Biochimica et Biophysica Acta, 943 (1988) 108–111
Elsevier

BBA 70566

BBA Report

Effect of alcohols on the phase transitions of dihexadecylphosphatidylcholine

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(Received 30 October 1987)
(Revised manuscript received 13 April 1988)

Key words: Phospholipid; Alcohol; Phase transition; Dihexadecylphosphatidylcholine

We have systematicallly investigated the effect of short chain alcobols (methanol to *n*-propanol) on the phase transitions of 1,2-dihexadecylphosphatidylcholine (DHPC), a lipid that forms a stable interdigitated gel phase ($L_{\beta I}$) in aqueous solution. The temperature of the low-temperature $L_{\beta I}$ to $P_{\beta'}$ phase transition of DHPC was found to increase with alcohol concentration, showing that alcohol interacts preferentially with the interdigitated phase relative to the non-interdigitated gel. The main transition of DHPC exhibited a biphasic effect of alcohol concentration similar to that previously observed with DPPC (Rowe, E.S. (1983) Biochemistry 22,3299–3305). As alcohol concentration is increased the lower $L_{\beta I}$ to $P_{\beta'}$ and main $P_{\beta'}$ to L_{α} transitions of DHPC merge at the threshold concentration of the biphasic effect, so that above this concentration there is one phase transition from $L_{\beta I}$ directly to L_{α}. This is analogous to DPPC above its biphasic threshold. Similar to DPPC, the transition between $L_{\beta I}$ and L_{α} exhibits marked hysteresis.

In recent years there has been an increased interest in the formation and properties of the interdigitated gel phase in model membranes. In this gel phase the acyl chains from opposing monolayers fully interpenetrate. The interdigitated ($L_{\beta I}$) gel phase has been observed in a variety of phospholipids [1–10]. In saturated PCs with identical chains, such as dipalmitoylphosphatidylcholine (DPPC), this phase is induced by a variety of additives, including methanol, ethanol, glycerol, chlorpromazine, benzyl alcohol [3,4,18], and thiocyanate ion [8]. In contrast, the diether PC 1,2-dihexadecylphosphatidylcholine (DHPC) exists in the interdigitated phase in the absence of any additive [9,10]. In a recent comparative study of DPPC and DHPC [9] using differential scanning calorimetry, X-ray diffraction, and ^{32}P-NMR techniques, it was shown that DHPC differs from DPPC in its thermal, structural, and dynamic properties. Upon heating, DPPC goes from the bilayer gel phase ($L_{\beta'}$) to the rippled $P_{\beta'}$ phase in a 'pretransition', and then through the main melting transition to the liquid-crystalline phase ($P_{\beta'}$ to L_{α}). In contrast, DHPC begins in the interdigitated gel ($L_{\beta I}$) phase, goes through a low-temperature 'pretransition' from the $L_{\beta I}$ phase to the rippled $P_{\beta'}$ phase, and then through the main transition from $P_{\beta'}$ to L_{α} [10,11]. Thus, the low-temperature transitions of the two lipids are not analogous, but the main transitions are analogous to each other in terms of the phases involved.

We have been interested in the effects of alcohols on the phase transitions in PCs [12–16]. It is now well established that the short chain alcohols such as methanol, ethanol and *n*-propanol induce the formation of the $L_{\beta I}$ phase in PCs

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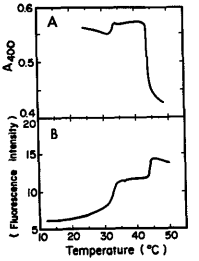

Fig. 1. (A) The effect of temperature on the optical density of DHPC liposomes (measured at 400 nm) in water. Note that the optical density increases at about 33°C, and at the main transition temperature (about 44°C) the optical density decreases sharply. (B) The temperature dependence of the fluorescence intensity (measured at 430 nm) of DPH-labelled DHPC liposomes. Note that the fluorescence intensity increases at about 33°C, and the main transition can be easily detected at about 43.5°C.

