Studies of the Lipid Phase Transitions of *Escherichia coli* by High Sensitivity Differential Scanning Calorimetry*

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Meyer B. Jackson and Julian M. Sturtevant

From the Department of Molecular Biophysics and Biochemistry and Department of Chemistry, Yale University, New Haven, Connecticut 06510

SUMMARY

High sensitivity adiabatic differential scanning calorimetry was performed on lipids, membrane vesicles, and whole cells of *Escherichia coli* enriched in particular unsaturated fatty acids by genetic means. Information concerning the shape of the transition is discussed. Transitions with an asymmetric shape reminiscent of a second order transition were observed. Comparison between the lipid transition observed in whole cells, membrane vesicles, and extracted lipids enriched in elaidate reveal some basic similarities. Studies of synthetic lipids were undertaken in an attempt to interpret the shapes of these transitions as a function of the lipid components of the membrane.

The lipids of the membranes of *Escherichia coli*, in common with those of other organisms, undergo phase transitions from a gel phase to a liquid-crystal phase (1, 2). Numerous studies relating the state of the membrane to the physiology of the organism have been reviewed by Cronan and Gelmann (3). The availability of mutants defective in fatty acid synthesis has permitted genetic manipulation of the physical state of the membranes through their chemical composition (4). The lipid phase transition has been monitored by X-ray diffraction (2, 5), differential scanning calorimetry (1), and fluorescence (6). In this communication we report a study of this transition by high sensitivity differential scanning calorimetry in which new detail about the transition of elaidate-enriched *E. coli* membranes was obtained. Some of these results have been presented in a preliminary communication (7).

MATERIALS AND METHODS

*Escherichia coli* strain K1080 was supplied by Dr. J. E. Cronan. This strain has a defect in unsaturated fatty acid biosynthesis and in β oxidation (4). The cells were grown in minimal salts medium E (6) supplemented with 0.5% glycerol, 0.3% casamino acids, 0.02% fatty acid, and 40 μM thiamin. The elaidate acid (or oleic acid) was added as a 10% solution in ethanol. (14C)Palmitate (1 to 2 μCi) (New England Nuclear) was added in some cultures to permit a quantitative measurement of the lipid concentration in a suspension of whole cells. Since the (14C)palmitate is incorporated exclusively into the lipids, the counts per min per mg of extracted lipid from the cells allows one to calculate the lipid concentration in the cell suspension.

In preparing samples for differential scanning calorimetry, **K1080 E. coli** was grown at 40° (33° if oleic acid was the supplementing fatty acid) to late exponential or stationary phase and harvested by centrifugation. A white layer of unknown nature on top of the pellet was removed by swirling or shaking the tube with 10 ml Tris/HCl, pH 8.0, until only the white layer was suspended and then discarded. Omitting this step gave inferior results. The cells were then washed repeatedly in the Tris buffer and the lipids were extracted according to the procedure described by Ames (9). The lipids were dried from chloroform, under vacuum, overnight or for several hours, and then suspended in 0.1 M potassium phosphate buffer, pH 6.6. For differential scanning calorimetry of whole cells, the cells were washed three times in Tris buffer and once in phosphate buffer, and approximately 0.2 g of pelleted cells were suspended in 1.5 ml of phosphate buffer. Membrane vesicles were prepared according to Kaback (10). All calorimetric experiments were performed in a Privalov calorimeter (11). Scan rates and concentrations are given in the figure legends.

Dielaidoyl phosphatidylethanolamine was synthesized according to procedures described by Rosenthal (12) and suspended in phosphate buffer. Dimyristoyl phosphatidylethanolamine was purchased from Calbiochem and purified by recrystallization from ethanol.

Fatty acid analysis of the methyl esters obtained from the lipids by transesterification in the presence of acetyl chloride was done by gas-liquid chromatography.

