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ABSENCE OF SUBTRANSITION IN RACEMIC DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES

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DL-Dipalmitoylphosphatidylcholine multilamellar vesicle suspensions were examined by the method of differential scanning calorimetry. A lack of the subtransition at 18°C was established. Such a subtransition is characteristic for L-dipalmitoylphosphatidylcholine suspensions. This lack is supposed to be the result of the impossibility of the racemic phospholipid mixture to form the low-temperature crystal structure L_c.

The thermotropic properties of multilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes have been widely investigated during recent years. Both phase transitions, main at approx. 41°C and pretransition at approx. 34°C, have been thoroughly characterized by a variety of physical methods: differential scanning calorimetry (DSC), X-ray diffraction, radiospectroscopy [1,2].

In 1980, Chen et al. [3] showed using highly sensitive DSC the presence of a third transition – the so called subtransition – which occurs at approx. 18°C. This transition is exclusively slowly reversible upon cooling and appears after the storage of the liposomes at 0°C for 3.5 days. The structural changes in the DPPC bilayers which correspond to the subtransition have been determined by X-ray diffraction and ^{31}P-NMR methods. According to these investigations, the subtransition corresponds to a bilayer crystal-$L_{\beta'}$ structural rearrangement. The low-temperature incubation ($t < 6$°C) converts the hydrated gel state, $L_{\beta'}$ (approx. 15 mol H_2O/mol DPPC) into a more ordered bilayer structure, L_c, characterized by a specifically ordered hydrocarbon chain lattice and a decrease in the interbilayer hydration (under 15 mol H_2O/mol DPPC). This conversion is a result of the changes in the mode of the molecular packing and is probably accompanied by a decreased hydration at the polar group interface [4–6].

Since only L-DPPC has been used so far, we decided to find out whether the presence of the other stereoisomer(D-DPPC) would influence the occurrence of the phase transitions observed, i.e., the packing properties of the phospholipid molecules. It could be expected that bilayers composed of racemic phospholipids would have different physical properties in comparison with bilayers composed of the pure L- or D-antipode. The present work shows that this difference can be registered by the DSC method.

L-DPPC and DL-DPPC were obtained from Fluka AG, Buchs, Switzerland. By means of thin-layer and gas chromatography, both phospholipids were found to be of over 99% purity. The multilamellar vesicle suspensions were prepared in 50 mM Hepes (pH 7.2). The DPPC was hydrated overnight, heated to 45°C for 1 h and shaken on a Vortex mixer for about 2 min. The lipid suspension was stored at 0°C for different periods of time, but at least for 3.5 days. All temperature scans were performed with the Privalov differential adiabatic scanning microcalorimeter [7]. The

noise level of the device is of the order of the line thickness in Fig. 1, and for this reason it is not indicated.

The phase transitions of the multilamellar vesicle suspensions of DPPC are presented in Fig. 1. Plot A is obtained after maintaining L-DPPC liposomes for 4 days at 0°C. Together with the pretransition at 34.5°C and the main transition at 40.7°C occurs the endothermal peak described by Chen et al. [3] with a maximum at 17.6°C. Plot B is a result of the reheating of the sample after cooling to 0°C in the calorimeter. The subtransition disappears, while the other two maxima are preserved. Plot C is a result of the scanning of DL-DPPC liposomes obtained and kept at the same conditions as those from L-DPPC. It is noteworthy that the subtransition endotherm is missing in this case, while the other two transitions are very pronounced. The preservation of the sample in the course of 21 days at 0°C does not lead to the occurrence of a subtransition. We used L-DPPC from two different batches and DL-DPPC from three different batches and obtained identical results. The characteristics of the phase transition in Fig. 1 are presented in Table I.