[13–18]. However, the effect of alcohols on a PC which exhibits a stable $L_{\beta 1}$ phase in the absence of additives has not been thoroughly investigated. In this paper we report the effect of short chain alcohols on the phase transitions observed in DHPC. Optical density and DPH fluorescence measurements have been used to detect the phase transitions.

The multilamellar lipid samples were prepared as described earlier [12] according to the method of Bangham et al. [19] to give the required phospholipid concentration. The optical density measurements were made by following the change in optical density at 400 nm as a function of temperature using a Varian/Cary 219 spectrophotometer, as described elsewhere [12]. The fluorescence measurements were made using a SLM 8225 spectrofluorimeter as described previously [15].

Fig. 1A shows the effect of temperature on the optical density of DHPC multilamellar liposomes in the absence of alcohol. Two transitions are observed, the temperatures of which are in good agreement with the values reported in the literature [9]. A small increase in optical density is observed at the low-temperature $L_{\beta 1}$ to $P_{\beta'}$ transition. This is in contrast to DPPC where there is a large decrease in optical density for its low-temperature $L_{\beta'}$ to $P_{\beta'}$ transition [15]. The difference in the optical density effects for the low-temperature transition for the two lipids is consistent with the fact that the low-temperature transitions are not analogous in the two lipids, as noted above. In contrast to the lower temperature transitions, the main transitions for both the lipids appear similar by optical density, consistent with the similarity of these transitions for the two lipids in the absence of alcohol in terms of the phases involved (i.e., $P_{\beta'}$ to L_α for both lipids).

Fig. 1B shows the temperature dependence of the fluorescence intensity for DHPC liposomes labelled with DPH. At the lower temperature transition from $L_{\beta 1}$ to $P_{\beta'}$ the fluorescence intensity at 430 nm increases, whereas in DPPC there is a small decrease in fluorescence at its low temperature transition from $L_{\beta'}$ to $P_{\beta'}$ (Nambi, P., unpublishes results). The increase in DPH fluorescence for the $L_{\beta 1}$ to $P_{\beta'}$ transition of DHPC is consistent with our previous observation that there is a decrease in DPH fluorescence intensity in DPPC for the alcohol-induced transition from $L_{\beta'}$ to $L_{\beta 1}$ [16].

The effect of ethanol on the temperature of the $L_{\beta 1}$ to $P_{\beta'}$ phase transition is shown in Fig. 2. It is seen that the $L_{\beta 1}$ to $P_{\beta'}$ phase transition temperature increases with increasing alcohol concentration. This indicates that ethanol interacts preferentially with the $L_{\beta 1}$ phase relative to the $P_{\beta'}$ phase, so that addition of ethanol shifts the phase equilibrium toward the $L_{\beta 1}$ phase. It may be noted that the effect of alcohols on the low-temperature

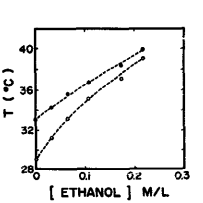

Fig. 2. The effect of ethanol on the low-temperature transition of DHPC as detected by spectrophotometry. ●, Heating scan; ○, cooling scan.

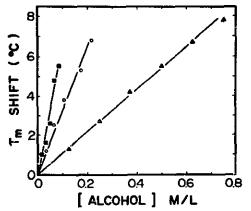

Fig. 3. The effect of different *n*-alcohols on the low-temperature transition observed by spectrophotometry (methanol, ethanol) and fluorescence (*n*-propanol). ▲, Methanol; ○, ethanol; ■, *n*-propanol.

transition of DPPC ($L_{\beta'}$ to $P_{\beta'}$), is to decrease the transition temperature [15], again demonstrating the non-analogy between the low-temperature transitions of the two lipids. Above an ethanol concentration of approximately 0.25 mol/l the low-temperature $L_{\beta I}$ to $P_{\beta'}$ transition approaches the main $P_{\beta'}$ to L_α transition and can no longer be resolved. Also shown in Fig. 2 are the midpoints of the transitions for cooling scans, demonstrating that even in the absence of alcohol there is considerable thermal hysteresis in the $L_{\beta I}$ to $P_{\beta'}$ transition for DHPC. Interestingly, the effect of alcohol is to reduce the magnitude of this hysteresis.

