Alcohol Induction of Interdigitation in Distearoylphosphatidylcholine: Fluorescence Studies of Alcohol Chain Length Requirements

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ABSTRACT Although it is now well established that the fully interdigitated phase is induced in saturated like-chain phosphatidylcholines (PCs) by a variety of amphipathic molecules including alcohols, no systematic study of the properties of the inducing molecules has been reported. To elucidate the stereochemical features that lead to the alcohol induction of interdigitation in PCs, we have investigated the induction of interdigitation in distearoylphosphatidylcholine (DSPC) by a series of alcohols. Our previously established DPH (1,6-diphenyl-1,3,5-hexatriene) fluorescence intensity method has been expanded (P. Nambi, E. S. Rowe, and T. M. McIntosh (1988), Biochemistry 27:9175–9182) and used to determine which of the alcohols induce interdigitation and to determine the threshold concentrations for each. We have found that each of the *n*-alcohols up to heptanol and several branched alcohols are capable of inducing interdigitation in DSPC; octanol and nonanol do not appear to induce interdigitation by these criteria. The threshold concentrations for interdigitation for each of these alcohols up to heptanol were found to be correlated with the membrane: buffer partition coefficients. The mole fraction of bound alcohol at the threshold concentration was similar for each of the alcohols up to pentanol. These results are discussed in terms of a general mechanism of the formation of the interdigitated phase.

INTRODUCTION

There is a wide variety of amphiphilic compounds that are capable of inducing the interdigitated $L_\beta I$ phase in saturated like-chain phosphatidylcholines (for review see Slater and Huang, 1988). Most of the work on this phase has been on its induction by ethanol (Rowe, 1992; Rowe and Cutrera, 1990; Nambi et al., 1988; Simon and McIntosh, 1984; Komatsu and Rowe, 1991; Ohki et al., 1990; Zeng and Chong, 1991; Zeng et al., 1993; Yamazaki et al., 1992). Additional compounds that induce the $L_\beta I$ phase in saturated like-chain PCs include glycerol, methanol, ethylene glycol, benzyl alcohol, chlorpromazine, tetracaine, and thiocyanate ion (McDaniel et al., 1983; McIntosh et al., 1983; Cunningham and Lis, 1986; Slater and Huang, 1988). In the absence of additives, dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) go into the interdigitated phase at increased hydrostatic pressure (Braganza and Worcester, 1986; Prasad et al., 1987). It has recently been shown that pressure and ethanol interact in inducing interdigitation in DPPC (Zeng and Chong, 1991). Several attempts at rationalizing the mechanism of the transition from the non-interdigitated to the interdigitated phase for these lipids have been made (Simon and McIntosh, 1984; Simon et al., 1986; Nambi et al., 1988; Rowe and Cutrera, 1990), based on the general concept of shielding of the hydrophobic terminal methyls from the aqueous interfacial environment by the inducer. However, to date no attempt to refine our knowledge of the properties of the inducers has been reported.

Fig. 1 shows an illustration of the sequence of phase changes that occur in DSPC as it is heated, in the absence and presence of ethanol. The lower pathway depicts the sequence that occurs in the absence of ethanol. It is now well established that in the presence of sufficient quantities of ethanol the lipid becomes interdigitated, following the upper pathway as temperature is increased. In our laboratory we have undertaken several studies to understand the thermodynamics of the transitions to and from the interdigitated phase, and to define the conditions under which interdigitation occurs (Nambi et al., 1988; Rowe and Cutrera, 1990; Zhang and Rowe, 1992; Rowe, 1992; Komatsu and Rowe, 1991; Komatsu et al., 1993). These previous studies have focused mainly on ethanol. We have recently developed a fluorescence method for the detection of the interdigitated phase that makes it possible to screen a large number of samples and determine their phase states (Nambi et al., 1988). In the present investigation we have used the 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence method to determine the stereochemical requirements for alcohols to induce interdigitation and have determined the relative efficacy of each of the alcohols in inducing interdigitation. These results are then interpreted in terms of the forces involved in the induction of interdigitation.

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MATERIALS AND METHODS

Materials

DSPC was purchased from Avanti Polar Lipids (Birmingham, AL). The fluorophore DPH was obtained from Molecular Probes (Eugene, OR). Ethanol was obtained from Publicker Industries Co. (Linfield, PA). 2-Methyl

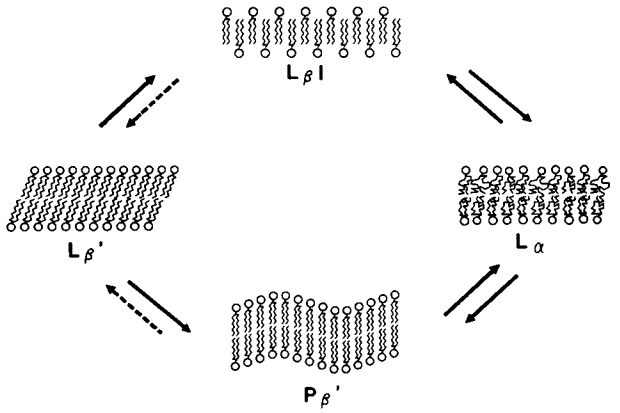

FIGURE 1 Schematic illustration of the phase sequences that are exhibited by DSPC as temperature is increased from left to right. The upper pathway is followed in the presence of alcohol above the threshold concentration; the lower pathway is followed in the absence of alcohol or at concentrations below the threshold concentration. The dashed arrows indicate the slow reversibility of these reactions, which leads to marked hysteresis in temperature scanning.

propanol was obtained from Fisher Scientific (Pittsburgh, PA). The other alcohols were from Aldrich Chemical Co. (Milwaukee, WI) or Supelco (Bellefonte, PA).

