biophys. Acta 947*, 139–171.