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THERMOTROPIC BEHAVIOUR OF DIPALMITOYLPHOSPHATIDYLCHOLINE LIPOSOMES CONTAINING RETINOIDS

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SUMMARY

Differential scanning calorimetry has been used to investigate the thermotropic behaviour of DPPC liposomes in the presence of different amounts of retinoids in a study of the sites concerned in the mutual lipid-retinoid interaction. The perturbing effect of retinal and retinol on DPPC liposome gel-liquid crystal phase transition has been related to the difference in the polar end group of retinoid. The hydrophilic polar group prevalence over the apolar tail has been evidentiated. Membrane fluidity increases by increasing the retinoid amount. These liposomes displayed a phase separation at high retinal or retinol concentrations.

INTRODUCTION

The structure of vitamin A is shared between a lipophilic ring system, attached to a long and rigid aliphatic chain, and an hydrophilic end polar group. This enables it to interact with the lipidic component of a biological membrane. Despite their involvement in sight processes, this and similar interactions have not yet been studied in depth.

Several attempts have been made to see whether vit. A, or retinoids in general, are incorporated by biological membranes as well as by simple systems, such as lipid monolayers, vescicles or liposomes. The action of both retinol (vit. A alcohol all trans), and retinal (vit. A aldehyde all trans) on membranes has been demonstrated by red cell haemolysis [1-3], and changes in the lysosomes permeability [4-6]. Baugham et al. showed that vit. A alcohol can penetrate monolayers of lecithin-cholesterol mixtures and suggested a complex formation between phospholipid and vitamin [7]. Absorbance measurements by Stillwell et al. on dimyristoyl- and dipalmitoylphosphatidylcholine (DPPC) liposomes in the presence of vit.A and retinoids showed that vitamin induced disturbance of the membrane structure, reduced the transition temperature and increased membrane permeability[8, 9].

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Leslie and Chapman reported a spectrophotometrical study of all-trans retinal incorporated in lipidic bilayer [10]

We here describe an investigation of the thermotropic behaviour of DPPC liposomes containing vit. A alcohol or aldehyde designed to establish the influence of end group hydrophilicity of the retinoid on lipidic membrane properties.

EXPERIMENTAL

Chemicals

Synthetic L- α dipalmitoylphosphatidylcholine, retinol and retinal were obtained from Sigma Chemical Co. as puriss. products. Phospholipid purity was checked by bidimensional TLC. Phospholipid phosphorous content was assayed photometrically as inorganic phosphate.

Preparation of liposomes

Preparation was carried away from direct light sources to prevent degradation of the light sensitive products. Solutions of DPPC and retinoids in $CHCl_3$: CH_3OH (1:1,v:v) were prepared and mixed in the appropriate ratio to obtain samples containing 8 mg DPPC and different amounts of retinoid. The solvent was removed at 35-40 °C under nitrogen flow and the residual traces were eliminated by lyophilizing for three hours.

To obtain the liposomes, 200 μl of 50 mM tris buffer solution at pH 7.4 were added to the lipidic film. Each sample was heated at 60 °C, vortexed for 3 minutes and then stabilized for 1 hour at 55 °C in a floating water bath and afterward subjected to thermal analysis. The pan content was then subjected to phosphate assay. Attempts to hydrate pure retinoids with an excess of tris buffer were unsuccessful.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was performed using a Mettler TA 3000 system equipped with a TC 10 TA control processor and a DSC 30 calorimetric cell. The system was calibrated in temperature and heat flow using palmitic and lauric acids. The sensitivity of the system was 1.7 mW full scale. 120 μl of each lipidic dispersion were sealed in a 160 μl aluminium pan and subjected to several heating and cooling cycles in the temperature range 10-55 °C at a scanning rate of 2 °C min^{-1}, after allowing the samples to equilibrate at 10 °C for 30'. Tris buffer was used as reference. The enthalpy changes were determined by integrating the calorimetric curves.

RESULTS AND DISCUSSION

The heating DSC curves of DPPC liposomes containing retinol or retinal at different concentrations are shown in figs. 1A, 1B respectively. It is evident that each retinoid interacts in a

characteristic way with the DPPC liposomes. The pretransition and the main transition peak for pure DPPC were observed at 35.7 and at 41.8°C respectively. Both retinoids suppress the pretransition peak, even at very low concentrations. The pure retinoids, when mixed with an excess of tris buffer, did not show any transition over the range 10-55°C.

