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Phase transitions in mammalian membranes

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SUMMARY

Reversible thermotropic phase transitions, centered at 0°C, have been detected in rat liver mitochondria and microsomes by differential scanning calorimetry. Water dispersions of the isolated lipids undergo similar transitions which arise from an order–disorder change in the fatty acid chains within bilayers. The transition temperature of the isolated lipids is slightly lower than that of the membranes in both mitochondria and microsomes. In the membranes, no change in the transition occurs following irreversible protein denaturation. The bulk of the lipids in the membrane participate in the cooperative melting process.

The lipids in the membranes and whole cells of *Mycoplasma laidlawii*[1,2], *Micrococcus lysodeikticus*[3], and *Escherichia coli* (ref. 4 and G.B. Ashe, personal communication) have been shown by calorimetry to undergo a thermotropic phase change like that of the extracted lipids. Since the phenomenon appears to be the same as the gel–liquid crystal transition of synthetic phospholipids in the lamellar phase, it provides strong evidence for an extensive bilayer in these membranes. Subsequent X-ray diffraction studies[5] support this notion. Similar phase transitions should occur in mammalian membranes containing lipid bilayers which are not rich in cholesterol[6]. We report here transitions in rat liver mitochondria and microsomes.

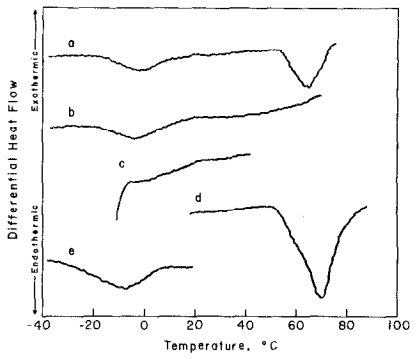
Sprague–Dawley Albino rats were decapitated and exsanguinated. The livers were gently homogenized in 1:5 (w/v) of cold buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.5) in a Potter–Elvehjem apparatus. Cellular debris was removed by filtration through cheesecloth and centrifugation at 1000 × *g* for 15 min. Mitochondria were sedimented at 8000 × *g* for 15 min, then resuspended and washed 3 times in buffer. The

supernatant from the mitochondrial fraction was centrifuged at 20 000 × *g* for 30 min to remove remaining contaminants, then the microsomes were sedimented at 80 000 × *g* for 90 min, and resuspended and washed 3 times in buffer. Lipids were extracted[7] under nitrogen, dried in the cold in a vacuum dessicator, then immediately suspended in buffer for calorimetry. Protein was determined by the Folin procedure[8] and phosphorus by the method of Chen *et al.*[9].

Mitochondria and microsomes were sedimented at 80 000 × *g* for 1 h in sucrose–Tris buffer, or buffer containing 50% ethylene glycol or 10% glycerol to prevent freezing. Samples of 110–120 mg (10–15% dry weight) were sealed in stainless steel calorimeter pans. References were the same suspending media used for samples, but included Sephadex G-100 to prevent convection. Thermograms were obtained with a modified Perkin–Elmer DSC-1B differential scanning calorimeter operated at 5 degrees/min and a full-scale range of 1 mcal/s. The calorimeter records differential power input necessary to heat both sample and reference at the same rate; a transition appears as a peak whose area is proportional to the heat of transition. Stearic acid was used as the calibrant.

Electron microscopy showed intact mitochondria, with about 5% contamination with lysosomes and peroxysomes. Mitochondria in glycol had intact inner and outer membranes and showed less contamination but some loss of matrix material. The microsomes appeared to be virtually uncontaminated, but few ribosomes were present. With a Clark electrode and succinate as substrate, the mitochondria consumed $1.1 \cdot 10^{-2}$ and the microsomes $9.8 \cdot 10^{-4}$ μatom O_2/min per mg protein, respectively, at 37°C. Glucose-6-phosphatase activity[10] was 0.416 μmole phosphate/min per mg microsomes, with no activity in the mitochondria.

Scanned upward from −40°C, whole mitochondria in glycol undergo a broad, reversible endothermic transition centered about 0°C (Fig. 1, a). The peak between 50



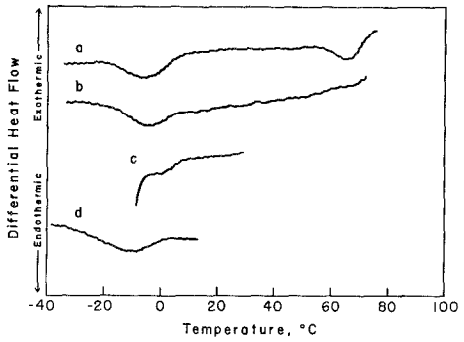


Fig. 1. Thermograms of rat liver mitochondria suspended in 50% ethylene glycol (a) before and (b) after protein denaturation; (c) in 10% glycerol; and (d) in buffer; and (e) extracted lipids in 50% ethylene glycol.