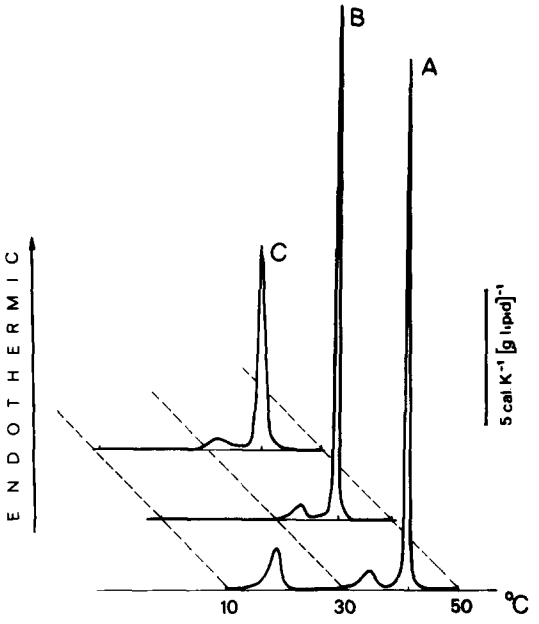

Fig. 1. Calorimetric transition curves for multilamellar suspensions of DPPC, observed at a scan rate of 0.5 K/min. Plot A, scan of L-DPPC liposomes after 4 days at 0°C. Plot B, reheating of the sample after cooling to 0°C in the calorimeter. Plot C, scan of DL-DPPC liposomes obtained and kept under conditions the same as those for L-DPPC.

The absence of a subtransition in DL-DPPC liposomes should be the result of the impossibility of the D-DPPC and L-DPPC mixture to form the structure L_c. The intermolecular interactions in a racemic mixture of L- and D-antipodes could be divided at least into two different classes, one of them being formed by LL and DD pairs, and another one by DL pairs. In particular, if a racemic phospholipid bilayer is considered, the question arises as to what extent the interactions between similar and antipode lipid molecules in it differ. This question cannot be easily answered. However, simple qualitative considerations and juxtaposing of space-filling models of L-DPPC and D-DPPC suggest that a noticeable influence of the chirality of the molecules on the interactions between their hydrocarbon chains could hardly be expected. It seems more likely that the difference in the interactions between similar and antipode phospholipids would be displayed in the polar headgroup area. In this way, it could be assumed that the mutual space orientation of the polar groups of L-

TABLE I

CHARACTERISTICS OF THE PHASE TRANSITIONS OF MULTILAMELLAR SUSPENSIONS OF DPPC

t_c, transition temperature; $\Delta t_{1/2}$ transition width; ΔH, transition enthalpy.

		Subtransition			Pretransition			Main transition		
		t_c (°C)	$\Delta t_{1/2}$ (°C)	ΔH (kcal/mol)	t_c (°C)	$\Delta t_{1/2}$ (°C)	ΔH (kcal/mol)	t_c (°C)	$\Delta t_{1/2}$ (°C)	ΔH (kcal/mol)
L-DPPC	I scan	17.6	2.2	3.28	34.5	2.0	1.34	40.7	0.4	8.78
	II scan	–	–	–	34.5	2.6	1.49	40.7	0.3	8.54
DL-DPPC		–	–	–	32.0	4.0	1.75	40.8	1.2	8.70

and D-isomers of DPPC does not favour the dense packing of the acyl chains characteristic for the L_c phase. It is easy to check this suggestion by X-ray diffraction methods. The relatively greater disorder in the racemic bilayers is reflected also in the characteristics of their main phase transition and pretransition. It is seen from the table that these transitions are less cooperative than the corresponding transitions in an L-DPPC bilayer. The cooperative units during the main transition calculated using the well-known formulae (see, for example, Refs. 2 and 3) consist of 110 and 300 molecules for DL-DPPC and L-DPPC, respectively. Recent experiments in our laboratory show that a specific thermal behaviour is displayed not only by DL-DPPC but to a significantly greater extent by DL-dipalmitoylphosphatidylethanolamine (unpublished data).

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