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Calorimetric Evidence for Phase Transitions in Spin-Label Lipid Bilayers†

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ABSTRACT: Dispersions of pure, spin-label phosphatidylcholines in aqueous buffer have been investigated with the Privalov high-sensitivity differential scanning calorimeter. The lipids studied are mixed-chain ones in which C-2 of glycerol bears a spin-label derivative of stearic acid and the fatty acid group at C-1 is palmitate. A well-defined phase transition is observed at 30.3–30.7 °C for the phosphatidylcholine labeled near the polar end of the stearate chain (label at C-5). A sharp transition (32–34 °C) is also observed for the lipid spin-labeled near the terminal methyl of stearate (label at C-16), but the thermodynamic parameters for this lipid depend strongly on the history of the sample. Calorimetric evidence for hysteresis in the phase transition of the C-16-labeled lipid is presented. In contrast to the above spin-label lipids, the lipid labeled at C-12 does not show a sharp transition in the region 5–35 °C. In general, therefore, the thermal behavior of the spin-label phosphatidylcholines resembles that of phosphatidylcholines bearing double bonds or branched methyl groups at similar locations on acyl chains. During synthesis of mixed-chain lipids, migration of acyl chains occurs. Methyl esterification procedures which are compatible with the acid-labile spin-label group are described. Gas chromatographic analysis of methyl esters shows that chain migration during synthesis gives 15–20% of the spin-label fatty acid at the glycerol C-1 position.

Considerable insight into the physical chemistry of phospholipid bilayers, with and without protein components, has been obtained in studies which employ lipid probes. These probes may contain paramagnetic groups for electron paramagnetic resonance (EPR)[1] studies (Seelig, 1970; Hubbell & McConnell, 1971; Gaffney & McConnell, 1974), chromophores for fluorescence measurements (Sklar et al., 1975; Radda, 1975), or nuclei, such as deuterium (Seelig, 1977) or fluorine (Sturtevant et al., 1979), suitable for NMR investigation. In some cases, the physical properties of the probe molecule may have important consequences for the course of the experiment. For instance, the α- and β-paranaric acids, which are fluorescent lipid probes, partition between fluid and solid lipid phases to differing extents (Sklar et al., 1975), and

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[1] Abbreviations: PSPC, 1-palmitoyl-2-stearoylphosphatidylcholine; P16MSPC, 1-palmitoyl-2-(16-methylstearoyl)phosphatidylcholine; 1-palm-2-(*m*,*n*)PC, a spin-label derivative of PSPC bearing the 1-oxy-2,2-dimethyloxazolidine group on the $n + 2$ carbon of the 2-stearoyl chain; DSC, diffferential scanning calorimetry; EPR, electron paramagnetic resonance; GC, gas chromatography; TLC, thin-layer chromatography.

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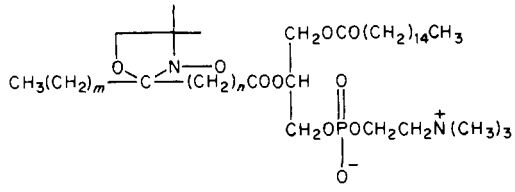
fluorination (Sturtevant et al., 1979) of phospholipids, for NMR studies, alters the order–disorder, phase transition temperatures from those of unsubstituted analogues. The results of studies by high-sensitivity differential scanning calorimetry of the thermal behavior of several spin-label phosphatidylcholines [1-palm-2-(*m*,*n*)PC] are presented here.



1-palm-2-(*m*,*n*)PC
[corresponding spin-label fatty acid: (*m*,*n*)FA]

Electron paramagnetic resonance studies of the thermal behavior of these spin-label lipids have been described previously (Chen & Gaffney, 1978). Knowledge of this thermal behavior has consequences particularly for measurements of lateral diffusion with high concentrations of spin-label lipid (Scandella et al., 1972) and for studies of the phase diagrams of binary lipid mixtures in which one component is a spin-label lipid (Ito et al., 1975).