Samples

The stock solutions of the lipids and of DPH were prepared in chloroform. The chloroform stock solutions of the lipid and the probe were kept in the freezer until ready to use. Appropriate volumes of the chloroform solution of the lipid and DPH were mixed to give a lipid-to-probe ratio of 500:1 before preparation of multilamellar liposomes according to the method of Bangham et al. (1967). Chloroform was removed by first evaporating with N_2 gas and then removing the final residual chloroform under vacuum. Doubly distilled water was added to the thin film containing the lipid and probe. Nitrogen was bubbled to remove any dissolved oxygen, and the lipid was hydrated at ~10°C above the chain-melting temperature of the lipid for at least 1 h. During this incubation, the sample was vortexed periodically. The concentration of lipid for the fluorescence experiments was 0.13 mM. The alcohols were added to the samples at least 30 min before beginning the scans. For this low-lipid concentration, which is an order of magnitude below the lowest alcohol concentration used, it can be assumed that the free alcohol concentration is the same as the total alcohol concentration.

Fluorescence

The fluorescence experiments were performed using the SLM 8225 spectrofluorometer as described previously (Veiro et al., 1987; Nambi et al., 1988). The excitation wavelength was 351 nm, and the emission was measured at 430 nm. A high-pass filter (cutoff light <370 nm) was used on the emission side to minimize scattered light. The lipid concentration was kept low to minimize the scattering contributions from the sample. Control experiments without the probe showed that the signal from stray scattered light was <2% of the signal from the samples with the probe at the same lipid concentration. The temperature was controlled by water circulating from an external programmable bath; it was measured by a thermistor immersed in a parallel cuvette, and the temperature data were read directly into the computer along with the fluorescence data. The rate of heating was 1°C/min.

RESULTS

Criteria for interdigitation-ethanol

By correlation of x-ray diffraction measurements with DPH fluorescence intensity, we have previously demonstrated the change in DPH fluorescence intensity that occurs for the L_{β}'-to-$L_{\beta}I$ transition at constant ethanol concentration as a function of temperature (upper pathway in Fig. 1), and during the P_{β}'-to-$L_{\beta}I$ transition in constant temperature experiments as a function of ethanol concentration (Nambi et al., 1988). The previous study focused on the lower-temperature transitions shown in Fig. 1; in the present study we have also followed the main transitions (the two higher temperature transitions to the right in Fig. 1) using DPH fluorescence.

Fig. 2 shows temperature scans of DPH fluorescence intensity in DSPC in the absence of ethanol and in the presence of a series of ethanol concentrations. Consideration of the two extreme scans, with no ethanol and with 0.98 M ethanol, respectively, demonstrates the results when the lipid undergoes the lower and upper sequences of phase changes in Fig. 1, respectively. In the absence of ethanol the L_{β}'-to-P_{β}' transition gives a relatively small decrease in DPH fluorescence intensity; in contrast the L_{β}'-to-$L_{\beta}I$ transition, in the presence of 0.98 M ethanol, gives a much larger decrease in intensity. (The correlation of this large change in DPH fluorescence intensity with the induction of the $L_{\beta}I$ phase was demonstrated for DPPC by x-ray diffraction (Nambi et al., 1988).) At the so-called "main transition," the P_{β}'-to-L_{α} transition in the absence of ethanol gives a decrease in DPH intensity; in contrast the $L_{\beta}I$-to-L_{α} transition in the presence of ethanol gives an increase in intensity. These data demonstrate two independent features that distinguish whether or not interdigitation is present, the magnitude of the decrease observed for the lower temperature transition and the sign of the change observed in the main transition.

Fig. 2 also shows scans for intermediate ethanol concentrations demonstrating the progression of these features as the pathway of the phase changes illustrated in Fig. 1 is shifted. It is interesting to note that for the intermediate concentration of 0.5 M ethanol, there is a suggestion of both a decrease and an increase in intensity at the main transition.

To establish quantitative means of determining the threshold alcohol concentration for the induction of interdigitation,

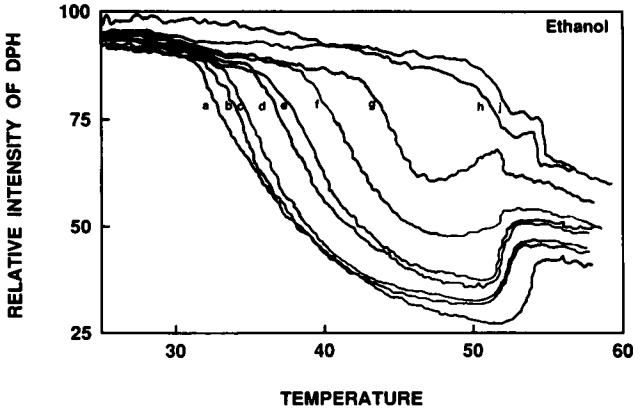

FIGURE 2 Relative DPH fluorescence intensity as a function of temperature in DSPC at a series of ethanol concentrations, which are as follows. *a*: 0.98 M; *b*: 0.87 M; *c*: 0.80 M; *d*: 0.72 M; *e*: 0.65 M; *f*: 0.59 M; *g*: 0.50 M; *h*:0.43 M; *j*:0 M.

it is useful to express the observations of Fig. 2 in a more quantitative way. Fig. 3 shows a plot of the fractional intensity remaining at the end of the lower temperature transition against the ethanol concentration. This plot shows clear sigmoidal behavior with a midpoint at 0.54 M ethanol.

