PHASE TRANSITIONS AND BILAYER STRUCTURE OF *MYCOPLASMA LAIDLAWII* B

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1. Introduction

Thermotropic mesomorphism (i.e., phase-changes induced by heat corresponding to the "melting" of the hydrocarbon chains within a bilayer configuration) have been demonstrated in dry phospholipids, monohydrates and phospholipid–water mixtures by Chapman and colleagues [1] using different physical techniques such as differential scanning calorimetry (DSC), differential thermal analysis (DTA), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), optical studies (birefringence) and X-ray diffraction. This transition is affected by the length and unsaturation of the hydrocarbon chains of the phospholipids, by the water content, by the nature of the polar groups [2] and by the presence of other molecules, like cholesterol. Cholesterol disturbs the normal packing of the hydrocarbon chains (a mixture 1/1 dipalmitoyl–lecithin/cholesterol does not show a thermal transition [3].

Recently Steim and colleagues [4–6] have reported the interesting result that intact organisms and isolated membranes from *Mycoplasma laidlawii* grown on unsupplemented tryptose show a reversible endothermic transition centered at approximately 40°and similar to the transition which is exhibited by the isolated lipids dispersed in water. This phenomena has not been found in other biological membranes such as erythrocyte membranes or myelin. (The cholesterol content of these systems is high, approximately 27% [7] and 45% [8] respectively of the lipid content, whereas in Mycoplasma membranes it is very small [9] maximum 1%.)

Engleman [10] recently using X-ray diffraction also presents evidence of a reversible transition in the same temperature range with the organisms and isolated membranes of *Mycoplasma laidlawii* which involve a characteristic change in the X-ray pattern from that corresponding to a rigid hexagonal packing of hydrocarbon chains to a less organized, liquid–crystalline state. No quantitative estimate of the amount of lipids involved in the transition was made from the X-ray results.

There are number of assumptions implicit in the interpretation of the thermal data [4–6] which we consider require consideration. One of the assumptions is also implicit in the interpretations of various workers studying membrane structure using techniques such as spin labels and fluorescent probes and is, therefore also of general interest.

The (reversible) transition found in the membranes correspond to 3.6 ± 0.4 cal/g of lipid and with the extracted total lipids in water 3.9 ±0.2 cal/g. A comparison of these values led Steim and coworkers to propose [6] that 90 ±10% of the lipids in these membranes are in an extended bilayer configuration organised in a Danielli–Davson sandwich structure [11] i.e., with polar groups of the lipids involved in electrostatic interaction with protein [4]. (The amount of protein associated with the *Mycoplasma laidlawii* amounts to 53% on a dry weight basis.)

We consider that the assumptions used in this deduction and calculation of the thermal data [4, 6] and [12] are:

a) that the lipids in the mycoplasma membrane aree arranged in the same random manner as occurs in the total lipid extract, *or*
b) that the heats of transition of each of the lipid class present are identical, i.e., including polar lipids and non-polar lipids,

and also

c) that there is no effect on the transition temperature of the lipids when interaction with protein occurs,

d) cooling a membrane, to the point where chain crystallisation occurs, does not lead to a process of squeezing out of non-polar protein groups.

We consider that assumption a) is inherently improbable and we have carried out experiments to examine the assumptions b) and c).

2. Experimental

Mycoplasma laidlawii B (strain PG9) was grown in tryptose medium, the organisms harvested and the membranes purified following the procedure of Razin and colleagues [13]. The purified membranes were freeze-dried and extracted three times with chloroform: methanol (2:1). The total lipids were fractionated by silic acid column chromatography, according to Shaw and colleagues [14]. Three fractions which make up the total original weight of lipids were collected: glycolipids (39%) phospholipids (46%) and neutral lipids, including the yellow-coloured carotenoids of the membranes (15%). The observed relative proportion of the different lipid classes are in good agreement with the work of Shaw [14]. The different lipids were further identified by NMR and IR spectroscopy. After eliminating any trace of solvent *in vacuo*, the different lipids were mixed with the appropriate amounts of water, heated to the reported transition temperature, thoroughly mixed and allowed to stabilize for several hours. The DSC spectra were recorded in a Perkin-Elmer DSC-1B differential scanning calorimeter. All the reported thermal scans were reproducible.

3. Results and discussion

The DSC spectrum of 50/50 mixture of the total lipids and water is shown in fig. 1A. There is a broad transition beginning at approx. 20° and centered around 40°, corresponding to a transition enthalpy of 3.8 ± 0.2 cal/g in good agreement with the value reported by Steim and colleagues [4, 6]. For the glycolipids fraction [14] (1–(*O*-α-D-glucopyranosyl)-2, 3-diacyl-D-glycerol: 1–(*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl)-2, 3-diacyl-D-glycerol (2:1) the DSC thermal scan shows a broad transition beginning at about 15° and centered at approximately 40° (fig. 1B) with a much larger heat involved in the transition: 8.3 ± 0.3 cal/g. With the phospholipid fraction (phosphatidyl-glycerol and phosphatidyl-glucose [14] a small transition occurs in the same temperature range, corresponding to 2.2 ± 0.2 cal/g (fig. 1C). Finally, the neutral lipids do not mix with water but a thermal scan of these lipids with a very small water content is presented in fig. 1D: it shows a broad transition that begins around 10° and is centered at approximately 25°, corresponding to 4.7 ± 0.2 cal/g. After remixing the different lipid fractions in chloroform/methanol, eliminating the solvent in vacuo and mixing the lipids with water, one obtains the same heat of transition temperature as the original total lipid fraction, fig. 1E, heat of transition: 3.3 ± 0.3 cal/g.