Fig. 3 shows the effect of methanol, ethanol and propanol on the low-temperature $L_{\beta I}$ to $P_{\beta'}$ transition of DHPC. It is seen that the longer the chain length of the alcohol, the more effective it is in shifting the phase equilibrium toward the interdigitated $L_{\beta I}$ phase. This result is consistent with our previous conclusions regarding the effect of alcohol chain length on the biphasic effect in DPPC [12,15]. At a certain critical alcohol concentration, different for each alcohol, the $L_{\beta'}$ to $P_{\beta'}$ transition approaches the temperature of the $P_{\beta'}$ to L_α transition (see below), and can no longer be resolved.

The effect of short chain alcohols on the main transition temperature of DHPC is shown in Fig. 4. The results with DHPC are similar to our earlier observations with DPPC [12,13], in which a biphasic effect of the alcohols on the main transition is observed. As with DPPC, above a threshold alcohol concentration, hysteresis is observed in the main transition and the T_m for the heating curves begins to increase with alcohol concentration. For DHPC the biphasic effect occurs at the alcohol concentration at which the low-temperature transition merges with the main transition. This suggests that above this threshold alcohol concentration, the DPHC no longer goes into the $P_{\beta'}$ phase upon heating, but rather goes directly from the $L_{\beta I}$ phase to the melted L_α phase. This interpretation is analogous to that for DPPC [13] in which it is believed that above the inflection point, the observed transition is directly from the $L_{\beta I}$ phase to the L_α phase. As is the case for DPPC, the effectiveness of the alcohol is increased by increasing the alcohol chain length. It should be noted, however, that for each alcohol the threshold alcohol concentration for the biphasic effect is considerably lower in DHPC than in DPPC.

In summary, we have shown that the effect of alcohols on the thermotropic phase behavior of DHPC is to shift the phase equilibria toward the interdigitated $L_{\beta I}$ phase relative to both the non-interdigitated $P_{\beta'}$ gel phase and the melted L_α phases. Our observatons are consistent with the earlier report by Simon et al. [18] for the same system, but the existence of the biphasic transition in DHPC at low alcohol concentration was not noted before. The observations for the main transition in DHPC are similar to the observations with DPPC [13]. As in DPPC, under conditions

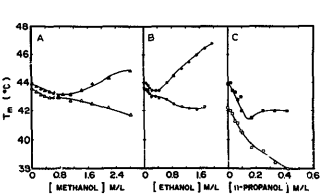

Fig. 4. (A) Effect of methanol on the main transition of DHPC measured using spectrophotometry. ▲, Heating scan; △, cooling scan. (B) Effect of ethanol on the main transition of DHPC measured using spectrophotometry. ●, Heating scan; ○, cooling scan. (C) Effect of *n*-propanol on the main transition of DHPC measured by fluorimetry. ■, Heating scan; □, cooling scan.

where the main melting transition is a transition from $L_{\beta I}$ to L_{α}, i.e., above the threshold concentration for the biphasic effect, the transition of DHPC exhibits marked thermal hysteresis. This comparison indicates the similar nature of the two lipids. The fact that substituting the ether linkage for the ester linkage changes the phase in the absence of alcohol from the bilayer to the interdigitated phase indicates that there is a fine balance in the physical forces that determine whether the gel phase in PCs will be interdigitated or not.

This research is supported by the Medical Research Service of the Veterans Administration and the National Institute of Alcohol Abuse and and Alcoholism (Grant AA-05371 to E.S.R.).

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