Fig. 4 similarly quantitates the difference in the fluorescence response to the main transition depending on whether it is the $L_\beta I$-to-L_α or the P_β'-to-L_α transition (i.e., the upper versus the lower pathway in Fig. 1). Here we have plotted the intensity before the main transition over the intensity at the end of the transition. If the fluorescence change is positive, then this ratio is >1; if it is negative, then this ratio is <1. This plot also shows a sigmoidal change that occurs over the same range of ethanol concentration as that shown in Fig. 3. The midpoint of this change also occurs at 0.54 M ethanol. The region over which the change in both intensity parameters occurs agrees well with the threshold ethanol concentration for interdigitation determined previously (Nambi et al., 1988), and by high-sensitivity differential scanning calorimetry (Rowe and Cutrera, 1990).

The agreement between the threshold ethanol concentration as observed at the two transition points means that there is no significant temperature effect on the threshold concentration. The exception to this may be the intermediate concentration at which there is first an increase and then a decrease at the main transition; this could be due to a sequence from L_β' to $L_\beta I$ to P_β' to L_α. As seen below, this type of scan appears only occasionally at only one concentration of alcohol, so it occurs over a very narrow alcohol concentration range. It has been previously reported, based on densimetric measurements, that such a phase sequence occurs in DPPC and DSPC in the presence of ethanol (Ohki et al., 1990).

Alcohol chain length dependence of interdigitation

Using the methodology described above we have investigated the effect of a series of *n*-alcohols on the phase behavior of DSPC.

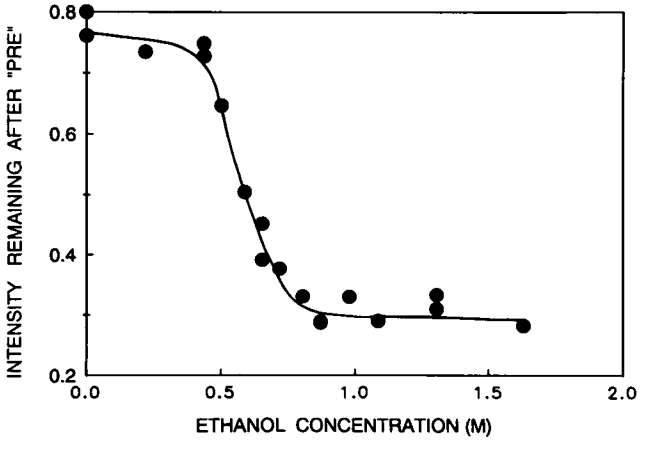

FIGURE 3 Fractional intensity remaining after the low-temperature transition in DSPC as a function of ethanol concentration. The *y* axis parameter is calculated as the ratio of the intensity at the end of the lower-temperature transition to the intensity at the beginning of the scan at 25°.

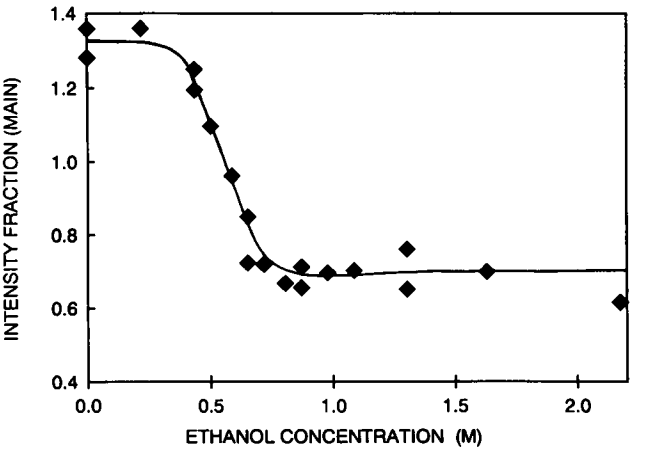

FIGURE 4 The intensity ratio of the contribution to the fluorescence change of the high-temperature (main) transition in DSPC as a function of ethanol concentration. The *y* axis parameter is the ratio of the fluorescence below the main transition to that after the main transition; a value >1.0 indicates a decrease in fluorescence for the main transition, whereas a value <1.0 indicates an increase in fluorescence for the main transition.

Methanol through pentanol

Fig. 5 *A–D* shows the series of fluorescence intensity scans for methanol, propanol, butanol, and pentanol, respectively. Qualitatively, for each of the alcohols the lower temperature transition exhibits a progression similar to that observed for ethanol, except that the alcohol concentrations are lower the longer the alcohol chain. The high-temperature transition also goes through a progression similar to that observed for ethanol, with the sign of the intensity change changing at about the same concentration as the change in the lower temperature transition. However, for butanol and pentanol, at the highest alcohol concentration used, the magnitude of the main transition decreases until it is virtually undetected at the highest alcohol concentration. From these data we conclude that each of these alcohols induces interdigitation, so that at the higher alcohol concentrations the sequence of phases is that in the upper path shown in Fig. 1.

Hexanol and heptanol

Fig. 6, *A* and *B*, shows the series of fluorescence intensity scans in DSPC in the presence of hexanol and heptanol. For both of these alcohols, the appearance of the changes in the low-temperature transition is similar to those with the smaller alcohols; plots of the intensity fraction of the lower temperature transition give a sigmoidal plot similar to Fig. 3 with threshold concentrations at 0.016 and 0.0065 M, respectively. The behavior of the lower-temperature transition suggests the induction of interdigitation by these two alcohols.

