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Differential Effects on Phospholipid Phase Transitions Produced by Structurally Related Long-Chain Alcohols†

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ABSTRACT: The thermotropic behavior of aqueous dispersions of dipalmitoylphosphatidylcholine and, in a few cases, dimyristoylphosphatidylcholine and distearoylphosphatidylcholine, was measured spectroscopically by using the spin probe, 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo[1]). From the resulting sigmoidal phase transition profiles, the main gel to liquid-crystalline transition temperature (T_m) was obtained, an estimate was made of the mean transition half-width ($\bar{W}_{1/2}$) and, where it was observed, the small pretransition (T_l) was also determined. The effects on these parameters of incorporating the long-chain alcohols, $C_{14:0}$, *cis*- and *trans*-$C_{14:1}$, $C_{16:0}$, and *cis*- and *trans*-$C_{16:1}$, were studied as a function of the concentration of alcohol. In DPL, the saturated alcohols produced a concentration-dependent elevation, the trans unsaturated alcohols, a smaller elevation, while the cis unsaturated alcohols produced a substantial depression of T_m. All six alcohols broadened the main transition. The latter effect was large in the case of the saturated alcohols but significantly smaller in the case of three out of four of the unsaturated alcohols. The unsaturated hexadecenols were also incorporated into DML and DSL. As with DPL, the trans isomer raised, while the cis isomer lowered, the main transition temperature. In each case, there was an increase in the mean transition half-width ($\bar{W}_{1/2}$). Spin-labeled phospholipid (PC(7,6)) was used to determine the order parameter of DPL vesicles in the presence and absence of 33 mol % *cis*- and *trans*-hexadecenol. Above T_m, both alcohols ordered the lipid membrane slightly, whereas, below T_m, the cis isomer disordered, while the trans isomer expelled the spin label from the lipid bilayer. In contrast to their effect on T_m, all three of the C_{16} alcohols shifted the pretransition (T_l) to higher temperatures such that $|\Delta T_l|$ was usually greater than $|\Delta T_m|$. The manner and extent to which the phase transition parameters were modified were found to depend not only on the length and shape of the added alcohol but also on the chain length of the lipid into which it was incorporated. The results are discussed in terms of a thermodynamic model describing the differential partitioning of the alcohols into the gel and liquid-crystalline phases of the respective lipids.

The thermotropic phase transitions of aqueous phospholipid dispersions have been the subject of numerous studies in the last few years (for a recent review, see Lee (1977)). Impetus for such studies derives from the conjecture that a large variety of biological phenomena, having a biomembrane as their locus, are mediated by lateral phase separations in the lipid portion of the membrane. For example, lateral diffusion (Cullis, 1976), transport (Thilo et al., 1977), and membrane fusion (Poste & Allison, 1973) have been shown to be markedly enhanced in the temperature region of the gel to liquid-crystalline transition of the membrane lipids. Also, breaks in Arrhenius plots of glucagon-stimulated adenylate cyclase (Houslay et al., 1976), calcium-dependent ATPase in sarcoplasmic reticulum (Hidalgo et al., 1976), and phospholipase activity of β-bungarotoxin (Strong & Kelly, 1977) attest to the importance of this phenomenon in modulating enzyme activity. Furthermore, certain bacterial membranes such as *Acholeplasma laidlawii* (Verkleij et al., 1972), *Halobacterium cutirubrum* (Esser & Lanyi, 1973), and *Escherichia coli* (Träuble & Overath, 1973; Jackson & Sturtevant, 1977), as well as erythrocyte ghosts at low temperature (Verma & Wallach, 1976), have, themselves, been shown to undergo thermotropic phase transitions. The role of perturber molecules in shifting and broadening the phase transition has attracted considerable attention recently both because of the information yielded concerning the transition process and because of the possible involvement of phase transitions in drug action. Thus, Jain & Wu (1977) and Lee (1977) have carried out an extensive study of the effects on the DPL phase transition of a wide variety of organic and inorganic compounds. In particular, the effects of incorporating aliphatic alcohols into both pure and mixed lipid dispersions have been examined by several biophysical techniques, including light scattering (Hill, 1974), fluorescence (Lee, 1976), differential scanning calorimetry (Hui & Barton, 1973; Eliasz et al., 1976; Jain & Wu, 1977; Jain et al., 1978), and dilatometry (MacDonald, 1978). Taken together, the above studies show that short-chain alcohols depress, whereas long-chain alcohols elevate, the main gel to liquid-crystalline transition temperature (T_m) of the phospholipids into which they are intercalated. In

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[1] Abbreviations used: DPL, dipalmitoylphosphatidylcholine; DML, dimyristoylphosphatidylcholine; DSL, distearoylphosphatidylcholine; PC(7,6), 1-acyl-2-[8-(4,4-dimethyloxazolidine-*N*-oxyl)]palmitoylphosphatidylcholine; Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl; $C_{14:0}$, tetradecanol; $C_{14:1}$, *cis*- or *trans*-9,10-tetradecenol; $C_{16:0}$, hexadecanol; $C_{16:1}$, *cis*- or *trans*-9,10-hexadecenol; T_m, temperature of gel to liquid-crystalline phase transition; $\bar{W}_{1/2}$, mean transition half-width; T_l, temperature of pretransition; *n*, hydrocarbon chain length; *S*, order parameter; ESR, electron spin resonance.