Experimental Procedures

Syntheses of Spin-Label Lipids. The spin-label fatty acids were prepared in this laboratory by published procedures (Seelig, 1970; Hubbell & McConnell, 1971; Chen & Gaffney, 1978) or were purchased (Syva Associates). Labels from either source were purified as the esters by repeated preparative thin-layer chromatography (TLC) (5% ethyl acetate in benzene) until no other spots were detected on analytical TLC by sulfuric acid charring. The (1,14)FA ester used for synthesis of the lecithin which was analyzed by gas chromatography was pure by the criterion of microanalysis. Acylation of 1-palmitoyllysolecithin was done by the anhydride/fatty acid sodium salt method (Hubbell & McConnell, 1971; Cubero Robles & Van den Berg, 1969). Because syntheses were carried out on a very small scale (10–20 mg of lysolecithin), it was difficult to obtain a thoroughly mixed sample by the standard heated flask rotation. A reaction time of 24 h was used to assure randomization of the components in the reaction melt. Decomposition of the spin-label fatty acids to nonparamagnetic products occurred if samples were heated much over 80 °C. The prolonged reaction time undoubtedly contributes to the observed extent of acyl chain migration (see below). Several other synthetic approaches were even less satisfactory. Using sodium oxide as catalyst (Cubero Robles & Van den Berg, 1969) led to partial decomposition of the spin-label fatty acid at temperatures below 80 °C. When 4-(dimethylamino)pyridine was used as a catalyst (Gupta et al., 1977), it was found difficult to remove the catalyst entirely from the product lecithin. Since our syntheses were completed, an improved acylation procedure giving little chain migration has been introduced (Mason et al., 1981a,b). The latter approach has not yet been applied to spin-label lipid synthesis. (Note that spin-label lecithins are mixtures of two diastereoisomers because the fatty acids are racemic mixtures of two optical isomers.)

Hydrolysis of Spin-Label Lecithin [1-palm-2-(1,14)PC]. *Crotalus adamanteus* snake venom (1 mg, Sigma) was dissolved in 1 mL of 50 mM calcium chloride and extracted 2 times with 2 mL each of ether to remove lipid material. The washed snake venom solution was then added to 1 mg of spin-labeled lecithin in 10 mL of ether. The reaction mixture was stirred with a magnetic stirring bar for 2 h at room temperature. After this time, 5 mL of ethanol was added, and the mixture was reduced in volume to 1 mL on a rotary evaporator. The supernatant and precipitated enzyme were separated by low-speed centrifugation, and the precipitate was suspended in 5 mL of chloroform/methanol (1:2 v/v), clarified by centrifugation (500 rpm for 20 min), and combined with the above supernatant. The volume of the supernatants was reduced to 1 mL, 4 mL of ethanol was added, and the suspension was centrifuged at 500 rpm for 5 min. The supernatant was reduced to 1 mL, and 5 mL of ether was added to precipitate lysolecithin which was collected by centrifugation at 500 rpm for 10 min. The lysolecithin precipitate was washed 2 more times with ether as above, and the ether solutions of the fatty acids were combined.

Preparation of Fatty Acid Methyl Esters. Esterification and transesterification procedures were carried out under neutral or basic reaction conditions because the spin-label 3-oxyoxazolidine group is degraded by acid treatment.

Transesterification of lecithin or lysolecithin was done in a 5-mL reaction flask with a Teflon-lined screwcap. The lipid (1 mg of spin-labeled lecithin or the lysolecithin fraction derived from 1 mg of lecithin) was dissolved in 0.5 mL of benzene mixed with 0.7 mL of methanolic sodium methoxide (0.5 N from Supelco Inc.), and the closed reaction vessel was heated to 80 °C in an oil bath for 1 h. The mixture was cooled in an ice bath for 10 min after which 2 mL each of ether and ice-cold water was added to the reaction vessel. The contents of the vessel were mixed by shaking and centrifuged in a table-top centrifuge for 10 min. The lower, water layer was separated, and the organic layer was washed with a second, 2-mL portion of ice-cold water. The ether layer was dried over sodium sulfate for at least 12 h.

Fatty acid fractions from phospholipase A_2 hydrolysis of spin-label lecithin were esterified with diazomethane which was generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (*Aldrichimica Acta*, 1970). Freshly distilled ethereal diazomethane solution (50–100 μL) was added to the fatty acid dissolved in 1 mL of anhydrous benzene (distilled from and stored over sodium), and the reaction mixture was kept at room temperature for 1 h. The reaction was terminated by the addition of 10% acetic acid in methanol until no yellow color remained, and the resulting solution was stored over sodium sulfate.