For both hexanol and heptanol, however, the apparent behavior of the main transition is different from that for the lower alcohols: instead of going to a change in sign for the main transition, the main transition seems to disappear in a concerted fashion, as the low-temperature transition undergoes its change. For these two alcohols, there is no concentration at which the intensity increases for the main transition. It is also to be noted that the magnitude of the intensity

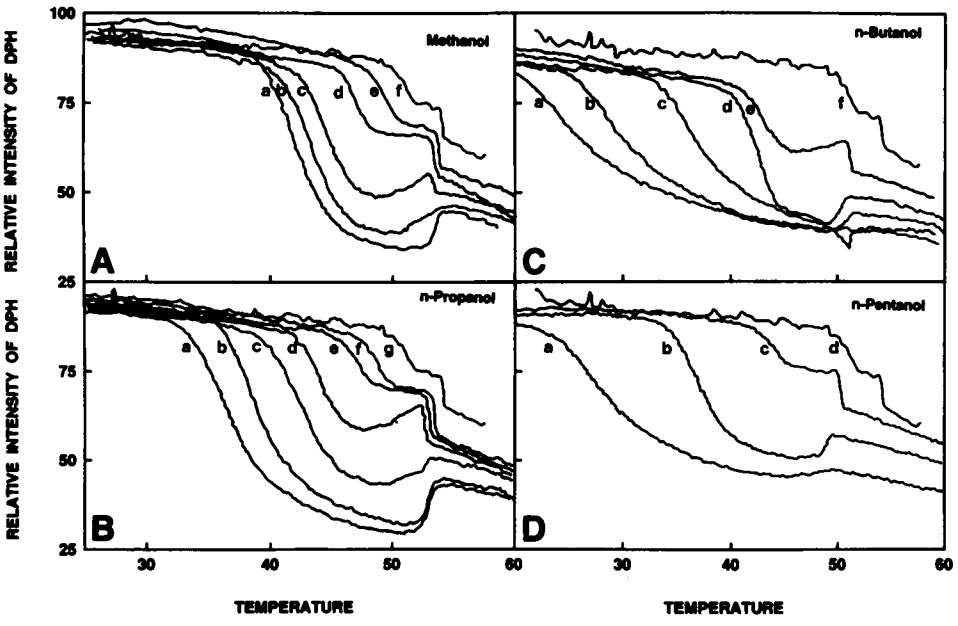

FIGURE 5 Relative DPH fluorescence intensity as a function of temperature in DSPC at a series of alcohol concentrations for four alcohols. (*A*) Methanol concentrations. *a*: 1.72 M; *b*: 1.56 M; *c*: 1.41 M; *d*: 1.25 M; *e*: 0.94 M; *f*: no alcohol. (*B*) *n*-Propanol concentrations. *a*: 0.27 M; *b*: 0.23 M; *c*: 0.20 M; *d*: 0.17 M; *e*: 0.13 M; *f*: 0.10 M; *g*: no alcohol. (*C*) *n*-Butanol concentrations. *a*: 0.27 M; *b*: 0.13 M; *c*: 0.081 M; *d*: 0.068 M; *e*: 0.041 M; *f*: no alcohol. (*D*) *n*-Pentanol. *a*: 0.045 M; *b*: 0.023 M; *c*: 0.011 M; *d*: no alcohol.

at its minimum (in the intermediate phase, i.e., $L_\beta I$,) is not as low as for ethanol, i.e., the decrease that occurs in the pretransition is less. The behavior of the DPH fluorescence intensity during the $L_\beta I$-to-L_α transition is not unique in this system; it is similar to that of DPPC in the presence of ethanol, a system in which interdigitation has been directly demonstrated by x-ray diffraction (Simon and McIntosh, 1984; Komatsu et al., 1993; Nambi et al., 1988). Therefore, the main transition behavior here is not inconsistent with the conclusion based on the behavior of the lower temperature transition, that hexanol and heptanol induce interdigitation in DSPC. Separate fluorescence depolarization experiments demonstrate that the main transition does indeed occur under these conditions (data not shown).

Octanol and nonanol

Fig. 6, *C* and *D*, shows the series of scans for DSPC in the presence of octanol and nonanol, respectively. These series are qualitatively different from those for the shorter-chain alcohols. One difference is that the low-temperature transition does not increase its magnitude with increasing alcohol concentration, but instead it becomes broader until it seems to disappear. The main transition persists up to quite high alcohol concentrations approaching the solubility limits of the alcohols. These data are not consistent with our criteria for interdigitation. Fluorescence depolarization experiments confirm that the main transition is present at these octanol concentrations (data not shown).

