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INTERDIGITATED HYDROCARBON CHAIN PACKING CAUSES THE BIPHASIC TRANSITION BEHAVIOR IN LIPID/ALCOHOL SUSPENSIONS

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It has been shown recently by Rowe ((1983) Biochemistry 22, 3299–3305) that ethanol has a 'biphasic' effect on the transition temperature (T_m) of phosphatidylcholine bilayers, reducing T_m at low concentrations but increasing T_m at high concentrations. Our X-ray diffraction data show that this reversal of T_m is a consequence of the induction of an unusual gel phase, where the lipid hydrocarbon chains from apposing monolayers fully interpenetrate or interdigitate. The properties of this interdigitated phase also explain the lipid chain length dependence of the reversal in the T_m versus ethanol concentration curves and the narrow width of the transition at high ethanol concentrations, as well as spectroscopic and calorimetric data from lipid suspensions containing other drugs such as methanol, benzyl alcohol, phenyl ethanol, and chlorpromazine.

The gel to liquid-crystalline phase transition has been extensively studied for a variety of lipid and lipid/small molecule suspensions. The energetics of this transition can provide information on the solvent properties of both the gel and liquid-crystalline states. Recently it has been found that several alcohols, including ethanol [1], methanol [2], and pentanol [2], exhibit a biphasic effect on the phase transition temperature of saturated phosphatidylcholine bilayers. That is, depending on concentration, these alcohols have two distinct effects on the main gel to liquid-crystalline phase transition temperature (T_m) of liposomes [1,2]. At low concentrations, there is a decrease in T_m with increasing alcohol concentration, whereas at higher concentration there is an abrupt reversal so that T_m increases with increasing alcohol concentration (see Fig. 1). In the case of ethanol, which has been the most thoroughly studied alkanol, the concentration where this reversal takes place depends strongly on the length of the lipid hydrocarbon chain [1]. In addition, the transition width becomes narrower in this higher concentration region [1]. The low concentration decrease in T_m has been successfully explained in terms of a usual freezing point depression whereby ethanol interacts preferentially with the liquid-crystalline phase [3,4]. However, the high concentration increase in T_m is not so readily explainable, although Rowe [1] has argued that the elevation in T_m may involve specific binding of ethanol at discrete sites to more than one lipid molecule on the surface of the bilayer.

In this paper we show that in the high concentration range ethanol induces an unusual gel phase, where lipid molecules from opposing monolayers fully interpenetrate or interdigitate (see Fig. 1). The properties of this interdigitated lipid phase can explain the increase in T_m at high ethanol concentrations, the chain-length dependence of the breakpoint in the T_m versus ethanol concentration curves, and the narrow width of the transition at high ethanol concentrations.

Fig. 2 shows densitometer traces of X-ray diffraction patterns recorded from di-

palmitoylphosphatidylcholine (DPPC) suspensions in ethanol/water concentrations on both sides of the breakpoint in the T_m curve for DPPC, which is at 50 mg ethanol per ml water [1]. For Fig. 2A, the ethanol concentration was 40 mg/ml while in Fig. 2B it was 60 mg/ml. At 40 mg/ml, both the lamellar repeat period of 64 Å and the double wide-angle reflection at 4.21 Å and 4.10 Å are identical to gel phase DPPC bilayer in water [5,6]. The double wide-angle reflection is characteristic of the $L_{\beta'}$ phase [5], where the lipid hydrocarbon chains are tilted relative to the plane of the bilayer. At 60 mg/ml and higher ethanol concentrations, the lamellar repeat period is only 48 Å, and the wide-angle pattern consists of a single sharp reflection at 4.09 Å (Fig. 2B). This pattern is characteristic of the interdigitated gel phase, which has been described for bilayers of dipalmitoylglycerol [7–9], DPPC [10,11], and β-DPPC [12]. In this phase the hydrocarbon chains are approximately perpendicular to the plane of the bilayer, accounting for the single sharp wide-angle reflection [5,10]. Moreover, the hydrocarbon chains from apposing monolayers are fully interpenetrated or interdigitated, so that the terminal methyl groups of the lipid chains abut the interfacial region on the opposite sides of the bilayer [7–12]. In this phase there are four hydrocarbon chains per lipid group at the interface [7,10]. This interdigitation accounts for the unusually small lamellar repeat period of 48 Å. Electron density profiles and schematic representations of the lipid molecules for both the normal $L_{\beta'}$ bilayer phase and the interdigitated phase are shown in Fig. 1.