RESULTS

Typical calorimetric scans are presented in the figures. Fig. 1 shows a scan obtained with a suspension of elaidate-enriched *Escherichia coli* lipids and a scan of a suspension of oleate-enriched lipids. The elaidate-enriched lipids have a narrower transition with a width at half-height of about 3.5°. The transition is highly asymmetric with a gradual onset and an abrupt end at 4°. The transition enthalpy averaged for many experiments was 86 cal (g of lipid)−1. The oleate-enriched *E. coli* lipids have a broader transition which occurs at a lower temperature. Some variation in scan behavior was seen, but the scans shown here are representative.

A scan obtained with elaidate-enriched whole cells is presented in Fig. 2A along with one obtained with lipids from the same cell growth (Fig. 2C). They have similar appearances, with the cell transition being broader, but with nearly the same temperature range and asymmetry of the lipid transition. The absorption of heat seen at higher temperatures is due to the irreversible denaturation of cellular material. Ambiguity in selecting the appropriate baseline for the scan with whole cells prevented an accurate determination of the transition enthalpy.

Many scans with whole cells were of poor quality due to exothermic processes probably associated with cellular metabolism. It was found that fewer washings of the cells in Tris buffer, and shorter handling before calorimetry, made the exothermic processes less likely. However, this prevented complete removal of fatty acid from the growth medium and is probably the reason why the scan of lipids extracted from the same cells is of poorer quality than the scan shown in Fig. 1.

Fig. 2B is a scan of membrane vesicles made from the same cells. This transition is also similar in shape to the whole cell and lipid transitions. Protein denaturation occurs at higher temperatures.

The fatty acid chain compositions of elaidate-enriched lipids showed that 80 to 90% and sometimes as much as 95% of the fatty acid chains of the lipids were elaidate. The oleate-enriched lipids contained 40 to 50% oleate. In both cases, there were no C16 unsaturated fatty acids. No correlation between the fraction of elaidate and the variations in the transition behavior were seen in the various preparations, nor was there...
A calorimetric scan of dielaidoyl phosphatidylethanolamine is shown in Fig. 3A. It bears a strong resemblance to the transition of elaidate-enriched lipids. The enthalpy is 6.3 Cal/g of lipid, and the temperature at the maximum is 37.5°. The asymmetry is very apparent although the transition is as narrow as that of other synthetic lipids studied in the same instrument (13).

A scan obtained with dielaidoyl phosphatidylethanolamine containing 13.2 mol % dimyristoyl phosphatidylethanolamine is shown in Fig. 3B. The asymmetry seen here is considerably less than in elaidate-enriched E. coli lipids.

All of the lipid transitions shown were reversible, although the reheats of E. coli lipids, vesicles, and cells did not retain all of the sharpness or enthalpy of the first heats.

DISCUSSION

The temperatures of the transitions reported here agree well with those given in previous reports (2, 5, 6). Asymmetry in the lipid transitions of biological membranes has been observed in Acholeplasma laidlawii (14) as well as in some previous studies of Escherichia coli lipids (15,16). The present experiments show this asymmetry much more clearly.

The transition is broader in whole cells than in extracted lipids, but the temperatures at which the maxima in specific heat occur are nearly the same and the asymmetry is retained. It is possible that in E. coli the lipids are distributed unevenly between the inner and outer surfaces of a membrane, the inner and outer membranes,1 or laterally within the plane of a membrane. This could give rise to differences between the melting behavior seen in extracted lipids and in whole cells and could account for the observations made here; however, the observed differences in heating scans between cells, lipids, and membrane vesicles are not large enough to support or refute the contention that nonuniform lipid distributions are widespread. Other possible explanations are that various membrane proteins have differing affinities for fluid and solid lipids (17), or that the membrane proteins are positioned in such a way that they disrupt the cooperativity of the lipid phase transition.

The growth of strain K1060 is profoundly affected by the fatty acid which supplements the growth medium (4). In the case of elaidate enrichment, 37° is found to be the minimum growth temperature. K1060, growing at 37°, would be growing below the end of the lipid transition and would have a substantial fraction of its lipids in the gel state. Experiments on a different strain grown with the minimal amount of unsaturated fatty acids (18) indicate that this strain can grow at a temperature well below the end of the lipid transition2 when the membranes are not completely fluid.

