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Membrane transitions in Gram-positive bacteria

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SUMMARY

Intact cells, membranes, and aqueous dispersion of lipids of *Micrococcus lysodeikticus* undergo a reversible thermotropic transition detectable by differential scanning calorimetry. The phenomenon is suggested to be a "melt" of fatty acid chains within lipid bilayers. The transition is the same in membranes and extracted lipids, but in whole cells it appears to occur at a slightly higher temperature, possibly because of the presence of cell wall. When observed in the calorimeter, protein denaturation and the lipid transition are independent events. At growth temperature, the fatty acid chains are in a melted state.

The lipids in the plasma membrane of *Mycoplasma laidlawii* have been shown by differential scanning calorimetry to undergo a reversible thermotropic phase change[1-3]. The temperature of the transition can be varied over a wide range by changing the fatty acid composition of the growth medium, but for any given culture it is very similar for living cells, isolated membrane preparations, and aqueous dispersions of protein-free lipids. Thermal denaturation of membrane protein has little effect on the position or shape of the lipid transition. Comparison of the heat of transition of membranes with that of an equivalent amount of membrane lipids suspended in water suggests that the majority of the lipids in the membranes contribute to melt[4]. The phenomenon was ascribed to an order–disorder melt of fatty acid chains within lipid bilayers, with the lipids remaining in the bilayer conformation both below and above the phase change[2]. This interpretation of the calorimetric results for *M. laidlawii* is supported by X-ray diffraction, which shows that the event as it occurs in membranes is quite similar to that seen in phospholipids in water[5,6]. Below the transition temperature, the fatty acid chains within the bilayers are in the α-crystalline state, where they are hexagonally packed and have rotational freedom about their long axes, while above the transition the high-angle diffraction is characteristic of liquid hydrocarbons. The melt is accompanied by a decrease in bilayer thickness and an increase in surface area

of the lipid molecules.

In principle, unless large quantities of cholesterol are present[7], such transitions should occur in any system containing lipid bilayers. Although detection could be difficult if melts are excessively broad or if extreme supercooling were to take place, it is reasonable to except that they occur with reasonable width and at accessable temperatures in many other membranes. In this report we extend the calorimetric observation of phase changes to a Gram-positive bacterium, *Micrococcus lysodeikticus*. The results are similar to those obtained with *M. laidlawii*.

M. lysodeikticus, obtained from Dr. P.L. Carpenter of the University of Rhode Island, was grown to stationary phase (24 h) in Difco brain-heart infusion at 37° with aeration. Growth was monitored by absorbance at 600 nm. Cells were harvested by centrifugation, washed twice with distilled water in the cold, and then suspended (0.3 g wet wt./ml) in 0.1 M Tris buffer, pH 7.5. Membranes were prepared by incubating with lysozyme (100–200 μg/ml) at 37° for about 30 min [8]. Following treatment with deoxyribunoclease I, the membranes were sedimented for 30 min at 20 000 x *g* and washed twice in 0.05 M Tris buffer, pH 7.5. For calorimetry, whole cells and membranes were packed at 100 000 x *g* for 1 h, either in 0.05 M Tris or 50% (v/v) ethylene glycol-distilled water. Lipids were extracted and washed by the method of Folch *et al.*[9]. Solvents were removed under a stream of nitrogen, then the lipids were dried to constant weight in a vacuum dessicator over P_2O_5. They were suspended in the same media used for cells and membranes and allowed to equilibrate for several hours before calorimetry.

Calorimetry was carried out with a modified Perkin–Elmer DSC-1B differential scanning calorimeter[2]. Sample pans, of approximately 170 μl capacity, were of gold-plated brass with screw tops. Sample weights of whole cells and membranes were 150–190 mg, containing 15–20% dry weight. Lipid samples were typically 5–10 mg at a concentration of 5–10%. References were either water or 50% ethylene glycol containing sufficient Sephadex (G-100) to prevent convection. Thermograms were obtained at a scan rate of 5 degrees/min and a full-scale sensitivity of 1 mcal/sec. The calorimeter was calibrated by melting stearic acid, and areas were measured with a planimeter.

Representative thermograms in 50% ethylene glycol of whole cells, membranes, and membrane lipids of *M. lysodeikticus* are shown in Fig. 1. Since the transitions begin at a low temperature, to observe the entire melt glycol must be added to prevent freezing. The calorimeter records the differential power input between sample and reference necessary to heat both at the same rate, so that a thermal event is recorded as a peak whose area is proportional to the enthalpy of the process. The scans are presented in the conventional manner, with endothermic peaks below the baseline.