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addition, it has been found that the alcohol chain length at which the shift in transition temperature (ΔT_m) changes from negative to positive is dependent upon the type of lipid used (Lee, 1976). However, the recent work of Jain et al. (1978) on the thermotropic behavior of DPL containing structural isomers of octanol underlines the importance of investigating the relationship between lipid phase transitions and the molecular geometry of perturber molecules. In this respect, unsaturated long-chain alcohols constitute an interesting and hitherto unstudied group of compounds.

Materials and Methods

DPL, DML, and DSL were purchased from Grand Island Biological, NY. All the alcohols used in this study were from Applied Science Labs. Inc., PA. Both lipids and alcohols were used without further purification. The spin-label 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) was synthesized by the method of Rozantsev (1970). PC(7,6) was synthesized via the spin-labeled fatty acid (Hubbell & McConnell, 1971) by condensing the latter with lysolecithin according to the procedure of Boss et al. (1975).

Experimental samples were prepared essentially as described by Trudell et al. (1974). To 100 mg of phospholipid was added 700 μL of 10 mM sodium phosphate buffer (pH 7.4)–0.1 M sodium chloride. An aliquot of 5 mM Tempo in distilled water (200 μL) was then added together with the appropriate amount of long-chain alcohol to give the required concentration (in the range 5–33 mol %). Phospholipid concentrations were always 10% w/v, and Tempo was 1 mM. Each sample mixture was heated 5–10 °C above its respective lipid phase transition temperature and then subjected to repeated vortexing and heating until a homogeneous dispersion was obtained (about 20 min). Samples were usually left overnight at the same temperature and revortexed before use if necessary. For ESR measurements, the lipid dispersions were introduced by syringe into 1-mm tubes sealed at one end, which were subsequently flushed with nitrogen, and finally sealed at the top with Seal-Ease (Clay Adams). The tubes were then placed in a Dewar situated in the cavity of a Varian EM 500 spectrometer. Temperature control was effected by a Haake E-52 water bath containing a heat-exchange coil through which hexadecane (mp 18 °C) was continuously pumped prior to passing through the Dewar. When it was necessary to operate at temperatures below 18 °C, *n*-decane was added to the hexadecane in order to lower its freezing point. Temperatures were stable to ±0.1 °C and were measured by a thermistor placed in the Dewar just above the sample.

The method utilizes the temperature-dependent partitioning of the spin label Tempo, between lipid and water, giving rise to a high-field doublet in the spectrum, from which the so-called solubility or f parameter (Shimshick & McConnell, 1973) can be obtained. Several scans were made at each temperature after allowing about 15 min for equilibration, and a range of about 10 °C above and below the main transition was studied. Mean values of f were plotted against temperature. The transition temperature, T_m, was taken as the midpoint of the steep section of the curve as defined by previous workers, and the same procedure was used for the pretransition, T_1, which was usually broader and less well defined. Samples were run in duplicate or, where the difference in T_m was greater than 0.1 °C, in triplicate.

The mean half-width, $\bar{W}_{1/2}$, was taken as half the temperature interval defined by the two points of intersection of a line drawn through the steep portion of the curve, with two lines fitted to the linear portions of the plot above and below the transition.

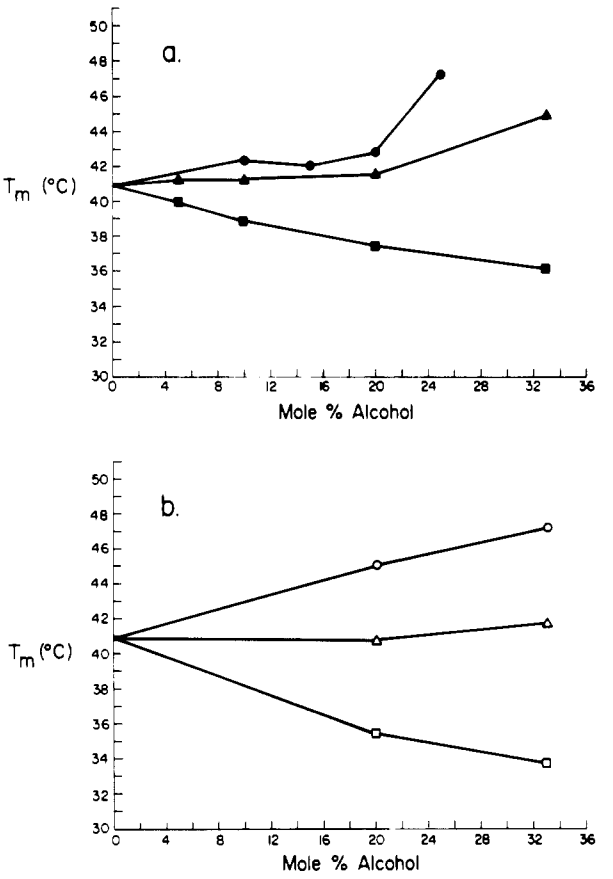

FIGURE 1: Effect on the main transition temperature (T_m) of DPL containing varying concentrations of long-chain alcohol, expressed as 100 × (moles of alcohol)/(moles of alcohol + moles of DPL). (a) Hexadecanol (●); *trans*-hexadecenol (▲); *cis*-hexadecenol (■). (b) Tetradecanol (○); *trans*-tetradecenol (△); *cis*-tetradecenol (□).