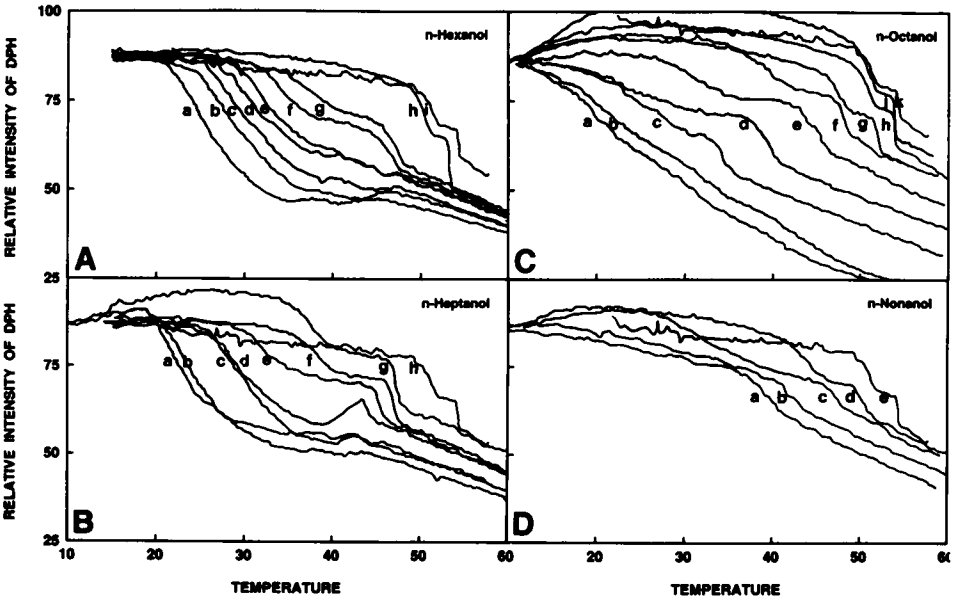

FIGURE 6 Relative DPH fluorescence intensity as a function of temperature in DSPC at a series of alcohol concentrations for four alcohols. (*A*) *n*-Hexanol concentrations. *a*: 0.196 M; *b*: 0.157 M; *c*: 0.155 M; *d*: 0.152 M; *e*: 0.137 M; *f*: 0.127 M; *g*: 0.0098 M; *h*: 0.0029 M; *j*: no alcohol. (*B*) *n*-Heptanol concentrations. *a*: 0.0077 M; *b*: 0.0069 M; *c*: 0.0065 M; *d*: 0.0060 M; *e*: 0.0052 M; *f*: 0.0043 M; *g*: 0.0026 M; *h*: no alcohol. (*C*): *n*-Octanol concentrations. *a*: 7.7×10^{-3} M. *b*: 4.6×10^{-3} M; *c*: 4.2×10^{-3} M; *d*: 3.8×10^{-3} M; *e*: 1.5×10^{-3} M; *f*: 7.7×10^{-4} M; *g*: 2.3×10^{-4} M; *h*: 2.3×10^{-5} *j*: 7.7×10^{-6} M; *k*: no alcohol. (*D*) *n*-Nonanol concentrations *a*: 9.0×10^{-4} M; *b*: 6.9×10^{-4} M; *c*: 3.5×10^{-4} M; *d*: 1.4×10^{-4} M; *e*: no alcohol.

Branched chain alcohols

The branched chain alcohols isopropanol, isobutanol, methylbutanol, and benzyl alcohol were also studied. The data for these alcohols are shown in Fig. 7, *A–D*. The scans from each of these series resemble those shown for the *n*-alcohols below heptanol with a synchronous change in sign of fluorescence changes of the main transition along with the increase in intensity change of the low-temperature transition. By our established criteria, each of these alcohols appears to induce interdigitation in DSPC. In the case of 2-methyl butanol, the changes are characteristic of the lower alcohols up to a point, but at the highest two concentrations shown, the magnitude of the lower transition becomes smaller again and the direction of change of the main transition again changes sign; it is not known what is occurring at these very high concentrations. It is interesting to note that for benzyl alcohol, although there is a change in sign for the main transition as is seen with the lower alcohols, at the highest alcohol concentration studied both the main transition and the low-temperature transitions disappear.

Threshold concentrations

The threshold concentrations for the induction of interdigitation by each alcohol in DSPC were determined from plots such as those shown for ethanol in Figs. 3 and 4. In each case, the threshold concentration is defined as the midpoint of the sigmoidal curves. There was no significant difference in the threshold concentrations determined from the behavior of the lower temperature transition or from the main transition behavior. The threshold concentrations determined for each alcohol in DSPC are shown in Table 1.

Figs. 8 and 9 show the intensity fraction plots for the lower and main transition parameters, respectively, for each of the *n*-alcohols from methanol to heptanol, plotted as log plots so that all could be shown together. This presentation of the data demonstrates the similarity of the curve shapes for each alcohol and the wide range of the threshold concentrations for the series of alcohols. The branched chain alcohols have similar-shaped curves (data not shown).

TABLE 1

Alcohols	Number of carbons	Threshold concentration (M)
Linear		
Methanol	1	1.40
Ethanol	2	0.59
n-Propanol	3	0.183
n-Butanol	4	0.074
n-Pentanol	5	0.018
n-Hexanol	6	0.016
n-Heptanol	7	0.0065
Branched		
Isopropanol	3	0.226
2-Methyl propanol	4	0.047
2-Methyl butanol	5	0.028
Benzyl alcohol	7	0.0123

Fig. 10 shows a plot of the log of the threshold concentration against the number of carbon atoms in each alcohol. It is noted that the branched saturated alcohols fit onto the linear plot as well as the *n*-alcohols. The threshold concentration of the aromatic benzyl alcohol is not as well correlated with carbon number as the saturated alcohols.

Using the partition coefficients for each alcohol along with the alcohol concentration at the threshold concentrations, the mole fraction of alcohol dissolved in the lipid at the threshold concentration can be calculated. Fig. 11 shows the mole fraction of alcohol in the lipid for each alcohol at its respective threshold concentration, calculated using partition coefficients determined by titration calorimetry or by the phase-transition shift method (Zhang and Rowe, 1992; Rowe, Campion, Leung, Parr, and Zhang, unpublished data). Although the partition coefficients used were those for the liquid crystal phase, and it is likely that the partition coefficients for

