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THERMAL-TURBIDIMETRIC STUDIES OF MEMBRANES FROM *ACHOLEPLASMA LAIDLAWII*

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SUMMARY

Light scattering measurements were made of aqueous dispersions of *Acholeplasma laidlawii* membranes as well as lipids extracted from these membranes. As the temperature was increased the turbidity values changed over a temperature range that was dependent upon the fatty acid residues present in the membrane lipids. These changes were reversible and followed a pattern similar to the changes observed in calorimetric studies. The advantage of this procedure is that it permits thermal studies using relatively small amounts of membranes and also permits the study of some interactions between the membrane components and solutes in the aqueous medium. In this way an interaction was observed with Ca^{2+} occurring only when the membrane had changed to the fluid state.

INTRODUCTION

Studies of the membranes of *Acholeplasma laidlawii* (formerly classified as *Mycoplasma laidlawii*) have demonstrated the existence of reversible, temperature-dependent changes affecting the hydrocarbon chains of the membrane lipids. The changes, detected by differential scanning calorimetry[1] and X-ray diffraction[2], have been attributed to a cooperative gel–liquid crystalline phase transition of the lipids in a bilayer structure. The temperature at which this transition occurs varies with the fatty acid composition of the lipids and ranges from below 0 °C for membranes with lipids containing predominantly unsaturated fatty acid residues, to 37 °C and higher for membranes enriched with saturated fatty acid chains. A previous report from this laboratory[3] described the use of turbidimetric measurements as a means of determining temperature-induced structural changes in dispersions of purified lecithins. In this communication, we report on the application of this method to the study of temperature-dependent changes in isolated membranes of *Acholeplasma laidlawii*. For these studies, membranes with lipids enriched in oleic acid, palmitic acid and stearic acid residues were investigated, since the thermotropic phase changes that these membranes undergo have been relatively well defined. The method described here permits the study of thermal changes using small amounts of membrane, on the order of 2–4 mg, and the analysis of changes resulting from the interaction of the membranes with solutes in the medium.

METHODS

A. laidlawii were grown statically at 37 °C in tryptose media containing defatted bovine serum albumin (Pentex Products) and a fatty acid supplement at a concentration of 40 mg/l. For these studies, oleic acid, stearic acid and palmitic acid (purchased from either Sigma Chemical Corporation or Calbiochemical) were used. After growth for 30 h, cells were harvested, washed and lysed by suspension in hypotonic buffer. Membranes were sedimented and further purified by centrifugation on 25–50 % discontinuous sucrose gradients followed by extensive washing with deionized water. Membranes were suspended in deionized water to concentrations of 5–10 mg protein/ml and stored at —20 °C. Fatty acid analysis of membrane lipids was performed as described elsewhere[4]. Generally, between 60–70 % of the fatty acid residues correspond to the supplement present in the media, although occasional preparations of membranes from stearic acid supplemented cultures contained only 40 % stearic residues.

Turbidimetric studies were performed as previously described[3]. In this procedure, a sample of the membranes containing 1–2 mg protein is dispersed in 10 ml of medium. For most experiments the medium was 0.025 M Tris buffer, pH 7.4, with salts added as indicated. Turbidity was measured with a Brice-Phoenix light scattering photometer. This apparatus was equipped with a heating stage through which water from a temperature controlled bath was pumped. A magnetic stirrer was mounted so as to rotate a small stirring pellet in the light scattering cell. Using 546 nm light, the intensities of the direct beam ($I_{0°}$), and the scattered light at 90° ($I_{90°}$) and at 45° ($I_{45°}$) were measured. The temperature of the system was measured by a thermistor probe in the sample. In the course of an experiment the temperature of the system was changed in one-half or one degree intervals, then maintained at that temperature for 3–5 min before measurements were made of the light intensities.

RESULTS

When membranes of *Acholeplasma* with lipids enriched in palmitic acid residues are heated, a continuous decrease in turbidity is observed from 10 to 38–40 °C. Following this decrease, the turbidity remains essentially constant in the temperature range from 40–44 °C (Fig. 1, Curve I and Fig. 2, Curve I). If the sample is cooled, the turbidity increases, showing changes in slope similar to the heating portion of the experiment, although at temperatures below 31 °C the turbidity reached is somewhat less than in the preceding heating sequence. In most instances each repeated heating cycle results in decreased turbidity values. The light scattered at 45° ($I_{45°}$) confirmed the changes in turbidity measured by the ratio $I_{90°}/I_{0°}$.