Samples for order parameter measurements were prepared in triplicate by evaporating down a chloroform/methanol solution containing 200 μL of DPL (50 mg/mL) and 50 μL of PC(7,6) (2.6 mM), together with the appropriate long-chain alcohol dissolved in methanol. After adding 0.5 mL of 0.9% NaCl, the sample concentrations were: lipid, 26 mM; spin label, 2.6×10^{-4} M; *cis*- and *trans*-hexadecenol, 6.5 mM. Homogeneous dispersions were obtained by vigorous vortexing at 50 °C. After overnight equilibration at 50 °C, the order parameters were measured at 53.6 and 29 °C on a Varian E-109 spectrometer, according to the method of Hubbell & McConnell (1971). Three scans per sample were taken and the order parameter was averaged.

Results

Main Transition. The main phase transition (T_m) for DPL was found to be 40.9 ± 0.2 °C, which is within the range of values quoted by other authors (for a comparative table of values, see Jacobs et al. (1977)). Effects on the DPL phase transition temperature of incorporating the three alcohols, hexadecanol and the geometric isomers of hexadecenol, are illustrated in Figure 1a, which shows a plot of T_m vs. alcohol concentration. The results for the C_{14} alcohols are presented in Figure 1b. The saturated alcohols both elevate the T_m of DPL, which is in agreement with results obtained from differential scanning calorimetry by Eliasz et al. (1976) and Jain & Wu (1977). The most striking aspect of these results is the fact that the cis and trans isomers produce completely opposite effects on the DPL phase transition temperature, i.e., cis isomers depress T_m, whereas trans isomers elevate T_m. There is a reasonably good linear relation between T_m and con-

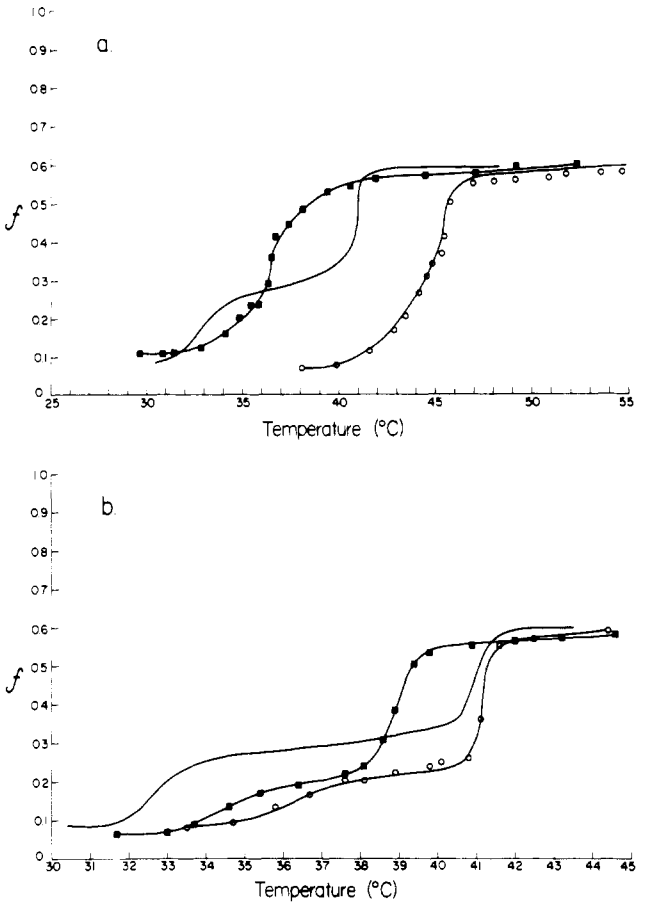

FIGURE 2: Phase transition profiles showing plot of f vs. temperature for DPL (no symbols) and DPL containing *trans*-hexadecenol (○) and *cis*-hexadecenol (■). (a) Thirty-three mole percent alcohol incorporation; (b) 10 mol % alcohol incorporation (note the expanded temperature scale).

centration for all the alcohols except hexadecanol and *trans*-hexadecenol above 20 mol %. The cis–trans difference is more clearly illustrated in Figure 2a which shows the transition profile for pure DPL and DPL containing 33 mol % of the *cis*- and *trans*-hexadecenols. The shifts in transition temperature, ΔT_m, in this case are almost equal in magnitude as well as opposite in sign, with a cis–trans difference of approximately 9 °C. A further feature of Figure 2a is the absence of any pretransition (T_l) in the alcohol-containing samples. However, at lower alcohol concentrations, the pretransition was observed, while the differential effect of the two isomers on T_m was maintained (Figure 2b). Comparing the hexadecenols with the tetradecenols (Figures 1a and 1b), it can be seen that *trans*-hexadecenol elevates T_m more effectively than *trans*-tetradecenol, while *cis*-tetradecenol depresses T_m more effectively than *cis*-hexadecenol. It is of interest to note that $|\Delta T_m|$ (cis–trans) is almost the same in both cases, for example, 4–5 °C at 20 mol %.