Thin-Layer Chromatographic Analysis of Lipid and Ester Samples. Completion of the snake venom hydrolysis was demonstrated by the absence of lecithin on a silica gel chromatogram (Analtech prepared plates, solvent system chloroform/methanol/water, 65:25:4). Complete conversion of fatty acids or lipids to methyl esters was demonstrated with silica gel plates run in solvents for lipids (as above), for fatty acids (benzene/ethyl acetate/acetic acid, 100:20:2), and for esters of fatty acids (benzene/ethyl acetate, 95:5).

Gas Chromatographic Analysis of Methyl Esters. Analyses were performed on a Packard gas chromatograph equipped with a digital programmer. The glass column was packed with 3% OV17 on 100/120 Gas Chrom Q and measured 6 ft by 2 mm i.d. Runs were with a temperature program of 150–230 °C at 25 °C/min followed by a hold at 230 °C for 25 min, and a nitrogen flow rate of 60 mL/min. The methyl palmitate standard was purified by recrystallization from ethanol. The spin-label standard was prepared by repeated thin-layer chromatography (5% ethyl acetate in benzene) of the commercially available ester [Syva Associates no. 617, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxyoxazolidine

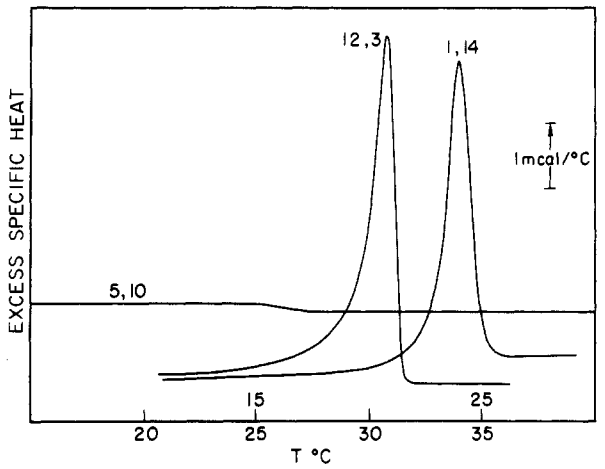


FIGURE 1: High-sensitivity differential scanning calorimetry (DSC) shows sharp phase transitions for two of three spin-label phosphatidylcholines. From top to bottom, the samples and the weight of lipid used for the measurement are the following: 1-palm-2-(1,14)PC, 1.16 mg; 1-palm-2-(5,10)PC, 1.983 mg; 1-palm-2-(12,3)PC, 1.860 mg.

methyl ester]. The instrument response was calibrated with a 1:1 (w/w) mixture of the pure esters, methyl palmitate, and the spin-label. Peak areas were measured as the height times the width at half-height. Under the above conditions, the 1:1 (w/w) calibration mixture of esters gave an average ratio of peak areas in seven runs of 1:0.40 [palmitate/(1,14) spin-label ester]. Thus, for an equi*molar* mixture of the esters, the instrument response is 1:0.57. This instrument response ratio was confirmed by mixing known amounts (based on the phosphate assay) of dipalmitoyllecithin and the spin-label lecithin and carrying them through the transesterification procedure. In this case, assuming that the spin-label lecithin contains one chain each of palmitate and spin-label fatty acid, the instrument response ratio for a 1:1 molar ratio of esters was found to be 1:0.54. The slightly different values for instrument response obtained in the two ways above amount to a 5% uncertainty in the absolute values of palmitate/(1,14) spin-label ester ratios in other runs. As an additional test that impurities were not introduced into samples during snake venom treatment a 1:1 mixture of the palmitate and spin-label esters was taken through the steps used for snake venom hydrolysis. GC analysis showed no difference in the ratio of esters before and after this treatment.

Differential Scanning Calorimetry (DSC). Lipids were prepared for differential scanning calorimetry (DSC) by dispersing 2 mg of dry lipid in 2 mL of 0.02 M phosphate buffer, pH 7.4, by agitation on a vortex mixer.