In a similar experiment, membranes of *Acholeplasma* enriched in stearic acid residues showed a rapid decrease in turbidity from 27 to 42 °C, followed by a constant range from 42 to 47 °C. Continued heating beyond 47 to 50 °C produced a sharp increase in turbidity. When this system was cooled, the turbidity values increased in a continuous manner giving higher readings than in the heating cycle. In another experiment with the same type of membranes the heating cycle was terminated at 46 rather than 50 °C. On cooling the turbidity changes paralleled the heating curve more closely than the sample that had been brought to 50 °C. The increased turbidity

that arises above 47 °C may be the result of irreversible changes in the proteins in the membrane structure.

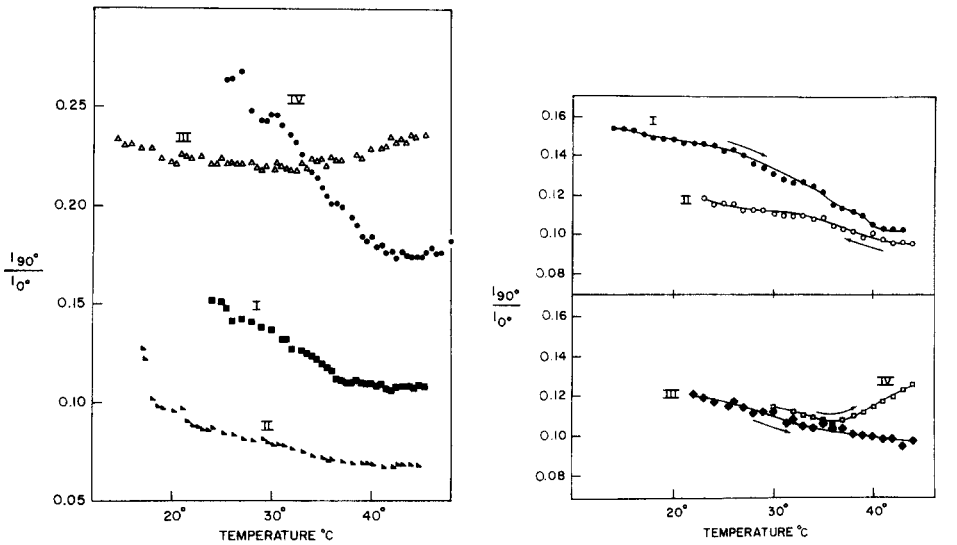

Fig. 1. Changes in turbidity on heating aqueous systems of *Acholeplasma laidlawii*. Each system of membranes containing 1–2 mg protein or 0.8–1.0 mg lipid extract was heated in 10 ml Tris buffer 0.025 M, pH 7.4. I, membrane enriched with palmitate; II, lipid extract of palmitate-enriched membranes; III, oleate-enriched membranes; IV, stearate-enriched membranes.

Fig. 2. A series of experiments with palmitate-enriched membranes. I and II, heating and cooling in buffer alone; III, reheating in buffer + 0.06 M NaCl; IV, reheating in buffer + 0.06 M NaCl and 3 mM $CaCl_2$.

Oleic acid enriched membranes as shown in Curve III, Fig. 1, exhibited a different behavior on heating; showing only a small decrease in turbidity from 14 to 20 °C. From 20 to 38 °C very little change occurred, followed by a small increase in turbidity from 38 to 45 °C.

To study the characteristics of the lipid components alone, the lipids were isolated by two cycles of extraction with chloroform–methanol (2:1, by vol.) from membranes of *Acholeplasma* grown in palmitic acid-enriched medium. The dried lipids obtained (0.8 mg) were used for a thermal study by first dispersing in 5 ml of water by gentle ultrasonic radiation and then diluting to 10 ml with Tris buffer (pH 7.4). As seen in Curve II, Fig. 1, although the turbidity values of the lipid dispersions are lower than for the membranes, their thermal changes follow a pattern similar to that shown by the whole membranes. At 17–18 °C a sharp drop in turbidity accompanies the early stages in the hydration of the lipids. A further decrease in turbidity takes place on heating until 37 °C with no further change observed when heating is continued to 44 °C.