The effects of incorporating *cis*- and *trans*-hexadecenol into liposomes prepared from DML and DSL were studied. T_m values for pure DML and DSL were 23.6 and 54 ± 0.3 °C, respectively. These values agree with those obtained by other methods (Jacobs et al., 1977). The data for all three lipids are compared in Figure 3a, which is a plot of T_m vs. lipid chain length (n) for the pure lipid dispersion (control) and those containing 33 mol % of either *cis*- or *trans*-hexadecenol. The differential effect of the isomers was observed in all cases, i.e., *trans*-hexadecenol elevated, whereas *cis*-hexadecenol depressed, the T_m of the respective lipid. However, as the lipid acyl

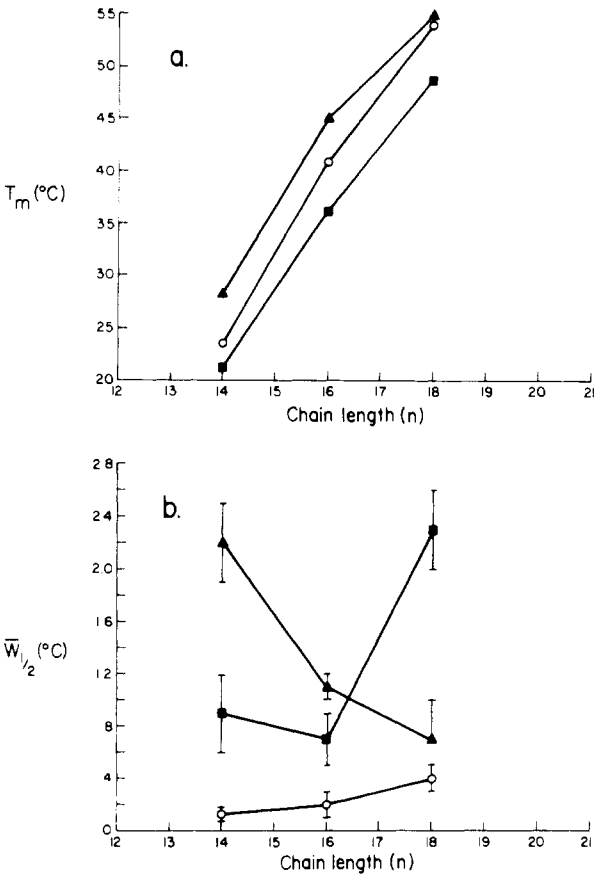

FIGURE 3: Relation between the number of carbon atoms in the lipid acyl hydrocarbon chain and (a) the main phase transition temperature (T_m); (b) the mean half-width of the transition ($\bar{W}_{1/2}$). Pure lipid (○); 33 mol % *trans*-hexadecenol (▲); 33 mol % *cis*-hexadecenol (■).

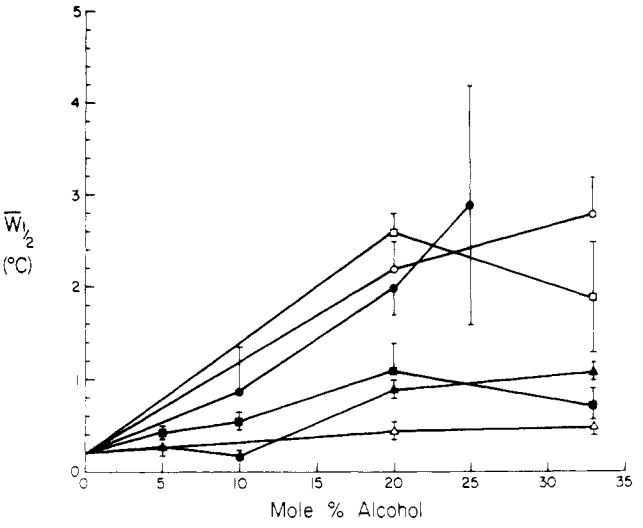

FIGURE 4: Effect of alcohol concentration on the mean half-width ($\bar{W}_{1/2}$) of the main phase transition of DPL. Hexadecanol (●); *cis*-hexadecenol (■); *trans*-hexadecenol (▲); tetradecanol (○); *cis*-tetradecenol (□); *trans*-tetradecenol (△).

hydrocarbon chain length was increased, the elevation in T_m produced by *trans*-hexadecenol became progressively smaller. Conversely, the depression produced by *cis*-hexadecenol increased twofold going from DML to DSL (Figure 3a).

