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CALORIMETRIC STUDIES OF LIPID PHASE TRANSITIONS IN NATIVE AND HEAT-DENATURED MEMBRANES OF BEEF HEART SUBMITOCHONDRIAL PARTICLES

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Summary

The lipids in beef heart submitochondrial particles undergo a broad reversible endothermic phase change centered at about −10°C. Following protein denaturation, a new reversible transition centered at about 20°C appears. The extracted lipids from these membranes exhibit thermal behavior that is essentially identical to the lipid transition in the intact membrane after protein denaturation. A role for this latent pool of higher-melting lipids is proposed.

Low-temperature gel-liquid crystal phase changes have been observed in the lipids of mitochondrial membranes from rat liver [1] and beef heart [2,3]. The free lipids are therefore extremely fluid at physiological temperature. Kinetic studies of mitochondrial enzyme activities [4,5] suggest, however, that large changes in activation energy occur in these systems between 0°C and 37°C. These changes in activation energy have often been attributed to transitions in the membrane lipids as detected by ESR spectroscopy [6—8].

We now present differential scanning calorimetric evidence for the existence of a latent pool of higher-melting lipids in beef heart mitochondrial inner membranes that is not in the free bilayer in the native state but is apparently released following protein denaturation.

Beef heart mitochondria were isolated and submitochondrial particles were prepared by sonic oscillation using a Branson Model 200 Sonifier [9]. Prior to sonication, the mitochondrial suspension was adjusted to 2 mM EDTA and saturated with nitrogen gas. The submitochondrial particles were

suspended in 50% glycerol at a protein concentration of 20 mg/ml and stored at −80°C. Protein was determined by using the Bio-Rad [10] method. Lipids were extracted under a nitrogen atmosphere according to the method of Bligh and Dyer [11]. Lipids were stored in chloroform solution in sealed ampules at −20°C.

Calorimetry was performed on a Perkin-Elmer DSC-2 differential scanning calorimeter modified for low-temperature operation. Samples were contained in 75 μl stainless-steel pans (Perkin-Elmer). Sephadex G-200 (Pharmacia) was used to prevent convection in the reference cell. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed as described by Weber and Osborn [12].

The lipids of beef heart submitochondrial particles undergo a broad thermotropic phase change between −15°C and 10°C (Fig. 1a). This transition is completely reversible; the sample can be heated and cooled repeatedly over the −40 to 30°C range with no significant alteration in the thermogram (Fig. 1b). These results are in general accord with previous calorimetric experiments [3]. Fig. 1c shows a thermogram over the −40 to 100°C interval which includes both the reversible lipid transition and a set of higher-temperature peaks corresponding to irreversible protein denaturation. The sample was then cooled immediately and rescanned (Fig. 1d). The appearance of the lipid transition is noticeably altered in comparison to the native state. Three major differences are that (a) the shape of the main lipid transition peak is changed, (b) the apparent enthalpy of the lipid transition decreases to approx. 60% of its original value, and (c) a new transition centered at about 20°C

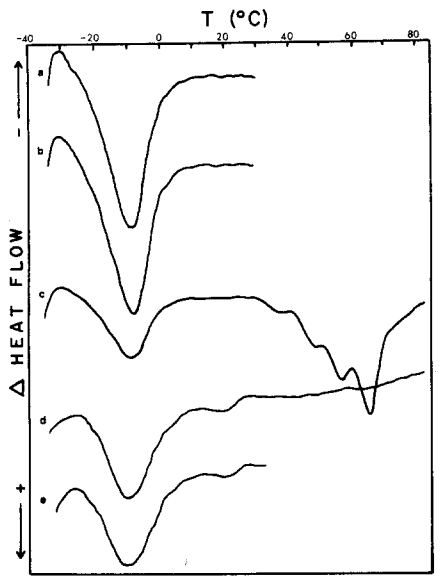

Fig. 1. Thermograms of submitochondrial particles in 50 mM 1,4-piperazinediethanesulfonic acid, pH 7.5, 50% ethylene glycol (Buffer) at a scanning rate of 5 K/min and a range of 0.2 mcal/s (in c, the range is 0.5 mcal/s). Five consecutive scans of a 75 mg sample were performed as follows: (a) first scan (−40 to 30°C); (b) second scan (−40 to 30°C); (c) third scan (−40 to 100°C); (d) fourth scan (−40 to 100°C); (e) fifth scan (−40 to 30°C). The temperature was quickly lowered −40°C following each scan and the succeeding thermogram was obtained as soon as thermal equilibration at −40°C was achieved.

appears. Like the lipid transition in the native membrane, the thermal behavior of the sample following denaturation is reproducible (Fig. 1e). The large decrease in the enthalpy of transition following protein denaturation may be attributable at least in part to the inability to obtain a true baseline on the low-temperature side of the main lipid peak. The inclusion of 50% ethylene glycol in the sample provides a freezing-point depression about 40 K. If the lipid transition is significantly broadened after denaturation, its apparent relative size might decrease. Alternatively, a larger fraction of the lipids may bind to the thermally disrupted membrane proteins, thereby reducing the pool of lipids in the free state.