Fig. 2. Thermograms of rat liver microsomes suspended in 50% ethylene glycol (a) before and (b) after protein denaturation; and (c) in 10% glycerol; and (d) extracted lipids in 50% ethylene glycol.

and 70°C arises from irreversible protein denaturation and vanishes after heating to 80°C. Protein denaturation does not appreciably affect the lipid phase change (Fig. 1, b). To determine whether glycol might introduce artifacts, mitochondria were scanned downward from 40°C in 10% glycerol. The half of the transition which is visible before freezing (Fig. 1, c) is identical to those seen in glycol. A larger protein denaturation peak in buffer without glycol is evident (Fig. 1, d); this difference could be due to loss of matrix protein. Fig. 1(e) shows the transition in isolated lipids in 50% glycol. Its temperature range is slightly lower than that of the mitochondria.

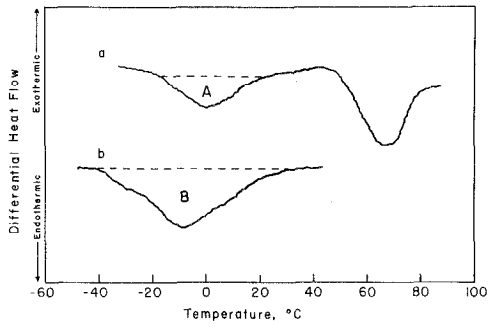


Fig. 3. Thermograms of (a) whole mitochondria and (b) extracted lipids in 50% ethylene glycol. A corresponds to the area under the lipid transition in the membranes and B to the area under the transition in the protein-free lipid extract.

Thermograms of microsomes are shown in Fig. 2. The experimental conditions of Fig. 2(a, b and c) are identical to those of Fig. 1. Again, the transition temperature range of extracted lipids in glycol (Fig. 2, d) is slightly lower than that in the membranes.

The magnitude of the transition in membranes can be estimated by comparing the heats of transition per unit mass of lipid in both membranes and isolated lipids. Fig. 3(a) shows the transition in mitochondria and Fig. 3(b) that of the lipids extracted from the same preparation. The dotted lines conservatively minimize the area (A) for the membranes and maximize the area (B) for the lipids. When normalized to lipid phosphorus content, the ratio of A (160 μg lipid P) to B (404 μg lipid P) is 0.80. Thus the membrane transition is at least 80% as energetic as that of the extracted lipids; a minimum value of 75% was similarly calculated for microsomes.

The lipids of beef heart mitochondria have been shown by X-ray diffraction[11] to undergo a broad transition between 10 and −10°C. Above 10°C the organization is lamellar, with mobile fatty acid chains; below −10°C the lamellar phase remains but the chains are rigid. The temperature region of the phase change corresponds to that observed here for rat liver mitochondria. In view of the similarity in lipid composition in the two systems, the transition in rat liver mitochondria constitutes strong evidence for the

existence of a bilayer. Similar transitions from gel to liquid crystal occur in synthetic phospholipids[12], where again the organization is lamellar both above and below the melting point. The calorimetric phenomenology is the same in microsomes. The overwhelming majority of the lipids in mitochondria and microsomes is phosphatidylcholine and phosphatidylethanolamine[13], both of which produce the lamellar phase in water. We conclude, therefore, that the membrane transitions indicate melting of the fatty acid chains of the phospholipids, that the majority of the lipids in both mitochondria and microsomes is in bilayers, and that at physiological temperature the interior of the bilayers is liquid-like.

The temperatures of lipid phase changes depend upon the length and saturation of the fatty acid chains, and upon the polar head group. Since mitochondrial and microsomal lipids are not drastically different, it is not surprising that the position and width of the transitions are nearly the same in both systems. The membrane transition temperatures are slightly higher than those of the free lipids. This discrepancy might indicate constraints imposed by protein[14], or it might arise because a small amount of lipid hydrophobically bound to membrane protein is released to contribute to the transition in the extracted lipids.

The comparison of heats of transition of membranes and extracted lipids emphasizes that the membrane transitions are major events, and that the bilayer must be rather extensive. A rigorous determination of the extent of bilayer would require the enthalpies of transition per unit mass of bilayer to be the same in both membranes and lipid extracts. Since the transition temperatures are similar, and since the enthalpies of transition of phospholipids do not differ drastically, this assumption is reasonable. If one assumes it is true, then at least three fourths of the lipids in mitochondria and microsomes are present as bilayers. An extensive bilayer does not rule out penetrating proteins, nor does it imply that proteins are extensively bound *via* polar interactions with the lipid head groups.

Uncoupling agents like dinitrophenol and valinomycin cause a drastic decrease in resistance in liposomes[15]. In view of the evidence presented here for the existence of bilayers in mitochondria, these liposome studies are quite relevant to physiological properties. In contrast, sharp breaks in Arrhenius plots of oxidation[16] and motion of spin-labeled fatty acids[17] in rat liver mitochondria do not correlate with the broad, low temperature melts observed calorimetrically, and cannot reflect the entire phase change.

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