Mean Half-Width of Main Transition. The effects of all six alcohols on the mean half-width ($\bar{W}_{1/2}$) of the main transition in DPL are shown in Figure 4. While we recognize that our definition of $\bar{W}_{1/2}$ is somewhat arbitrary (see Materials and Methods) and does not correspond to the temperature

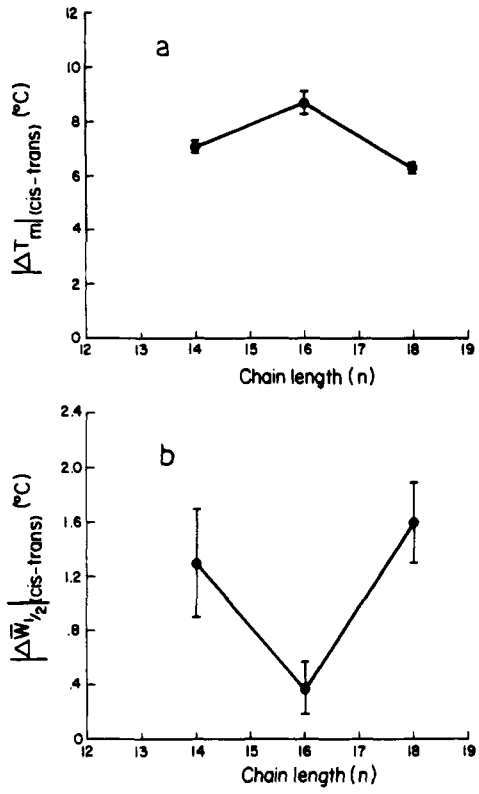

FIGURE 5: An illustration of the "cis–trans" effect. (a) The absolute difference in transition temperature $|\Delta T_m|$ (cis–trans) for 33 mol % *cis*- and *trans*-hexadecenols incorporated into phospholipids of varying acyl hydrocarbon chain length. (b) The absolute difference in mean transition half-width ($|\Delta \bar{W}_{1/2}|$ (cis–trans)) for the same two unsaturated alcohols under identical conditions.

range over which the transition is observed to occur calorimetrically, the relative effects produced by the alcohols probably reflect at least qualitatively the real differences between them. Two main features of these results stand out. Firstly, all of the added compounds have the effect of broadening the gel to liquid-crystalline transition in a concentration-dependent manner. This accords with previous studies which showed that short-chain *n* alcohols have no effect on the transition width (Jain & Wu, 1977; McDonald, 1978), whereas long-chain *n* alcohols cause an increase (Jain et al., 1978; Lee, 1976). Secondly, one can see from Figure 4 that the alcohols tend to fall into two groups with regard to the extent to which they increase, $\bar{W}_{1/2}$. Both saturated compounds and *cis*-tetradecenol produce a large increase in $\bar{W}_{1/2}$, whereas the other three unsaturated alcohols all exert a much smaller effect, especially *trans*-tetradecenol.

The mean half-widths of the main transition of the lipids increased in the order DML, DPL, DSL (0.1, 0.2, and 0.4 °C, respectively). Figure 3b also shows the effect of 33 mol % of *cis*- and *trans*-hexadecenol. The main feature of this plot is that the unsaturated isomers show opposite trends in their transition-broadening efficacy. *trans*-Hexadecenol is progressively less effective at broadening the main phase transition as the lipid chain length increases, whereas *cis*-hexadecenol tends to show the opposite effect.

Lipid Chain Length and the Cis–Trans Effect. The relationship between T_m and lipid chain length shown in Figure 3a shows reasonable but not perfect linearity, with all three regression coefficients greater than 0.989. One of the reasons for the deviation from linearity can be seen in Figure 5a which is a plot of the difference in T_m for the two isomeric alcohols, $|\Delta T_m|$ (cis–trans), vs. *n*. The interesting feature of this plot

Table I: Order Parameter (*S*) for DPL in the Absence and Presence of 20 mol % *cis*- and *trans*-Hexadecenol, above and below T_m Determined by Using PC (7,6) Spin-Label[a]

additions:	none	*cis*-$C_{16:1}$	*trans*-$C_{16:1}$
$S_{53.8°C}$	0.273 ± 0.006	0.285 ± 0.004	0.298 ± 0.010
ΔS	0	0.012	0.025
$S_{41.4°C}$	0.389 ± 0.007	ND[b]	0.385
ΔS	0		−0.004
$S_{29°C}$	0.624 ± 0.004	0.510 ± 0.007	NM[b]
ΔS	0	−0.114	

[a] Standard deviations are provided when more than two samples were averaged. [b] ND, not determined; NM, not measurable.

is that it is biphasic and maximal for $n = 16$. Thus, the sensitivity of the lipid to distinguish between *cis*- and *trans*-hexadecenol is optimal when both the lipid and alcohol chains contain equal numbers of carbon atoms. Where chain mismatching occurs between lipid and alcohol, i.e., with DML and DSL, the cis–trans difference is attenuated.

A similar effect characterizes the alcohol-induced broadening of the main transition. Figure 5b shows a plot of the difference in mean half-width for the isomeric alcohols, $\Delta \bar{W}_{1/2}$ (cis–trans), vs. *n*. Again, the plot is biphasic, but in this case the difference is minimal for DPL, and increases with DML and DSL. Further studies would determine whether or not this chain-matching phenomenon is a general rule for acylphosphatidylcholines containing unsaturated alcohols.