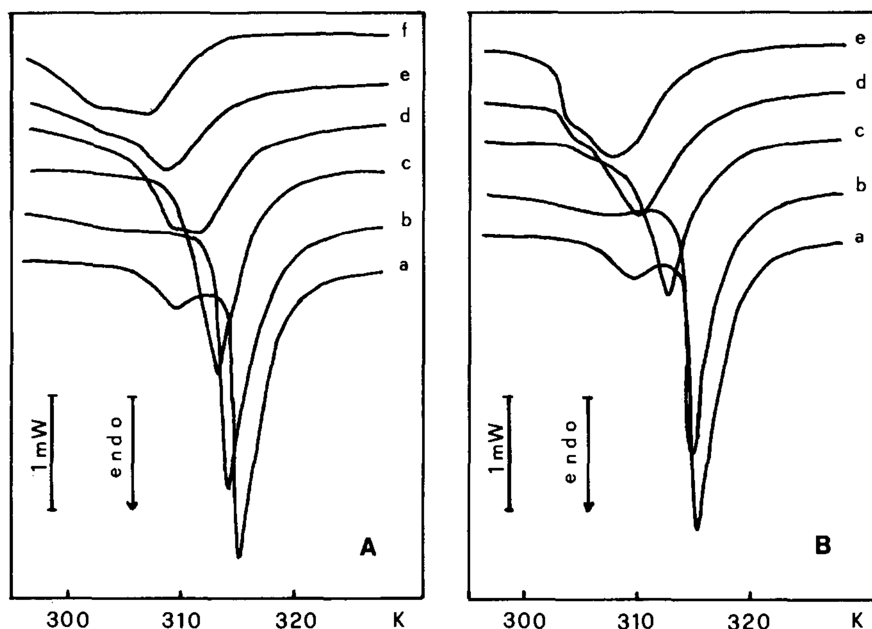

Fig. 1 Typical DSC heating curves of DPPC liposomes containing retinoids.
A) Retinol at molar fractions:
a=0; b=0.015; c=0.12; d=0.24; e=0.33; f=0.50.
B) Retinal at molar fractions:
a=0; b=0.015; c=0.12; d=0.24; e=0.33.

Increasing the vitamin concentration in liposomes results in: i) a decrease in DSC main peak temperature (tab.1); ii) broadening of the peak; iii) an irregular change in the onset temperature; iiii) for molar fractions higher than 0.3, the appearance of a shoulder or a second peak at a temperature 4-5 °C lower than that of the main peak (fig 1A, 1B).

Small variations of enthalpy changes as a function of the concentration (tab. 2) as well as negligible differences between the heating and the cooling mode were observed. The lowering of T_{trs} values with the concentration means an increase in the membrane fluidity, considering as "fluidity" the amplitude, in a qualitative sense, of motion of the lipid fatty acid chains. The appearence of a second peak as well as the nearly constant enthalpy changes (ΔH) suggests the onset of phase separation with one domain richer in phospholipid and a second domain richer in retinol or retinal.

TABLE 1
Main transition peak temperature (T_{trs}) of DPPC liposomes for different molar fractions of retinol and retinal. The figures are the mean values obtained from DSC heating curves. The percentual standard deviation was better than 0.5.

Molar Fraction	T_{trs}(K)	
	Retinol	Retinal
0.00	315.2	315.2
0.015	314.9	315.0
0.03	314.7	314.7
0.06	314.8	314.2
0.09	314.2	313.7
0.12	313.5	313.2
0.24	311.7	310.7
0.33	309.4	309.4
0.40	309.1	308.6
0.50	306.7	308.6

TABLE 2
Main transition enthalpy changes (ΔH_{trs}) in of DPPC liposomes for different molar fraction of retinol and retinal. The figures are the mean values calculated from DSC heating curves. The percentual standard deviation was better than 5.

Molar Fraction	ΔH_{trs} KJ mol^{-1}	
	Retinol	Retinal
0.00	35.1	35.1
0.015	34.7	33.4
0.03	33.9	33.9
0.06	34.7	33.4
0.09	33.0	32.6
0.12	33.4	30.5
0.24	34.7	32.2
0.33	34.7	31.3
0.40	34.7	31.1
0.50	34.7	31.3

By calorimetric investigation of the βcarotene-DPPC-tris buffer system we found that unlike the retinoids βcarotene is unable to fluidify DPPC liposomes. This behaviour confirms that the polar group is more effective than the apolar tail and cyclohexyl ring [S.Gurrieri and F.Castelli, unpublished data] . These results are coherent with Arvidson's studies [11] with polarized light spectroscopy on dioleoylphosphatidylcholine in the presence of βcarotene and retinal; the βcarotene long axis

distribution is perpendicular to the lipidic chains, whereas the retinal is oriented with the polar group located on the water bilayer interface and the hydrophobic tail directed towards the center of the bilayer.

In addition to an interaction between the ring system common to both retinoids and the DPPC aliphatic chain, an important role would thus appear to be played by the polar moiety of the retinoid molecules. A similar interaction between the phospholipid and vit. D_3 in liposomes was pointed out in our previous work [12]. Moreover, a large increase in the intramembrane fluidity of rod outer - segment membranes (CROSM) induced by the cyclohexyl ring of retinal is reported by Wessel [13].

The different thermotropic behaviour of retinol and retinal at pH 7.4 could be explained looking at the different polarity of the polar headgroup. All the retinoids interact electrostatically with the polar moiety of the lecithin remaining at the surface of the bilayer. They thus produce only a shift of the gel-liquid crystal thermal transition towards lower temperatures. The lack of meaningful enthalpy change is an indication of a rather superficial interaction, without effective distruption of the chain packing. A similar interpretation, on the basis of calorimetric investigations, is reported by Cater and al. [14] to explain the thermotropic behaviour of liposomes of DPPC-morphine derivatives and by Chapman et al. for some phospholipid-protein system [15].

Vit.A aldheyde and, to a greater extent, vit.A alcohol are involved in hydrogen bonding either to the carbonyl oxygens or free phosphate oxygen of DPPC, producing a reorganization of the lipidic polar group with a less efficient packing of the lipid chain and hence a lower transition temperature.

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