Differential scanning calorimetry (Mabrey & Sturtevant, 1978) was performed on a highly sensitive instrument designed by Privalov et al. (1975). The scanning was at a rate of 0.5 °C/min, in most cases, and in the ascending mode only. For construction of the "cooling curve" for a lipid phase transition, the sample was cooled from above the transition temperature to a specific temperature and then scanned with increasing temperature in the normal manner. The heat absorbed in each scan was assumed to be proportional to the fraction of lipid which underwent transition during cooling.

The transition temperature was defined as the temperature of the maximum rate of heat absorption, i.e., the peak of the DSC curve.

Results

DSC measurements were made on phosphatidylcholines bearing fatty acid chains with the 1-oxy-2,2-dimethyloxazolidine group at one of three different positions on one of the two fatty acid chains of the lipid. The lipids all contained palmitate primarily at C-1 of the glycerol backbone and the spin-label fatty acid primarily at C-2 of glycerol. Figure 1 shows representative DSC scans for each of the lipids, and Table I lists the thermal data obtained from measurements on each sample. Clearly, the lipids which bear the spin-label group near either the polar head or the terminal methyl ends of the fatty acid chains have phase transition temperatures within the temperature range of the main order–disorder transitions of several well-characterized synthetic phosphatidylcholines. The DSC curves for the transitions of the spin-label lipids shown in Figure 1 are more asymmetric than those obtained previously for saturated chain phosphatidylcholines (Mabrey & Sturtevant, 1978). For comparison with the curves in Figure 1, the DSC scan of a branched chain phosphatidylcholine [1-palmitoyl-2-(16-methylstearoyl)phosphatidylcholine, P16MSPC] is shown in Figure 2. The branched chain lipid was chosen as an analogue of the lipid spin-labeled at

Table I: Thermal Data for the Phase Transitions of Spin-Label Phosphatidylcholines, the Parent Lipid (PSPC), and a Branched Chain Lipid (P16MSPC)[a]

lipid	ΔH (kcal/mol)[b]	T_m (°C)[c]	$\Delta T_{1/2}$ (°C)[d]
1-palm-2-(12,3)PC	7.3	30.7	1.00
	8.4	30.3	1.05
1-palm-2-(1,14)PC	9.2–10.4	34.5–33.9	1.7–1.2
	16.5–16.6	32.6–32.4	1.0–1.6
	13.8–14.4	32.8–32.3	1.4
PSPC[e]	8.33	48.98	0.14
P16MSPC	10.2	34.6	1.2

[a] All spin-label PC's studied are acylated at C-1 primarily with palmitic acid and at C-2 primarily with a spin-label derivative of stearic acid. The numerals in parentheses in the name of spin-label lipid give the number of methylene groups between the carbon bearing the spin-label and the carbonyl carbon (first numeral) or the terminal methyl carbon (second numeral) and the spin-label site. Thus, (12,3) designates an 18-carbon chain with the paramagnetic 1-oxy-2,2-dimethyloxazolidine group on carbon 5. PSPC is 1-palmitoyl-2-stearoylphosphatidylcholine; P16MSPC is 1-palmitoyl-2-(16-methylstearoyl)phosphatidylcholine. [b] Results of measurements made on several different synthetic batches of the lipids are listed vertically. The range of ΔH and T_m values shown for each batch of 1-palm-2-(1,14)PC corresponds to the extremes obtained in repeated scans of the same sample. All thermal parameters are accurate to ±0.2 in their respective units except for PSPC data which are more accurate. [c] Temperature of maximal excess specific heat. [d] Width of the DSC transition curve at half-maximal excess specific heat. [e] Data are taken from Chen & Sturtevant (1981).

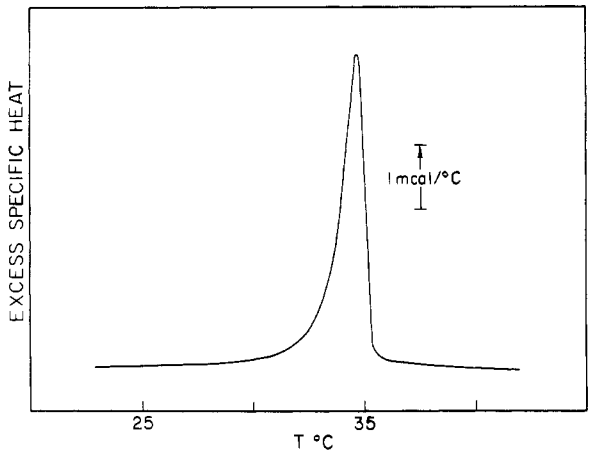


FIGURE 2: DSC data are shown for the branched chain lipid 1-palmitoyl-2-(16-methylstearoyl)-PC (1.208 mg of lipid used).