DPL Order Parameter. The effects of 20 mol % *cis*- and *trans*-hexadecenol on DPL membrane order are shown in Table I. The order parameter (*S*) for pure DPL multilammellar liposomes at 29 and 53.6 °C are 0.624 ± 0.004 and 0.273 ± 0.006, respectively. From the table, one can see that, above the T_m of DPL, both alcohols ordered the lipid bilayers to a small but significant extent. Although the trans compound was twice as effective as the cis compound, the absolute magnitude of the effect is so small that, within the experimental error, the values of ΔS must be regarded as similar. At 29 °C, however, *cis*-hexadecenol produced a marked lowering of the order parameter, while the trans isomer excluded the spin label from the gel phase, giving rise to a large free-solution signal and a lipid spectrum which was too small to allow measurement of the order parameter. Additional spectra for *trans*-hexadecenol were therefore run at 47.6, 41.4, and 35 °C. Between 35 and 41.4 °C there was a dramatic change in the spectrum such that, at the lower temperature, a large amount of spin label was excluded from the lipid bilayer, while, at the higher temperature which is in the region of the phase transition, the order parameter was similar to the control value. The low temperature exclusion of spin label was found to be reversible, upon reheating.

Pretransition. A premelting transition for DPL was found at 32.7 ± 0.2 °C and for DSL at 47.5 ± 0.2 °C. These values are somewhat lower than those obtained by calorimetry (34 ± 0.2 and 49.1 ± 0.2 °C, according to Hinz & Sturtevant (1972); 34.5 °C according to Janiak et al. (1976)). This discrepancy results from the relaxation rate of the pretransition being long relative to the scan rate of the calorimeter, but short compared with our equilibration times (Lentz et al., 1978). In agreement with Marsh et al. (1977), we did not observe a pretransition for DML above 12 °C, using the method of Tempo partitioning. Since alcohol concentrations greater than 10 mol % caused the disappearance of the pretransition, our data are extremely limited and are restricted to the C_{16} alcohols at 5 and 10 mol % in DPL. In contrast to their behavior with regard to the main transition, all three of the alcohols shifted T_1 to higher temperatures. Furthermore, changes in T_1 were,

on average, greater in magnitude than the corresponding changes in T_m. Thus, at 10 mol %, hexadecanol raised the pretransition 5.1 to 37.8 °C. For the hexadecenols at 5 and 10 mol %, respectively, the pretransitions were found as follows: cis 32.4 and 34.4 °C; trans 35.0 and 36.5 °C.

Discussion

Main Transition. The most important qualitative feature of our results is that, irrespective of the alcohol chain length, or the lipid acyl hydrocarbon chain length, the cis unsaturated alcohols depress T_m, whereas the corresponding trans analogues elevate T_m. Hill (1974) has shown that the shift in T_m brought about by small solutes may be related to the solubility in the liquid-crystalline phase. Although we do not have partition coefficients for these isomers, data of *cis*- and *trans*-butene in egg lecithin (Miller et al., 1977) together with solubility data for *n*-butane (Battino, 1971) have shown that the net effect of introducing one double bond is to increase the amount of solute in the lipid phase by virtue of an increase in the aqueous saturation concentration. However, no significant solubility difference was found between the unsaturated isomers. Although one should exercise caution in extrapolating data from simple olefins to long chain olefinic alcohols, it would seem that lipid solubility per se provides an insufficient explanation for the differential effects of *cis*- and *trans*-hexadecenol on the T_m of DPL. However, since the butene experiments were conducted above the T_m of egg lecithin, one should consider the possibility that the alcohols partition differentially into the gel phase of the lipid.

The studies of Eliasz et al. (1976), Lee (1976), Jain & Wu (1977) and McDonald (1978) have established an alcohol chain length dependence of ΔT_m with a so-called cutoff at C_{12}; i.e., alcohols with less than 12 carbon atoms depress the T_m of DPL, whereas longer alcohols elevate T_m. No entirely satisfactory explanation has been found for this effect, although a number of suggestions have been advanced (Lee, 1976). We suggest here that the reason for the crossover from negative to positive values of ΔT_m can be found from thermodynamic considerations if one allows for partitioning of the alcohols into *both* the gel and liquid-crystalline phases of the lipid. By assuming for simplicity that the main phase transition is a first-order process (Lee, 1977) and that at low concentrations the added alcohols are incorporated into the bulk phase of the lipid rather than into localized regions, then the following expression applies (Pitzer & Brewer, 1961)

$$\Delta T \simeq -\frac{RT_m^2}{\Delta H_A} \ln\left(\frac{n^l}{n^g}\right) \quad (1)$$

where ΔH_A is the enthalphy change associated with the transition and n^l and n^g are the mole fractions of lipid in the liquid-crystalline and gel phases, respectively. Since the process is endothermic, ΔH_A is always positive and thus, for alcohols which partition preferentially into the liquid-crystalline phase of the lipid, $\ln (n^l/n^g)$ will be positive and the transition temperature will be lowered. However, for alcohols which partition perferentially into the gel phase, the converse will be true. It also follows from the above expression that, if an alcohol dissolves equally in both phases, T_m should remain unchanged. An indication of the validity of eq 1 is provided by the data of Sklar et al. (1977) who showed that *trans*-parinaric acid partitions preferentially into gel phase liposomes and that it raises the phase transition of DPL. It is, therefore, probable that differential partitioning provides an explanation of our own results for the isomers of tetradecenol and hexadecenol. Since the butene data suggest that there should be little difference between the partition coefficients of cis and trans isomers above the phase transition, it is likely that the critical factor in determining the sign of ΔT_m will be the relative gel state solubilities of the added alcohols. Such a prediction is open to direct experimental test. Indeed, preliminary results from our laboratory indicate that the partition coefficient of hexadecanol does increase with decreasing temperature in the region of the phase transition.