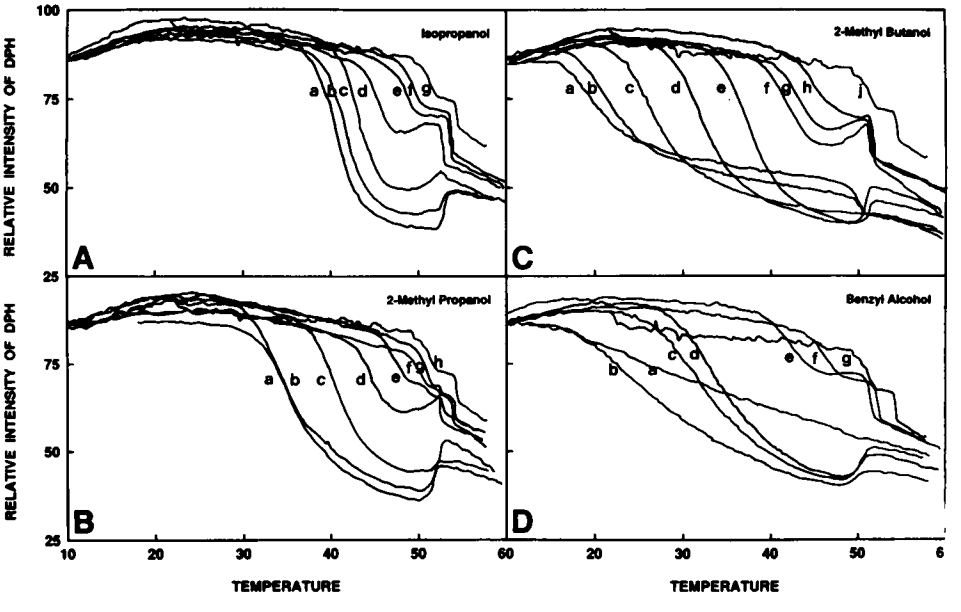

FIGURE 7 Relative DPH fluorescence as a function of temperature for a series of alcohol concentrations for four branched alcohols. (*A*) Isopropanol concentrations, from right to left: 0.0 M; 0.13 M; 0.17 M; 0.20 M; 0.23 M; 0.27 M; 0.30 M. (*B*) 2-Methyl propanol concentrations, from right to left: 0.0 M; 0.0067 M; 0.014 M; 0.027 M; 0.040 M; 0.054 M; 0.081 M; 0.108 M. (*C*) 2-Methyl butanol concentrations, from right to left: 0.0 M; 0.0057 M; 0.0113 M; 0.0227 M; 0.034 M; 0.045 M; 0.068 M; 0.091 M; 0.114 M. (*D*) Benzyl alcohol concentrations, from right to left: 0.0 M; 0.0046 M; 0.0092 M; 0.014 M; 0.019 M; 0.028 M. The trace that cuts across the other traces and appears to be nearly a straight line is the highest concentration at 0.037 M.

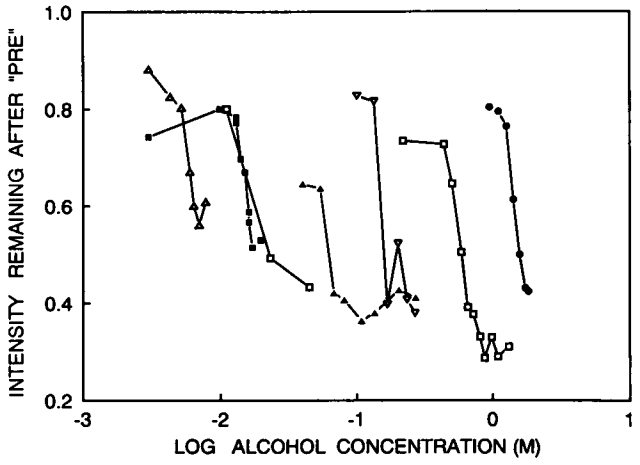

FIGURE 8 Fractional intensity remaining after the low-temperature transition in DSPC as a function of alcohol concentration for the series of *n*-alcohols; from right to left the alcohols are methanol, ethanol, propanol, *n*-butanol, *n*-pentanol, *n*-hexanol, and *n*-heptanol. The *y* axis parameter is calculated as the ratio of the intensity at the end of the lower temperature transition to the intensity at the beginning of the scan at 25°.

the $L_{\beta}I$ phase are lower than those for the liquid crystal phase (Zhang and Rowe, 1992), this calculation is useful for comparative purposes. As shown here, in spite of the very large range of threshold concentrations, the estimated amount bound is very similar, especially for the alcohols with five or fewer carbons.

DISCUSSION

Alcohol chain length dependence of interdigitation

This study demonstrates that alcohols up to chain length of seven carbons, as well as all of the branched alcohols studied,

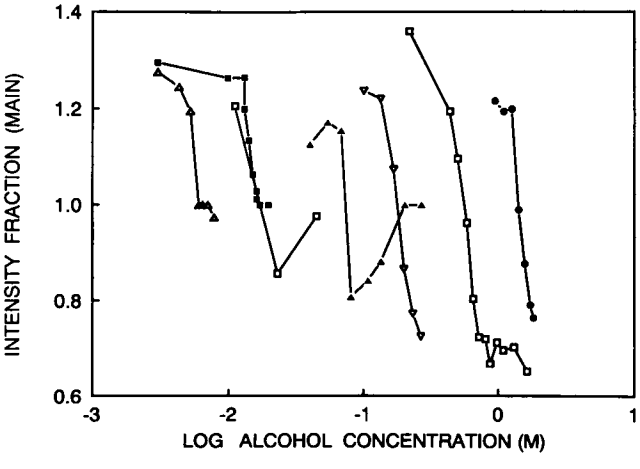

FIGURE 9 The intensity ratio of the contribution to the fluorescence change of the high-temperature (main) transition in DSPC as a function of alcohol concentration for the series of *n*-alcohols; from right to left the alcohols are methanol, ethanol, propanol, *n*-butanol, *n*-pentanol, *n*-hexanol, and *n*-heptanol. The *y* axis parameter is the ratio of the fluorescence below the main transition to that after the main transition; a value >1.0 indicates a decrease in fluorescence for the main transition, whereas a value <1.0 indicates an increase in fluorescence for the main transition.