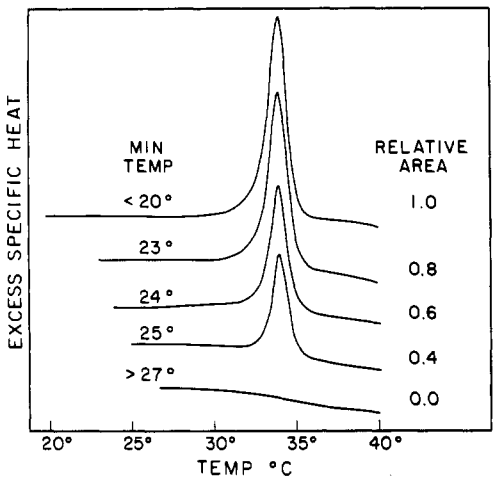


FIGURE 3: Hysteresis in the phase transition of 1-palm-2-(1,14)PC is revealed by DSC. The temperature listed to the left of each scan is the minimum temperature to which the sample was cooled before it was scanned to increasing temperatures. The relative area under each DSC curve is shown on the right side of the plots.

the same carbon atom because both types of lipid are mixtures of diastereoisomers. The widths at half-height for the transitions of the spin-label lipids are considerably wider than the widths for many known saturated straight-chain lipids of single or mixed chain composition (Mabrey & Sturtevant, 1978; Chen & Sturtevant, 1981; Mason et al., 1981a,b). For instance, the parent lipid PSPC has a $\Delta T_{1/2}$ of only 0.14 °C compared with widths from 1.0 to 1.7 °C for the spin-label lipids. The branched chain lipid P16MSPC, however, shows thermal behavior much closer to that of the spin-label lipids, as demonstrated by the data in Table I.

An earlier report of paramagnetic resonance evidence for phase transitions in bilayers of pure spin-label lipids (Chen & Gaffney, 1978) revealed marked hysteresis in the phase transition of 1-palm-2-(1,14)PC when the heating and cooling curves were compared. An exactly analogous study of this hysteresis could not be conducted with the Privalov DSC instrument because only scans in the heating direction can be made. As an alternative approach to construct the cooling curve for DSC data, the sample was cooled, from above the phase transition temperature to various temperatures from 3 to 10 °C below the transition temperature, and again scanned to increasing temperatures. A similar approach has been used to detect hysteresis in the phase transitions of fluorinated lipids (Sturtevant et al., 1979). The results of this procedure for 1-palm-2-(1,14)PC are shown in Figure 3. Even though the sample was cooled to well below the transition temperature (~34 °C) in each case, the areas under the scanning curves differed. In fact, when the sample was cooled to only 27 °C, no endothermic transition was observed at all. The curves in Figure 3 are put into quantitative form and compared with EPR data for the same lipid in Figure 4. Although the branched chain lipid P16MSPC shows a phase transition resembling that of 1-palm-2-(1,14)PC in temperature and width, it does not exhibit hysteresis in heating and cooling curves. Hysteresis is much less pronounced in the DSC curves for the lipid labeled near the head group, 1-palm-2-(12,3)PC, but it can be detected.

For determination of the degree of chain migration that occurred during synthesis of the spin-label lecithins, an analysis by gas chromatography was made on one of the samples of 1-palm-2-(1,14)PC, the lipid bearing the spin-label group near the methyl end of the fatty acid chain. This sample of spin-label lecithin was prepared from highly purified and crystalline