The lipid phase transition in all three samples is broad, beginning a little below 0° and ending by 30°. Thermal gradients in the calorimeter, which do not exceed 3°, are negligible compared to the observed width of the transition. In addition to the reversible lipid melt, protein denaturation gives rise to several incompletely resolved peaks at higher temperatures in the whole cell and membrane samples. In both whole cells (Fig. 1, a and b) and membranes (Fig. 1, c and d), comparison of the scans taken before and after protein denaturation reveals that protein denaturation is irreversible. However, within the precision of the instrument, protein denaturation does not affect the position, shape, or area of the peaks arising from the lipid transition. In the high-temperature region of the thermograms a slight upward deviation of the baseline occasionally occurs. This baseline curvature results from

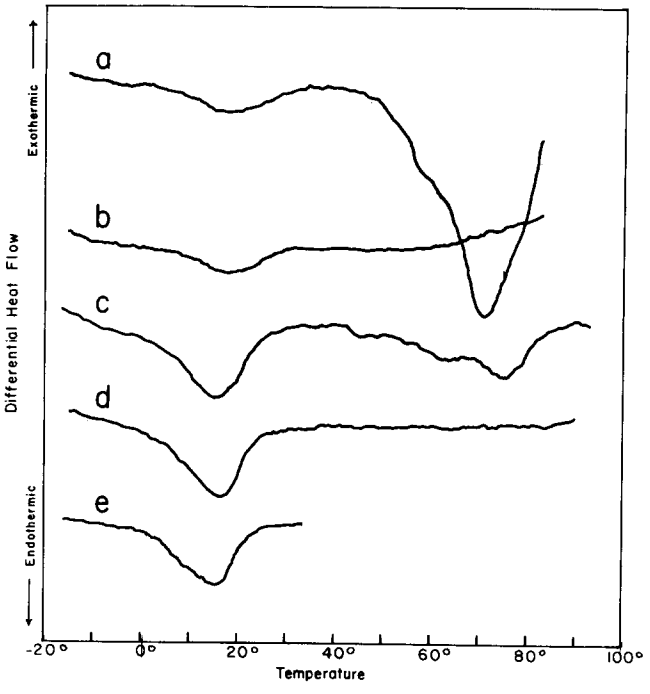

Fig. 1. Thermograms, taken in 50% ethylene glycol, showing (a) whole cells of *M. lysodeikticus* before protein denaturation and (b) cells after denaturation; (c) isolated membranes before protein denaturation and (d) after denaturation; and (e) protein-free membrane lipids. Scans a and b are from a different culture than the others.

differences in emissivity of the sample and reference cell surfaces and is not indicative of a thermal event. Note that the lipid peak in the whole cells occurs at a slightly higher temperature than in the isolated membranes. The two samples come from different cultures grown at different times under the same conditions, and are typical of the small variations observed frc culture to culture.

A suspension of protein-free membrane lipids produces a peak (Fig. 1, e) whose shape and temperature range appears almost identical to that observed in both the first and second scans (Fig. 1, c and d) of the membranes. Although accurate area determinations are difficult, such measurements lead to a value of 4.0 ± 0.4 cal/g for the enthalpy of transition of extracted membrane lipids dispersed in 50% ethylene glycol. One may attempt to assess the extent of the lipid melt within the membranes if one assumes that in the membranes those lipids undergoing the phase change have the same enthalpy of transition as the extracted-lipid dispersions. Thus a comparison of the enthalpy of transition of membranes with that of an equivalent amount of membrane lipids suggest that at least two-thirds of the lipids present in the membrane contribute to the melt. Given the above assumption, this estimate is a lower limit. Such estimates, in combination with qualitative observations of the relative magnitude of the lipid and protein peaks, indicate that the membrane transitions are not minor effects.

Thermograms of whole cells, membranes, and membrane lipids, all in Tris buffer without ethylene glycol, are presented in Fig. 2. Since freezing must be avoided, and some time is necessary after the beginning of a scan to establish steady-state conditions, the entire lipid transition cannot be recorded. Fig. 2 (a) includes the typical initial transient deflection which renders the beginning of the thermogram unusable and introduces some distortion of the low temperature end of the peak. Nevertheless, enough can be seen to establish its position and approximate size. Comparison of the samples in Tris with the previous ones in ethylene glycol shows that the peaks occur over the same temperature region in both solvents. With Tris, as with ethylene glycol, peaks are obtained for the lipid change and for protein denaturation in both whole cells (Fig. 2, a) and membranes (Fig. 2, c). On the second scan of the same samples, the reversible lipid melt remains but the irreversible protein peak has vanished (Fig. 2, b and d). Again the transition of protein-free lipids (Fig. 2, e) is very similar to that of the membrane preparation. Although the results in Tris buffer are essentially the same as in ethylene glycol, for whole cells the behavior appears to be slightly different in the two solvents. Close inspection