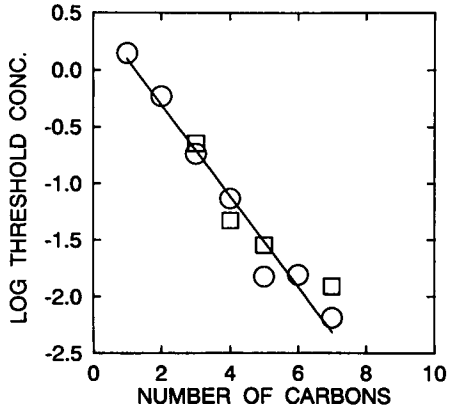

FIGURE 10 Log of the threshold concentration for the induction of interdigitation in DSPC for the series of alcohols, plotted against the number of carbons in the alcohol. (○) *n*-alcohols; (□) branched alcohols: isopropanol, 2-methyl propanol, 2-methyl 1-butanol, and benzyl alcohol.

induce interdigitation in DSPC. Octanol and nonanol do not give the same fluorescence characteristics as the lower alcohols, suggesting that they are not inducing interdigitation. If this suggestion is verified using x-ray diffraction, it will indicate that there is a chain length cutoff for the induction of interdigitation. The origins of this putative cutoff are discussed below.

Threshold concentrations for interdigitation

The threshold concentrations for the induction of interdigitation vary with the number of carbons in the alcohol in a similar fashion as do the membrane: buffer partition coefficients. This is illustrated by the estimated amounts bound at the threshold concentrations shown in Fig. 11. The absolute numbers shown in Fig. 11 should be considered as maximum values, as the partition coefficients used were those for

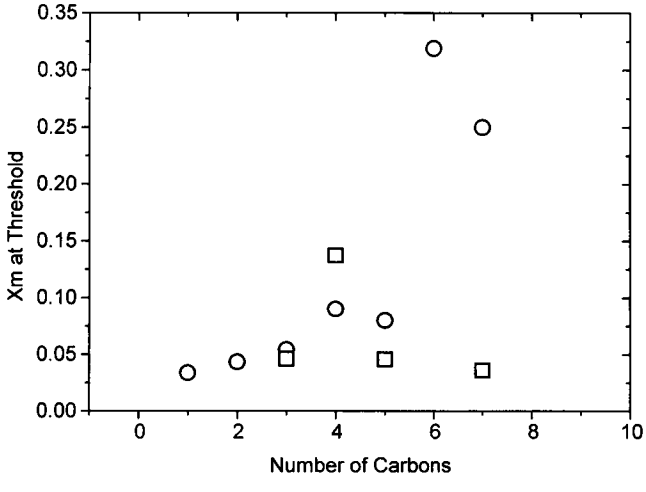

FIGURE 11 The mole fraction of alcohol in the lipid at the threshold alcohol concentration for the induction of interdigitation in DSPC for each alcohol, plotted against the carbon number of the alcohol. (○) *n*-alcohols; (□) branched alcohols: isopropanol, 2-methyl propanol, 2-methyl 1-butanol, and benzyl alcohol.

the liquid crystal phase, and we expect the partition coefficients for the $L_\beta I$ phase to be lower by a factor of approximately two (Zhang and Rowe, 1992). The similarity of the number of alcohols bound for each of these alcohols at the threshold concentration suggests that it is the number of alcohols bound, rather than any change in "potency" with increasing size, that causes interdigitation to occur. However, it must be pointed out that this conclusion rests on the assumption that the dependence on carbon number of the partition coefficients for the $L_\beta I$ phase will parallel those for the liquid crystal phase. Future measurements of these partition coefficients for the $L_\beta I$ phase are planned.

General mechanism of the induction of interdigitation

The stereochemical and thermodynamic influences that lead to interdigitation in saturated like-chain PCs have been discussed previously by ourselves and others (Rowe and Cutrera, 1990; Nambi et al., 1988; Simon and McIntosh, 1984; Simon et al., 1986). Simon and co-workers showed by x-ray diffraction that the packing of the acyl chains in the $L_\beta I$ phase is closer, i.e., the van der Waals' interactions are greater in the $L_\beta I$ phase than in the L_β' phase. In their analysis these authors considered the packing in the acyl chain region and the free energies from exposure of the terminal methyls to the interfacial region, and concluded that an important driving force was the greater van der Waals' interactions in the acyl chain region, which counteracted the unfavorable exposure of the terminal methyls to the solvent. Their analysis is consistent with the fact that there is a decrease in threshold concentration for induction of $L_\beta I$ with increasing lipid chain length (Rowe, 1983, 1985; Guy and Rowe, unpublished results). It was later shown by Nambi et al. (1988) and Rowe and Cutrera (1990) that the appearance of interdigitation was temperature dependent such that there is a low-enthalpy transition from L_β'-to-$L_\beta I$ at a temperature that replaces the L_β'-to-P_β' transition (see Fig. 1), so that the $L_\beta I$ phase competes with the P_β' or ripple phase rather than the lower-temperature L_β' phase (Nambi et al., 1988). This led to the suggestion by these authors that the steric crowding of the headgroups in the L_β' phase, which leads to the P_β' or ripple phase in the absence of alcohol, is also an important driving force toward the $L_\beta I$ phase, given that the area per headgroup is greatly increased in the $L_\beta I$ phase compared with either the L_β' or the P_β' phase. This conclusion is also supported by the finding that the $L_\beta I$ phase is not induced by ethanol in the smaller-headgroup phosphatidylethanolamines (Rowe, 1985, 1987). The interdigitated phase can be induced in DSPC by increased hydrostatic pressure (Braganza and Worcester, 1986; Prasad et al., 1987), showing that it is a possible configuration of the lipid in the absence of additives. However, the presence of amphiphilic solutes greatly enhances its stability, and the present study has further explored the solutes that induce this phase.