Since E. coli lipids are 60 to 70% phosphatidylethanolamine (19), the elaidate-enriched lipids of the strain studied here have dielaidoyl phosphatidylethanolamine as their major chemical species. In comparing the calorimetric scans of elaidate-enriched K1060 lipids and of synthetic dielaidoyl phosphatidylethanolamine, many similarities are evident. The

1 Overath et al. (23) have shown that the inner and outer membranes of E. coli have the same fatty acid composition and transition temperatures.

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temperatures of the transitions are nearly equal, suggesting that the cells have reduced their transition to the lowest obtainable temperature by incorporating as much elaidate as possible. Another striking similarity is the asymmetry evident in the two transitions. The occurrence of asymmetric transitions similar to the one observed here for dielaidoyl phosphatidylethanolamine appears to be a general property of phosphatidylethanolamines (13).

Purified synthetic lecithins, in common with most pure crystalline substances which undergo a phase transition, have melting heat capacity curves which are symmetrically broadened derivative curves of the step functions in enthalpy expected for first order transitions. The melting of phosphatidylethanolamines clearly deviates from this behavior. In addition, it is difficult to construct a phase diagram (13), with all components completely miscible in both phases, which can predict melting behavior of the sort seen here with elaidate-enriched lipids. The shapes of these transitions thus suggest unusual phase behavior and possibly a second order transition.

A second order phase transition is distinguished from a first order phase transition in the lowest order of the derivative of the free energy in which one sees a discontinuity with temperature. Thus, a first order transition has a latent heat, but a second order transition has a discontinuity in the heat capacity without having a latent heat. Such transitions are also called A transitions, and some elementary discussion of these phenomena can be found in Ref. 20.

Discontinuities in any property are in actual systems always smeared out to some extent, and for this reason we cannot identify with certainty the order of these transitions. The asymmetry could be due to a pretransitional heat uptake as is seen in some liquid crystals (21). It is also possible that the many components of the E. coli lipids, and impurities in the synthetic lipids, give rise to an asymmetric broadening of a first order transition. In this connection, it is significant that repeated recrystallization from ethanol and chloroform/acetone of synthetic phosphatidylethanolamines, including dielaidoyl phosphatidylethanolamine, did not change the shape or temperature of the transition once the lipid was purified to constant behavior. Thus, an impurity is probably not the cause of the asymmetry in dielaidoyl phosphatidylethanolamine or other phosphatidylethanolamine transitions.

To aid in analyzing the asymmetry of such transitions, we shall introduce a quantitative index of asymmetry. A horizontal line is drawn on a differential scanning calorimetry scan at half the maximal heat capacity. This line intersects the heat capacity plot above and below the temperature at which the maximum in heat capacity occurs ($T_{\text{max}}$). We shall divide the temperature difference between $T_{\text{max}}$ and the lower intersection temperature by the difference between the upper intersection temperature and $T_{\text{max}}$. This ratio will be used as an index of asymmetry.

Synthetic dielaidoyl phosphatidylethanolamine has an asymmetry index of between 2.5 and 3.5. The scan in Fig. 1 has an asymmetry index of 8.0. An attempt to simulate the fatty acid chain composition of elaidate-enriched E. coli lipids with a mixture of two phosphatidylethanolamines is shown in Fig. 3. In this mixture, and in other mixtures, the asymmetry index was slightly lower than in pure dielaidoyl phosphatidylethanolamine, and we conclude that this experiment was not successful in reproducing the asymmetry of the E. coli lipid transition. The oleate-enriched E. coli lipids, in which the fatty acid chains were much more heterogeneous, had a rounder and more symmetrical transition curve. It may be that the shape of the unimolecular E. coli lipid transition is a result of the polar group heterogeneity and that the fatty acid chains are homogeneous enough not to influence the shape of the transition significantly. Diversifying the fatty acid chains gives the result seen with oleate-enriched E. coli lipids where the transition is broader and more symmetrical.

Further study of the phase behavior of the lipids of E. coli is important since it is still not known whether the two coexisting phases seen by x-rays during melting (4) have different compositions (22), and knowing the composition of the two phases may provide important information about the impact that the transition has on the functioning of membrane proteins.

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