Experiments were performed to investigate whether any changes in the chemical characteristics of the membrane arose as a consequence of the thermal transition. In the present series, various salt solutions were added to dispersions of palmitate enriched membranes and the thermal transitions were observed. Fig. 2 shows a series of consecutive turbidimetric measurements made with the same sample of palmitate-

enriched membranes. These membranes were first heated in dilute phosphate buffer (Curve I). After cooling from 44 to 22 °C (Curve II), the system was brought to 0.06 M in NaCl and reheated as shown in Curve III. Both of these heating and cooling cycles exhibited the typical turbidity changes. After cooling again to 30 °C, $CaCl_2$ was added to bring the concentration to 3 mM. On heating now (Curve IV) the turbidity decreased to 36–37 °C, then a sharp increase in turbidity ensued followed by flocculation at the temperature at which the hydrocarbons have completed the transition to the fluid form. Similar experiments in Tris buffer gave like results in 3 mM $CaCl_2$ indicating that the presence of inorganic phosphate was not necessary for the increase in turbidity observed. Membranes enriched with oleic acid residues exhibited a different behavior. When $CaCl_2$ (3 mM) was added to this system at 17 °C an increase in turbidity resulted. On heating now, the turbidities showed small changes similar to the changes observed with oleate-enriched membranes in the absence of Ca^{2+}.

DISCUSSION

The results presented here suggest that the thermotropic gel–liquid crystal phase transition of the hydrocarbons of membrane lipids can be detected by measurements of the turbidity of membrane suspensions. Evidence that indicates that this transition is responsible for the decrease in turbidity observed can be summarized as follows: (1) Membranes enriched in palmitic residues and stearate residues show the temperature-dependent decrease in turbidity while membranes enriched with oleate residues do not. This result is in agreement with studies showing that membranes with lipids enriched with long-chain saturated fatty acid shows a broad transition terminating in a range above 35 °C while membranes enriched with the corresponding unsaturated fatty acids have a transition near 0 °C. (2) The isolated lipids from enriched membranes show similar changes in turbidity in the same temperature ranges as the whole membranes. In explaining the change in turbidity of the suspensions of membranes or membrane lipids several possibilities can be considered. There may be a change in the refractive index of lipids associated with the increased fluidity at the higher temperature. Cherry and Chapman[5] suggested that at least for dioleoyl lecithin, the refractive index of the fully hydrated lipid is lower than for the lipid alone. There is no data available on this point for lipids of *Acholeplasma*. Some other studies, however, are suggestive of the manner in which the refractive indices of the membranes could change. Barer *et al.*[6] studied the refractive index of a variety of cells and parts of cells and found that in the case of amoeba in active movement, changes in the refractive index of the pseudopodium occur which indicates an increase in the water content of this region. Similarly, a decrease in the turbidity of the membranes we studied could result from changes in the refractive index that accompanies an increase in the hydration of the polar heads of the lipids. This possibility has been raised in the previous work on phospholipids[7] and is supported by X-ray diffractions studies of *Acholeplasma* membranes[2] which showed an increase in the spacing of the hydrocarbon chains above the transition temperature. This increase in spacing could result in a greater exposure of the lipids to the aqueous medium with increased hydration. An alternate explanation for the changes observed in the turbidity of the membranes enriched in saturated fatty acids is a change of the

shape of the whole membrane as a consequence of (1) the increase in the fluidity of the hydrocarbon chains and (2) a rearrangement of other membrane components, specifically the proteins. The rearrangement could be a consequence of the change in the spacing of polar head groups as well as a decrease in the thickness of the bilayer. These changes within the bilayer are analogous to the changes that accompany the thermal transitions of phospholipids.

Finally, the studies on the effect of calcium salts suggest that the gel–liquid crystal transition alters the accessibility of some polar groups to the aqueous surroundings. X-ray diffraction studies of brain lipids in water[8] showed that the presence of Ca^{2+} results in a sharp decrease in the water between lipid layers. A recent study of the thermal transitions in dipalmitoyl lecithin–water systems gives further insight into the chemical changes that are associated with these transitions. Using a fluorrescent probe to indicate the changes in the polar groups at the lipid surface, Trauble[9] showed that above the transition temperature, Ca^{2+} was bound more strongly than below the transition. Further, the effect with Ca^{2+} was much greater than with univalent salts. These changes are interpreted as resulting from structural changes in the lipid which make ionic groups available for reaction at the lipid water interface. It is possible that a similar interaction takes place with the lipids within the *Acholeplasma* membranes. Additionally, Ca^{2+} can produce an aggregation of membrane particles.

These results suggest that measurements of thermal turbidity may be a useful means for studying the interaction of salts or membrane acting agents with intact membranes as well as with their lipid dispersions.

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