The role of the inducer within the framework above is to decrease the unfavorable contribution due to the exposure of the terminal methyls to the aqueous interfacial region by binding in that region and making it more hydrophobic. It has also been suggested by Simon et al. (1986) that the amphiphilic inducer must be relatively small, so that it penetrates the acyl chain region to some degree, separating the headgroups (in the L_β' phase) but not extending very far into the bilayer, so that if the lipid were to stay in the L_β' or P_β' phase, there would be a void created below the amphipathic molecule. The fact that methanol induces interdigitation as readily as the other alcohols up to heptanol, however, suggests that it is not necessary for an inducer to penetrate significantly the acyl chain region to induce interdigitation. We have found that there appears to be a chain length cutoff for the *n*-alcohols at eight carbons, for the 18-carbon DSPC. This is consistent with the suggestion that longer chains can penetrate far enough into the bilayer so that perhaps they can increase the area per headgroup without creating a potential void within the bilayer. However, the eight carbons of octanol are still considerably shorter than the acyl chains of DSPC, so this is not a complete explanation for such a cutoff.

The finding that the mole fraction of bound alcohols at the threshold concentration is similar for each of the alcohols from methanol to pentanol, as well as the branched alcohols, isopropanol, 2-methyl propanol, 2-methyl butanol, and the aromatic benzyl alcohol, indicates that a molecule of methanol is as effective as a molecule of *n*-pentanol in inducing interdigitation. This suggests that the primary effect of the inducer in causing interdigitation is in the interfacial region where the alcohol hydroxyl apparently replaces water. That alcohol brings a hydrophobic moiety with it, which then disrupts the interfacial water and favors an increase in area per lipid headgroup. Although some of these alcohols are long enough to penetrate partially into the acyl chain region, the finding that methanol is as effective as pentanol suggests that the most important effect leading to interdigitation is in the disruption of the water in the interfacial region of the gel-phase lipid, accompanied by an increase in area.

Interpretation of fluorescence intensity change

The results reported here provide additional support for the method of measuring DPH fluorescence intensity to detect the interdigitation of PCs. In addition to the change in fluorescence intensity at the transition from L_β' to $L_\beta I$, correlated with the x-ray diffraction results, which we reported previously (Nambi et al., 1988), we have found that there is also a dramatic characteristic difference in the fluorescence intensity change at the "main transition" depending on whether it is the P_β'-to-L_α transition or the $L_\beta I$-to-L_α transition (the lower path versus the upper path in Fig. 1). Thus, the DPH intensity measurement gives two independent indications of whether the lipid has become interdigitated or not.

The observations presented here of a greater decrease in fluorescence for the L_β'-to-$L_\beta I$ transition than for the L_β'-to-P_β' transition and a change in sign of the fluorescence intensity change for the main transition originate in a lower DPH fluorescence intensity for the $L_\beta I$ phase than for the P_β'

phase (i.e., the two alternate pathways in Fig. 1). We previously proposed that the reduced DPH fluorescence intensity in this phase is due to a redistribution of the DPH from sites parallel to the lipid surface to an orientation parallel to the acyl chains (Nambi et al., 1988). This orientation then brings the fluorophore in closer proximity to the aqueous environment of the interfacial region. This interpretation was supported and elaborated by Kao et al. (1990) in their observations of DPH fluorescence in the interdigitated lipid C(18):C(10) phosphatidylcholine. They found that there was an increase in DPH fluorescence intensity in the main transition of this lipid as it went from the mixed interdigitated gel to the partially interdigitated liquid-crystal phases, and they also interpreted this change in fluorescence as being due to a redistribution of the DPH.

Our data on alcohol chain lengths shows that the magnitude of the DPH fluorescence change at the "main" transition is also dependent on the alcohol chain length. As seen by comparison of Figs. 5, 6, and 7, for the shorter-chain alcohols there is an increase at the "main" transition, whereas for the longer alcohols the "main" transition has seemed to disappear. By considering the changes at both transitions, it is clear that what is changing with alcohol chain length is the DPH intensity in the $L_\beta I$ phase: for the longer-chain alcohols, the magnitude of decrease at the L_β'-to-P_β' transition is decreased at the same time that the magnitude of the increase at the $L_\beta I$-to-L_α transition is decreased. This indicates that the longer the alcohol, the greater is the fluorescence intensity of the DPH fluorophore in the interdigitated phase; in fact for the longest alcohols it approaches the intensity in the L_α phase. There is not an obvious explanation for this observation. One possibility is that the alcohol is directly affecting the DPH fluorescence by shielding the DPH from the aqueous solvent with the longer alcohols providing more shielding than the shorter ones. This would suggest that there are direct contacts between the DPH molecules and the bound alcohol, or that the longer alcohols prevent penetration of water into the bilayer better than the shorter ones. As shown in Fig. 11, the mol % of alcohol in the membrane at the threshold concentration is as low as 5%, which is relatively low, but it could possibly provide shielding of some of the DPH molecules. Another possibility is that there are subtle variations in the structure of the $L_\beta I$ phase depending on the stereochemistry of the inducer; e.g., if the thickness of the structure were increasing with alcohol chain length, the DPH molecules may be buried to a greater extent by the interdigitated phase induced by longer-chain alcohols. These subtleties of the structure of the interdigitated phases can be resolved by x-ray diffraction studies of these phases.

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