The induction of the interdigitated phase explains the biphasic nature of the T_m versus concentration curves shown in Fig. 1 as follows. The linear decrease in T_m at low concentrations is explained by more ethanol partitioning in the liquid-crystalline than the gel phase [1,3,4]. The sudden break in the curve, at about 50 mg/ml ethanol for DPPC, is caused by the onset of the interdigitated phase. The induction of this phase causes the break in the T_m curve since the interdigitated phase has twice as many hydrocarbon chains per lipid headgroup at the interface (and therefore more ethanol binding sites) than the usual $L_{\beta'}$ gel phase. Consequently the partition coefficient becomes greater in the gel phase than the

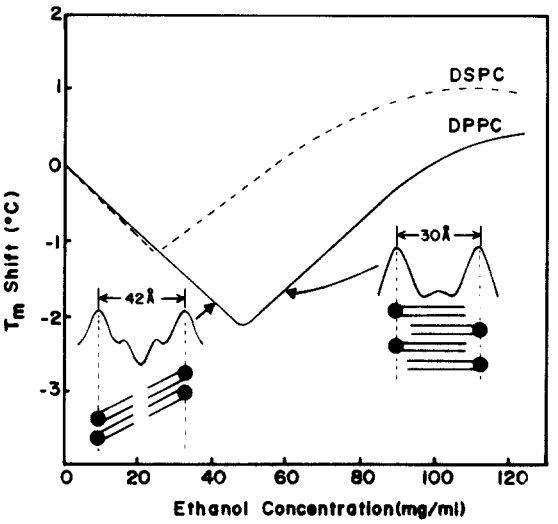

Fig. 1. Change in transition temperature for suspensions of distearoylphosphatidylcholine (DSPC) (dashed line) and DPPC (solid line) as a function of ethanol concentration. Data taken from Rowe [1]. The break in the DPPC curve occurs at an ethanol concentration of 50 mg/ml. Also shown are electron density profiles for DPPC in the $L_{\beta'}$ phase at 40 mg/ml and in the interdigitated phase at 60 mg/ml. The electron density profiles were generated from the diffraction patterns given in Fig. 2 and the same phase angles as in Refs. 6, 10 and 11. Below each profile is a schematic view of the structure, with the black dots representing the lipid headgroups and the straight lines representing the lipid hydrocarbon chains.

liquid-crystalline phase and therefore causes T_m to increase with increasing ethanol concentration [1,3,13] until a concentration is reached where the interdigitated phase becomes saturated with ethanol. At this point the T_m versus concentration curves become independent of ethanol (at about 100 mg/ml). X-ray diffraction patterns recorded at 120 mg/ml are identical to those recorded at 60 mg/ml, indicating that there is no change in the structure of the interdigitated phase at higher ethanol concentrations.

Ethanol apparently induces the interdigitated phase in a manner similar to other surface active molecules such as benzyl alcohol, tetracaine, chlorpromazine, methanol, phenylbutanol, and glycerol [11]. All of these molecules share two important properties. First, they all can displace water from the interfacial region, and second, they do not extend too deeply into the bilayer interior [11]. That is, these amphiphilic molecules anchor to the

interface by virtue of their polar moiety, with the non-polar part of the molecule intercalating between the gel state hydrocarbon chains. However, since for these particularly small molecules the nonpolar moieties are short compared to the lipid hydrocarbon chains, this interfacial location would potentially cause voids between chains in the bilayer interior [11]. Since the energy of formation of holes in hydrocarbons is extremely large, the chains must eliminate the formation of potential voids [14]. For the surface active molecules listed above, the lipid molecules respond to their addition by forming the interdigitated phase.

The chain-length dependence of the T_m curves in Fig. 1 can be explained by consideration of the energetics of the usual $L_{\beta'}$ bilayer and the interdigitated phase. There is energy gained in going from the $L_{\beta'}$ phase to the interdigitated phase since the van der Waals energy is greater in the more closely packed interdigitated phase. On the other hand, there is an energy cost in going to the interdigitated phase since the terminal methyl groups become exposed to water and since the attractive interaction between the zwitterionic headgroups is diminished [15]. The energy due to rotational isomerism would be expected to be similar for both the $L_{\beta'}$ and interdigitated phases. The van der Waals energy gained, ΔE_g, will be proportioned to the number of CH_2 groups in the lipid hydrocarbon chains [16], whereas the energy cost, ΔE_c, exposing hydrocarbon to water and separating lipid headgroups will be independent of chain length (see below). Thus, since the energy gained upon interdigitation is directly related to the length of lipid hydrocarbon chains while the energy cost is independent of chain length, it becomes energetically favourable for long-chain lipids to interdigitate at smaller ethanol concentrations than shorter chain lipids.