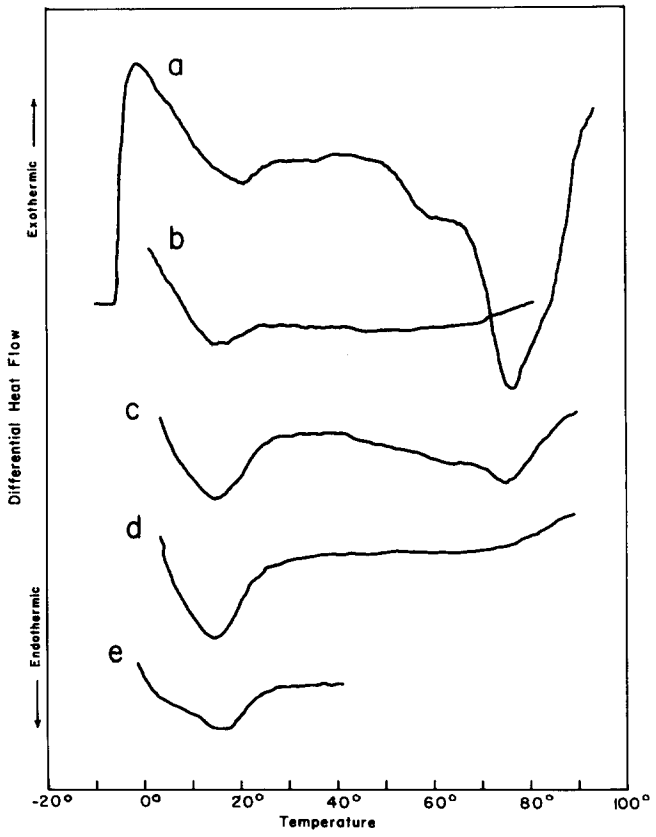

Fig. 2. Thermograms, taken in Tris without ethylene glycol, showing (a) whole cells of *M. lysodeikticus* before protein denaturation and (b) cells after denaturation; (c) isolated membranes before protein denaturation and (d) after denaturation; and (e) protein-free membrane lipids. All samples were from the same culture.

of the scans of cells in Tris without ethylene glycol (Fig. 2, a and b) reveals that the lipid transition occurs at a slightly higher temperature before protein denaturation than after. Heating the cells beyond denaturation lowers the peak of the lipid transition to the position characteristic of isolated membranes and extracted lipids. Since the transitions are near the initial transient deflection, it is possible that this lowering of the lipid peak is an instrumental artifact. However, the effect has occurred consistently in scans of cells from several different cultures of bacteria. For some of these scans an internal standard of methyl palmitate was used to verify that the temperature calibration did not change between the first and second scans. Interaction between the membrane and wall might be responsible for the higher transition in whole cells in Tris buffer. Exposure to high temperatures on the first scan could disrupt such interactions, so that the transition is lower on the second scan. Whole cells in ethylene glycol do not show the effect; in this case disruption of wall–membrane association might result from initial plasmolysis upon exposure to ethylene glycol.

If it is assumed that the protein-free lipids in buffer are in the lamellar phase, then, in analogy with similar transitions known to occur in *M. laidlawii* and synthetic phospholipids[2,10], the calorimeter results provide strong evidence that the majority of the lipids in both isolated membranes and live cells in *M. lysodeikticus* are arranged in bilayers. The existence of the bilayer melt in whole cells indicates that in membranes it is not an artefact of preparation. When cells are grown at 37° the fatty acid chains within the bilayers are in a liquid state, well above their melting point. In cells there appears to be some perturbation of the melt by the cell wall. Note, however, that in membranes the lipid transition and protein denaturation as observed calorimetrically are independent events, and that the lipid melt in membranes is not dependent upon protein conformation. Although the results are inconsistent with extensive hydrophobic lipid–protein association, they do not eliminate limited hydrophobic interaction with penetrating protein molecules. They also do not imply that the bilayer is continuous, or that the lipids the bilayer are electrostatically bound to protein. It is reasonable to expect reversible membrane transitions to occur in other Gram-positive bacteria, whose membranes and walls are morphologically and chemically similar to those of *M. lysodeikticus*. The membranes of *M. lysodeikticus* are rich in branched-chain fatty acids[11], so that transitions for other Gram-positive organisms having a similar fatty acid composition may occur in the same temperature region observed for this organism.

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