The addition of a foreign molecule to a lipid bilayer in the gel state will disrupt the tight packing of the acyl chains and consequently weaken the strong intermolecular forces resulting from the all-trans conformation of the lipids (Lee, 1977). However, this free energy loss will be compensated for by the degree to which the added alcohol can interact with the phospholipid. If the alcohol closely resembles the lipid in chain length and configuration, it is possible that such interactions may be maximized to produce a state which is even more stable than the natural gel state. This enhanced stability may also arise partly from hydrogen bonding between the OH group of the alcohol and some suitably oriented part of the phospholipid head group and partly from the relief of Coulombic repulsions between head groups (Maybrey & Sturtevart, 1976; Jacobs et al., 1975). However, these effects should be similar for all the long-chain alcohols, so that the origin of the differential effects of the unsaturated alcohol isomers must lie in the acyl region of the bilayer and can most readily be understood in terms of the stereochemistry imposed by the presence of a double bond in the 9,10 position of the hydrocarbon chain. The trans unsaturated alcohols should closely resemble the all-trans conformation of *n*-hexadecanol, except for a small lateral displacement, or jog, of the long axis at the double bond so that, in each case, minimal lattic rearrangement is required for insertion of the alcohol. Thus, van der Waals forces in the acyl region are conserved, and gel-state partitioning is favored. On the other hand, a cis double bond, especially in the 9,10 position (Barton & Gunstone, 1975), prevents the alcohol from adopting a structure resembling that of an all-trans chain, even when gauche bonds are allowed. Incorporation into the gel phase will, therefore, not be favored since it would result in a large reduction in van der Waals interactions.

The difference between the C_{14} and C_{16} isomers in DPL can also be rationalized by similar arguments. *trans*-Tetradecenol is shorter than *trans*-hexadecenol by two methylene groups, so that van der Waals interactions between alcohol and DPL in the gel state will be correspondingly less extensive for the former than for the latter. Equally, *cis*-tetradecenol should partition less into the gel state than *cis*-hexadecenol. In fact, one can see from the T_m vs. concentration plots in Figures 1a and 1b that the results for the C_{14} isomers are obtained simply by a downward rotation, about the T_m axis of the corresponding hexadecenol plots. The behavior of the saturated alcohols seems to be more complex. From the foregoing chain-matching arguments, one would reason that hexadecanol would partition better into gel phase DPL than tetradecanol and, thereby, produce a larger elevation in T_m. Our data show the converse to be true. This may simply reflect the fact that, in general, saturated alcohols are less lipid soluble than unsaturated alcohols, and hexadecanol is less soluble than tetradecanol.

The model can, however, be applied to the effect of varying the lipid chain length. The fact that *trans*-hexadecenol becomes less effective at elevating T_m as the lipid chain length increases suggests again that partitioning into the gel phase becomes relatively less favorable, presumably because of the

greater lateral compression within the lipid, caused by increasing van der Waals forces between the chains. In fact, an extrapolation of the plot in Figure 3a suggests that the T_m of diarachidoylphosphatidylcholine ($n = 20$) will probably be slightly depressed by 33 mol % *trans*-hexadecenol.

Relative distribution of the alcohols between the gel and liquid-crystalline phases can only provide a partial explanation for our results. At high alcohol concentrations, alcohol–alcohol interactions should become frequent and the assumptions in eq 1 become invalid. This is clearly demonstrated with hexadecanol and *trans*-hexadecenol, where the relation between T_m and concentration becomes nonlinear above 20 mol %. Mabrey & Sturtevant (1977) have provided evidence that excess palmitic acid in DPL leads to the formation of a 1:1 complex, but such effects were not noted with hexadecanol or hexadecane, both of which were found to elevate T_m at concentrations equal to the highest we studied. However, eq 1 provides a firm thermodynamic basis for our data, particularly in the range where the phase transition has not been excessively broadened.