To obtain some idea of the magnitude of ΔE_g and ΔE_c between the $L_{\beta'}$ and interdigitated phase, we present the following analysis. To calculate ΔE_g we use Equation 5 of Nagle and Wilkinson [16] and compare the internal energies of a bilayer in the $L_{\beta'}$ phase which has a double wide-angle reflection at 4.21 Å and 4.10 Å (meaning each hydrocarbon chain has four neighbours at a distance of 4.86 Å and two neighbours at a distance of 4.73 Å), and the fully interdigitated phase which has a single wide-angle reflection at 4.09 Å (meaning each hydrocarbon chain as six nearest neighbours at a distance of 4.72 Å). The van der Waals energy gained by going to the closer packed interdigitated phase is 0.13 kcal/mol per CH_2 group. Thus, for DPPC, $\Delta E_g = -4.25$ kcal/mol. The energy cost exposing the lipid terminal methyl groups to water is given $\Delta E_c = \gamma \cdot \Delta A \cdot N_A$ [15] where γ is the interfacial surface tension, ΔA is the surface area of the two terminal methyl groups exposed to interfacial water, and N_A is Avogadro's number. The value of γ has been estimated to range from 30–50 dyn/cm [14,17]. Although ethanol decreases γ in a concentration-dependent manner, this change is small over the considered concentration range [18]. Moreover, since the breaks in the T_m curve for long chain lipids occurs at very small ethanol concentrations (less than 10 mg/ml), this reduction in γ is not a primary factor in inducing the interdigitated phase. For ΔA we use the surface area of half a sphere whose volume

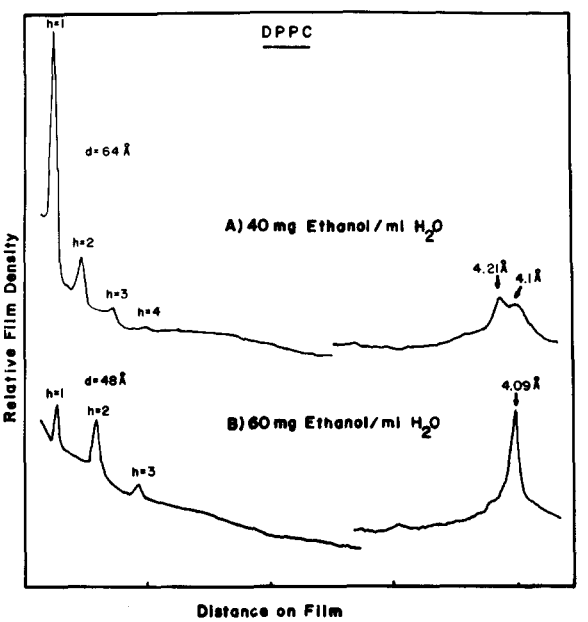

Fig. 2. Densitometer traces of X-ray diffraction patterns of DPPC in ethanol solutions of (A) 40 mg/ml and (B) 60 mg/ml. These traces were obtained as outlined in Ref. 6. Both patterns were recorded at 20 °C with lipid concentrations of 5 mg lipid per 20 mg of solution. In trace (A) there are four orders of a lamellar repeat period of 64 Å and a double wide-angle reflection at 4.21 Å and 4.10 Å. In trace (B) there are three orders of a lamellar repeat period of 48 Å and a single sharp wide-angle reflection at 4.09 Å.

is equal to a terminal methyl group. The volume of a methyl group is approximately twice that of a methylene group. The volume of a CH_2 group is equal to 1.25 Å times the cross-sectional area of one chain [5], which is $2(4.09\ Å)^2/(3)^{1/2}$. Using these values we calculate the exposed area for two exposed CH_3 groups to be $\Delta A = 64\ Å^2$. Thus, independent of chain length, ΔE_c ranges from 2.8 to 4.6 kcal/mol for the above range of surface tensions. Although there are other factors which could contribute to the stabilization of the interdigitated phase such as lipid headgroup interactions and rearrangements of water molecules, it nevertheless can be seen that the gain in van der Waals energy on chain interdigitation for DPPC is comparable to the energy loss of exposing hydrocarbon to water.

The sharper transition width observed for high concentrations of ethanol [1] is also explained by the induction of the interdigitated phase. In the interdigitated phase, the hydrocarbon chains are more highly ordered, as evidenced by the sharper wide angle pattern (Fig. 2). The more perfect ordering of the lipid molecules causes a narrower phase transition [13].

Since the interdigitated phase can be induced by a variety of small molecules [7–11], and, depending on chain length, the internal energies of the usual $L_{\beta'}$ phase and the interdigitated phase are comparable, there may be several addition examples of calorimetric or spectroscopic data on lipid/drug interactions which could be explained by gel state chain interdigitation. For example, benzyl alcohol [19,20], phenyl ethanol [19], and chlorpromazine [21], all produce breaks in curves of T_m versus drug concentration, and we have found that all of these molecules induce the interdigitated phase at concentrations above these break points [11]. The slopes of the T_m versus drug concentration curves before and after the break depend on the solubility of the drug in the liquid-crystalline phase relative to solubility in the usual gel state and the interdigitated state, respectively.

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