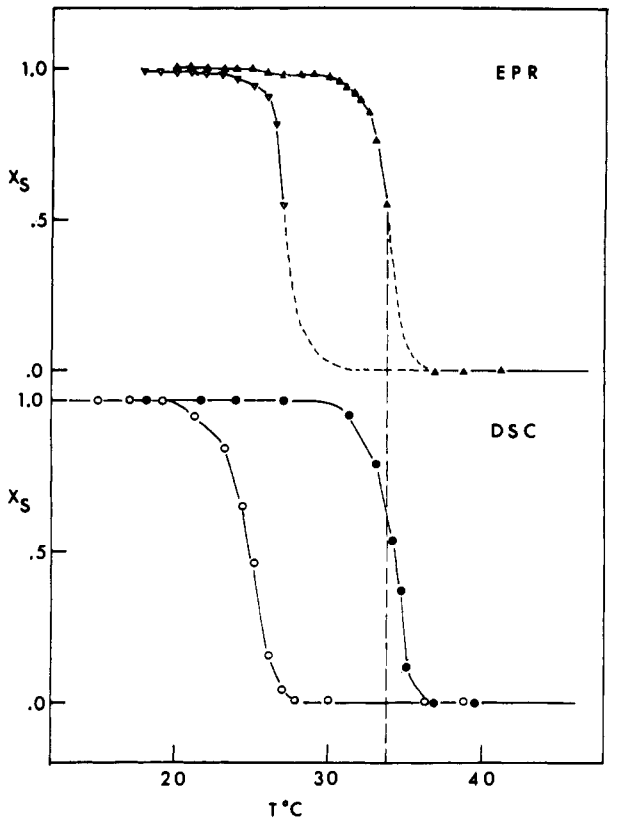


FIGURE 4: EPR and DSC data for hysteresis in the phase transition of 1-palm-2-(1,14)PC are in agreement for the heating curves but differ in the cooling curves. The mole fraction of lipid in the solid phase, X_s, is computed from raw EPR data as discussed earlier (Chen & Gaffney, 1978). Closed triangles give the heating curve, and open triangles give the cooling curve. For the higher temperature DSC curve, the value of X_s corresponds to the fractional area under the DSC plot from the low-temperature onset of the transition to the temperature of measurement. The DSC curve at lower temperatures was constructed from a set of data similar to that shown in Figure 3. The full area under the DSC curve is assumed to correspond to X_s. Open circles give data for the DSC cooling curve, and closed circles give those for the heating curve.

Table II: Fatty Acid Composition of 1-palm-2-(1,14)PC[a]

sample[c]	chromatography fraction[b] B	C
lecithin	0.56	0.55
lysolecithin	0.21	0.16
fatty acid	0.87	0.84

[a] The fatty acid composition is given in units of mole fraction of the spin-label fatty acid ester [(1,14) ester] in the methyl esters prepared from the indicated lipid sample. (See text for details of GC analysis of esters.) All values are approximately ±0.05 and represent the average of two or more separate methyl ester preparations. [b] The fractions, B and C, were obtained from chromatography on a silica gel column. Fraction B was the middle of the eluted lecithin peak, and fraction C was the late portion of that peak. An early fraction, A, decomposed during snake venom treatment although it did give results identical with fraction B results upon transesterification of the lecithin. [c] Conversion of the spin-label lecithin into lysolecithin and fatty acid components is described in the text. Under Discussion, the composition of the fatty acids esterified at glycerol C-1 is assumed to be identical with the composition of the lysolecithin component. Similarly, the C-2 fatty acid composition is taken as identical with that of the fatty acid recovered after snake venom hydrolysis of the lecithin.

spin-label fatty acid. Three fractions from silica gel chromatography of the labeled lecithin were collected. Table II gives GC data for the fatty acid composition of the lecithin,

and the lysolecithin and fatty acid fractions obtained from it by snake venom hydrolysis, for two of the fractions. These data show that mixing of the palmitate and spin-label fatty acid chains, as a result of acyl chain migration during synthesis, occurred to the extent of 15–20%. Acyl chain migration may account for some of the variability in ΔH (see Table I) and for the breadth of the transitions of the spin-label lipids.

Discussion

The spin-label lipids discussed here may be considered derivatives of 1-palmitoyl-2-stearoylphosphatidylcholine. The parent lipid has an order–disorder transition at 48.98 °C (Chen & Sturtevant, 1981). Two of the spin-label analogues have transition temperatures 15–19 °C lower. Introduction of a methyl group at the same position as the nitroxide group (C-16) in one of the spin-label lipids studied produces a similar reduction of the phase transition temperature (to 34.6 °C). The effects on the phase transition temperature of spin-label groups at various positions on a fatty acid chain are similar to the effects of variation in double-bond position (Barton & Gunstone, 1975) or in fluorine position on fluorinated phospholipids (Sturtevant et al., 1979). That is, the lipids labeled (or with double bonds) in the middle of the chain have much lower transition temperatures than do lipids with label groups (or double bonds) near either the polar or the terminal methyl ends of the fatty acid chain. It seems, therefore, that it is more appropriate to compare the behavior of the probe lipids to unsaturated, or branched, analogues of the parent saturated lipid than to the parent itself.