Width of the Transition. The effects on the mean transition half-width ($\bar{W}_{1/2}$) are harder to interpret. Since one phase arises out of the other, there is a finite temperature range in which both phases coexist, and the assumption that the gel too liquid-crystalline phase transition is first order can only be regarded as a first approximation (Lee, 1977). Theoretical treatments suggest that the temperature range is inversely related to the number of lipid molecules involved in the cooperative unit (March et al., 1976; Mabrey & Sturtevant, 1976; Mountcastle et al., 1978). If a compound added to the lipid acts in such a way that the cooperative unit is destabilized, then the average size of the lipid clusters involved in the transition will be decreased, with a resulting broadening of the transition creating a distinct two phase region. Direct proof of such a model is difficult and as yet it has little predictive value. Nor is there, at present, an adequate theory which relates the structure of an added alcohol to its ability to affect transitions cooperatively. In DPL, one might conclude from Figure 4 that saturated alcohols have a much greater disordering effect on the cooperative unit than unsaturated alcohols, although the large broadening effect of *cis*-tetradecenol is hard to explain. Alternatively, from Figures 1 and 4, one can see that at 20 mol % there is a reasonable correlation between the absolute change in transition temperature $|\Delta T_m|$ and the transition half-width, $\bar{W}_{1/2}$, i.e., the ranking order for $|\Delta T_m|$ is $C_{14:1cis} > C_{14:0} > C_{16:1cis} > C_{16:0} > C_{16:1trans} > C_{14:1trans}$, whereas, for $\bar{W}_{1/2}$, the corresponding order is $C_{14:1cis} > C_{14:0} > C_{16:0} > C_{16:1cis} > C_{16:1trans} > C_{14:1trans}$. If one considers the effect of lipid chain length on the broadening of the transition induced by the hexadecenols (data from Table I), one again finds a reasonable correlation between mean half-width and absolute shift in transition temperature. Thus a plot of $|\Delta T_m|$ vs. $1/\bar{W}_{1/2}$ is linear ($R = 0.967$) at 33 mol % incorporation, although *cis*-hexadecenol in DPL shows anomalous behavior.

Premelting Transition. The nature of the pretransition (T_l) is still a matter of uncertainty. Ladbrooke & Chapman (1969) and Lee et al. (1974) suggested that reorientation of the choline head groups might be responsible for the transition; the more recent studies of Janiak et al. (1976) and Gaber et al. (1978) have demonstrated a change in the angle of tilt and lattice arrangement of the acyl hydrocarbon chains at T_l, and, although the former authors suggested that this might be mediated by specific water/choline head-group Interactions, the nuclear magnetic resonance studies of Seelig (1977) indicate the noninvolvement of the choline group in the pretransition of phosphatidylcholine. Our data for the three C_{16} alcohols in DPL (Table I) show two main features with respect to T_l. At 10 mol %, all three of the alcohols shifted T_l to higher temperatures and, on average, changes in T_l were greater than corresponding changes in T_m. Secondly, the magnitude of the effects were in the order *n*-hexadecanol > *trans*-hexadecenol > *cis*-hexadecenol. According to our model, this represents the probable order of decreasing gel state solubility for the three alcohols. In fact, at 10 mol %, there is a very good correlation between $|\Delta T_m|$ and $|\Delta T_l|$ such that $T_m - T_l = k$, with a regression coefficient of 0.997. For pure DPL, k was found to be 8.2 ± 0.3 °C, and for 10 mol % alcohol concentration the mean value of k for the three alcohols was 4.6 ± 0.15 °C. Such a result is important in that it suggests that, at a fixed concentration of added alcohol, the absolute magnitude of shifts in the pretransition (ΔT_l) are uniquely determined by corresponding changes in T_m. Thus although all three alcohols stabilize the phase below T_l, the degree to which they do so is also reflected in their ability to alter the stability of the gel phase relative to the liquid-crystalline phase.

Once again, our results suggest the relative importance of the acyl hydrocarbon chain in these processes since, for all three alcohols, the interaction in the head-group region should be similar.

Bilayer Fluidity. Our measurements of the order parameter changes induced in DPL by *cis*- and *trans*-hexadecenol (Table I) add support to the hypothesis that the origin of the differential effects on T_m is to be sought in the stability of the gel phase of the lipid. Neither alcohol had a marked effect above the phase transition, although both produced a slight increase in order. Thus, there is no correlation between the effects on fluidity in the liquid crystalline phase and the direction in which T_m is shifted. Below the phase transition, the order parameter must be interpreted with caution because of the tendency for the bulky spin label to be excluded from the gel phase, and the possibility of a heterogeneous distribution of the alcohols and spin label in the bilayers. Nonetheless, the dramatic displacement of the label from the bilayer in the presence of *trans*-hexadecenol is consistent with the postulated stabilizing effect of the alcohol on the phospholipid gel phase, while the observed disordering in the presence of *cis*-hexadecenol may reflect the postulated destabilizing effect of this isomer. Similar arguments have recently been advanced by Usher et al. (1978) to account for the differential fluidizing effects of fatty acids above and below the T_m of DML.

Possible Biological Implications. It is tempting to speculate that a cis–trans isomerization might be employed in vivo as a phase switch for modulating protein function through control of the lipid environment. The cis–trans photoisomerization of rhodopsin (Wald, 1968) is an obvious example, and it is an interesting possibility that a light-induced interconversion of geometric isomers might be utilized in some circumstances to modulate boundary lipids. A related suggestion has been made by Verma et al. (1972) who demonstrated a photoinduced fluidity change in PC bilayers containing retinal or chlorophyll *a*. Furthermore, Rousselet & Devaux (1978) have recently shown that, in membrane-bound rhodopsin, spin labels attached to the protein sulfhydryl groups interact with phospholipids spin labeled in the head group only after bleaching. Whether this results from a conformation change in rhodopsin (for which there are now several lines of evidence (Ostroy, 1977)) or from a photoinduced "melting" of the boundary lipids, which would allow the exogenous lipids close approach to the protein, is an open question in this case. The general

concept of a phase switch thus remains an intriguing possibility at present.

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