Thus, from these results we see that assumption b) that the heats of transition of each lipid class should be the same is not correct. Indeed, earlier work has already shown that transition temperatures can differ with different phospholipid classes even when they contain the same hydrocarbon chains [15]. (If it were argued that the enthalpy value for the phospholipid fraction is low due to poor crystallisation of the hydrocarbon chains and it should really have a value similar to that

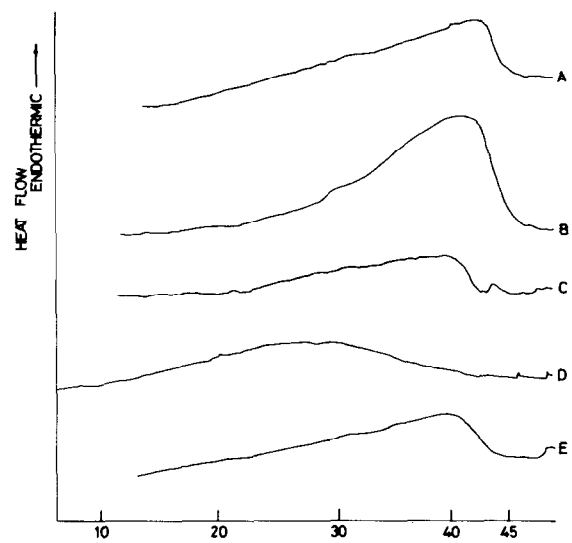

Fig. 1. DSC thermal heating scans 50/50 lipid-water mixtures of lipids extracted from Mycoplasma laidlawii B: A: total lipid; B: glycolipid; C: phospolipid; D: neutral lipid; E: reconstituted total lipid. (The heating rate is 8° K/min. All scans are on range 2 except for C which is on a range twice more sensitive.)

of the glycolipid, i.e., about 8 cal/g this would also affect the quantitative comparison with the membrane because this is almost twice the enthalpy value given by the membrane).

Next we have studied the effects of electrostatic lipid–protein interaction on the temperature of the gel–liquid crystal transition using the Mycoplasma lipids. The Mycoplasma phospholipids are negatively charged and readily interact with the basic protein cytochrome *c*, forming a complex which precipitates and can be disrupted by high ionic strengths. The DSC thermal scan of this complex shows a reversible transition beginning at about 12° and centered around 30°, ten degrees below the transition temperature of the phospholids (fig. 1C) and the total lipids transition (fig. 1A). The first scan showed an irreversible transition, beginning at approximately 55°, probably corresponding to the denaturation of cytochrome *c*. The total lipids also form a complex with cytochrome *c* which shows an overall transition again shifted 10° compared with the single lipid transition. These results demonstrate that electrostatic interactions between lipid and protein can affect the gel–liquid crystal transitions of the lipid. Thus we also find that assumption c) that electrostatic interaction between lipid and protein does not affect transition temperatures, is not correct.

The fact that the transition of total lipid shows a transition temperature and an enthalpy change similar to that observed in intact membrane of *Mycoplasma laidlawii* is therefore, by no means a conclusive proof that all (i.e., 90 ± 10%) the total lipids in the membrane are in an extended bilayer configuration, nor is it necessarily consistent with electrostatic linkage of lipid and protein, i.e., the Danielli–Davson membrane model.

We can of course, speculate about the meaning of our present results. If all the glycolipids are arranged together in the membrane a thermal transition of these lipids *alone* could account for the heat and transition temperature observed in the mycoplasma laidlawii membrane with the glycolipids *not* involved in electrostatic interaction with protein. This would leave the remainder of the lipid, some 61% to be organised in different configurations with lipid–lipid and lipid–protein interactions occurring. This conclusion would be in harmony with the X-ray results and also with the fact that the phase transition temperature observed with the *Mycoplasma laidlawii* can be raised or lowered dependent on the supplementary diet of fatty acid provided with, in each case the phase transition temperature of the membrane matching the lipid transition temperature. It is also consistent with the results of studies with black lipid films which have demonstrated that single lipid bilayers do not exhibit the functional properties of biological membranes and that similar properties only appear after the penetration and modification of this structure by proteins and antibiotics [16]. The concept that different lipid classes may be involved in different ways in lipid–lipid and lipid–protein interactions to make up a membrane structure is also an appealing one.

These results show that it is necessary to be cautious in making quantitative predictions of the amount of bilayer structure present in membrane systems using the thermal calorimetric technique. Even the correlation of a lipid transition with the existence of bilayer structure in a membrane assumes that no squeezing out process as mentioned in assumption d) takes place although we have little experimental evidence for or against this particular assumption as yet.

Finally, the fact that the transition temperatures of certain membrane lipids can be affected by electrostatic interaction with protein means that one must be cautious about the interpretation of comparisons made of hydrocarbon mobility of membranes using probe molecules such as spin labels or fluorescent probes where the comparison made is between the isolated lipids and the intact membranes.

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