Marked hysteresis in the phase transition of 1-palm-2-(1,14)PC can be detected by both EPR (Chen & Gaffney, 1978) and DSC techniques. [Hysteresis of similar magnitude has been observed for the transition of a fluorinated lecithin (Sturtevant et al., 1979).] The DSC and EPR techniques are in agreement for the temperature at which the transition occurs when the sample is heated. However, there are differences in cooling curves obtained by the two techniques. The differences may result from the fact that the samples could not be handled in exactly the same way. Alternatively, they may result from errors in estimating the mole fraction of solid lipid from the DSC curves. The exact area under the heat absorption curve for the partially cooled samples used to construct the DSC cooling curve is necessary for accuracy. Since the DSC trace varies in a nonlinear manner on either side of the transition, it is difficult to draw an accurate base line for area determination. [Note also the slight nonlinearity in the trace for 1-palm-2-(5,10)PC shown in Figure 1.] The estimates of the fraction of molecules in the solid phase shown in Figure 4 could be low by as much as 20% if other reasonable estimates of the base line are used. The result of these considerations is that the true DSC cooling curve could be centered at a higher temperature than our estimate, by as much as a 1 °C or more. In contrast, determination of the transition curve for heating is almost independent of the choice of base line for a reasonably symmetrical heat absorption peak. For the same reasons, any hysteresis less than 2–3 °C wide indicated by DSC measurements [as observed for 1-palm-2-(12,3)-PC] should be accepted with reservation.

Spin-label lipids have been applied in the past in many different ways to provide information about the structure and dynamics of model and biological membranes. In some applications, the physical properties of the probes themselves may make a substantial contribution to the result, and in others, the probe properties are virtually irrelevant. Cases where the information provided in this report on the phase transitions of spin-label lipids is clearly of importance include use of spin-label lipids to detect the phase transition of a host lipid and some studies of lateral diffusion of lipids or of phase separations which require that high mole fractions of spin-label lipid be introduced into membranes (Scandella et al., 1972; Ito et al., 1975). In both types of experiment, the degree of miscibility of the spin-label lipid with the unlabeled lipids is a factor in interpreting the results. We have shown that the phase transitions of PSPC derivatives bearing a 16-methyl or a spin-label group at C-16 are similar in shape, width, and temperature, although different in that only the spin-label lipid exhibits hysteresis. The temperature dependence of the EPR spectra of a 0.5 mol % mixture of the C-16 spin-label PC in the 16-methyl-PC as host gives a sharp response to the phase transition of the latter (data not shown). The onset and termination temperatures for EPR signal responses to the P16MSPC phase transition are about 32 and 34 °C, respectively, where DSC changes range from about 32 to 35 °C. The midpoint of the EPR change is 33.2 °C, and the peak of the DSC plot is at 34.6 °C. However, the DSC peak is quite asymmetric, and the temperature at which half of the area of the DSC curve is reached is closer to 34 °C. Thus, in this case, the EPR spectra provide a reasonable approximation to the information provided by DSC. The spin-label data would have to be subjected to detailed line-shape analysis in order to determine the precise temperature at which half of the probe molecules had responded to the host phase transition because spectral amplitudes are a nonlinear function of the number of molecules involved when two different line shapes overlap. The responses of the EPR spectra in mixtures containing a higher mole fraction of the spin-label lipid would depend on the phase diagram of the system. Although we have made a preliminary study of the phase diagram of 1-palm-2-(1,14)PC and dimyristoyllecithin by EPR and DSC, the data have not been analyzed in detail because the system has three lipid components when one considers the two diastereoisomers of the spin-label lipids. Qualitatively, however, the thermal data for this system are complicated and undoubtedly show evidence of some immiscibility of the lipids in both the fluid and solid phases. Nevertheless, at 0.5 mol % of the spin-label PC, the EPR spectra show sharp changes in a range of 0.5 °C centered around 23 °C, again a reasonable approximation to the DSC data for the phase transition of dimyristoyllecithin. The hysteresis in the phase transition temperature of 1-palm-2-(1,14)PC may even be an advantage in studies of phase separations induced by divalent ions (Ito et al., 1975) or by proteins (Jost & Griffith, 1980) because it could help to identify a separate phase rich in the spin-label PC. In our examination of dimyristoyllecithin mixtures with 1-palm-2-(1,14)PC, we found hysteresis in the EPR spectra responses for mixtures which contained greater than 50 mol % of the latter component.

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Effect of Hydrogen Ion Concentration on Rhodopsin–Lipid Interactions†

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ABSTRACT: This study concerns the role titratable chemical groups play in rhodopsin–lipid interactions. Tempo partitioning techniques are used to construct DMPC–rhodopsin partial binary phase diagrams. Such diagrams are constructed at pH 7.0, where rhodopsin has a net negative charge, and at pH 5.0, where rhodopsin is virtually isoelectric. It is found that at pH 5.0, increases in rhodopsin content induce a depression in temperature of the solidus curve. At pH 7.0, the solidus curve is not a strong function of temperature. Additional studies, involving nitroxide-labeled palmitic acid, methyl palmitate, and lecithins, indicate that the pH-dependent changes in the phase behavior of DMPC–rhodopsin systems are due to the titration of rhodopsin's carboxylic acid groups.

A great deal of knowledge has emerged over the last decade concerning the motional state of membrane components in rhodopsin–phospholipid systems. Model systems, involving lecithin–rhodopsin recombinant membranes, have been particularly useful in this regard and the results from studies of such systems provided the background for this work.

Hong et al. (1975) have found that rhodopsin incorporation into lecithin bilayers resulted in an increase in the average viscosity of the bilayer as sensed by several nitroxide-labeled lecithin probes.

Fischer & Levy (1981) reported experiments involving DMPC[1]–rhodopsin recombinants that demonstrated several new features concerning the effect of rhodopsin on the motional state of lipids. First, proton spin–lattice (T_1) relaxation measurements involving DMPC–rhodopsin recombinants demonstrated that each rhodopsin molecule immobilized approximately 50 choline quaternary amine groups. Second, experiments involving proton T_1 studies of phospholipid chain terminal methyl groups as well as spin-labeled fatty acids with the nitroxide group placed in the center of the DMPC bilayer indicated that rhodopsin had a smaller effect on molecular motion in the center of the bilayer than on that in the outer half of the hydrophobic region of the bilayer.

Experiments utilizing rhodopsin–lecithin systems in which the rhodopsin has been covalently labeled with various ESR probes have also been performed (Davoust et al., 1980). These experiments demonstrated both a restriction of probe mobility in fluid membranes and the existence of a highly immobilized spectral component that was induced by decreases in temperature and/or lipid content.

Kusumi et al. (1980) have also performed experiments involving spin-labels convalently bound to rhodopsin in DMPC membranes. Utilizing saturation transfer techniques, they found that rhodopsin experienced relatively free rotational motion in the fluid phase. This rotational motion became more restricted as the temperature was lowered below the gel-to-liquid-crystalline phase transition temperature.

The above-mentioned experiments involving rhodopsin have measured the effect of a protein on various types of molecular motion within the membrane. In contrast, relatively few studies have addressed questions concerning the specific nature of protein–lipid interactions. For example, what chemical groups are involved in the rhodopsin–phospholipid interactions that produce immobilization? The ESR studies reported in this article address such questions. We have systematically examined the effect of pH on the phase behavior of rhodopsin–DMPC systems. This was accomplished by using Tempo partitioning techniques (Shimshick & McConnell, 1973) to identify fluidus and solidus curves. We have also examined the effects of pH on the behavior of several spin-labeled amphiphiles in DMPC and DMPC–rhodopsin membranes. The results of these two kinds of studies begin to define the role that titratable groups play in rhodopsin–lipid interactions.

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[1] Abbreviations: DMPC, L-α-dimyristylphosphatidylcholine; Tempo, 2,2,6,6-tetramethylpiperidinyl-1-oxy; PC, phosphatidylcholine; FA, fatty acid; DTAB, dodecyltrimethylammonium bromide; ME, methyl ester.

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