Since the protein components of beef heart submitochondrial particles have been shown to aggregate at low temperatures [3,13], the possibility of lipid phase separations was investigated. Samples of native membranes incubated at −40°C for up to 24 h show no change in the appearance of the lipid transition. Denatured membranes similarly treated, however, exhibit a striking alteration in the lipid melt (Fig. 2), which is resolved into three components. When the scan is repeated immediately after cooling to −40°C, its appearance once again returns to its original form.

In an attempt to determine the origin of the higher-temperature reversible endotherm, dispersions of the extracted membrane lipids were analyzed calorimetrically (Fig. 3). The phase change in the extracted lipids is virtually

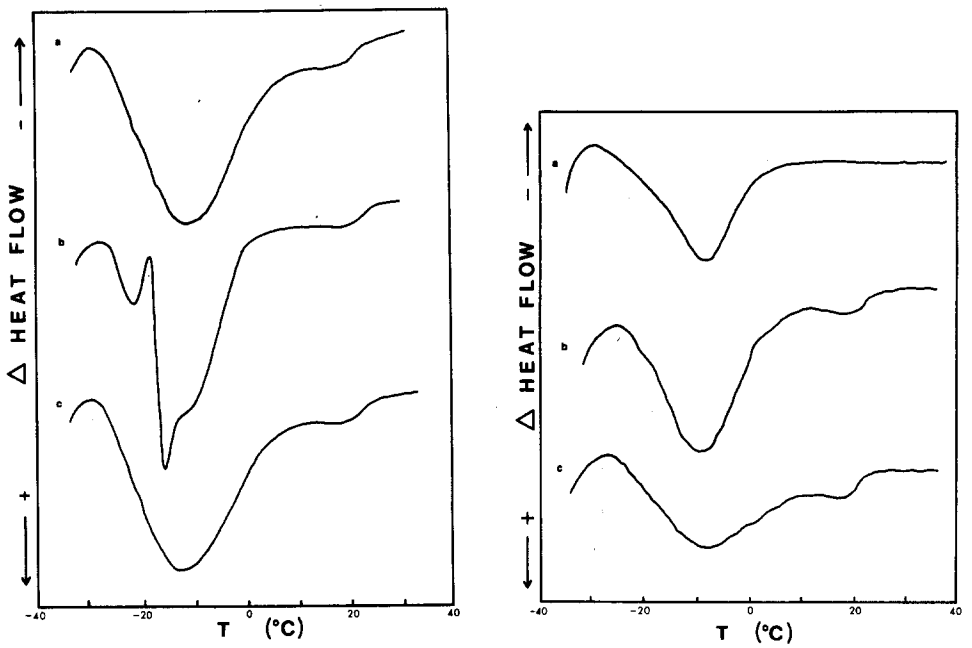

Fig. 2. Phase separations observed in a single sample of denatured submitochondrial particles in Buffer at a scanning rate of 5 K/min and a range of 0.2 mcal/s. The sample was first incubated at −40°C for 5 min prior to scanning (a), then incubated at −40°C for 12 h prior to scanning (b), and finally rescanned immediately following thermal equilibration at −40°C (c).

Fig. 3. Comparison of the lipid transitions of membranes and lipid extracts suspended in Buffer at a scanning rate of 5 K/min, as follows: (a) native membranes (range = 0.5 mcal/s); (b) denatured membranes (range = 0.2 mcal/s); (c) extracted lipids (range = 0.2 mcal/s).

the same as that observed in the denatured membrane. When subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, the extracted lipids showed a very small amount of low molecular weight protein contamination (about 1—2 wt%).

Changes in the slope of Arrhenius plots (often referred to as 'breaks') have been observed for a multitude of membrane-bound enzymes. One such system is mitochondrial succinate oxidase, which was shown to undergo a large change in activation energy from 16.5 kcal/mol below 23°C to 4.2 kcal/mol above that temperature [4]. This break temperature coincided with a change in the molecular motion of a nitroxide-labeled fatty acid in the mitochondrial membrane which was interpreted as a phase change in the bulk lipids [6]. More recently, two breaks were observed in Arrhenius analyses of both succinate oxidase activity and the ESR motional parameter of a fatty acid spin label; the break temperatures (8 and 24°C) were correlated with the lower and upper limits, respectively, of a broader phase change in the mitochondrial membrane lipids [7].

The relationship between Arrhenius plot breaks in mitochondrial membrane-bound enzymes and the gel-liquid crystal phase transition in the bulk lipids is rather tenuous since most direct physical measurements of mitochondrial lipids indicate that they are extremely fluid at temperatures above 10—15°C [1—3]. It has been suggested, moreover, that studies of the temperature dependence of the behavior of nitroxide spin probes in lipid and membrane systems may sometimes lead to Arrhenius plot breaks despite continuous changes in the rate of probe motion and ordering [14]. Mitochondrial membranes from rat brown adipose tissue studied using a variety of spin probes showed no discontinuities in Arrhenius analyses of molecular motion or partitioning [15] in spite of the presence of breaks in respiratory activity and anion transport in these membranes [16].

It is therefore difficult to rationalize the involvement of phase changes in the free lipids in the bilayer with the sharp changes in activation energy which occur at temperatures above the gel-liquid crystal phase transition in the native membrane. Our results indicate that there is a small pool of lipids in beef heart inner mitochondrial membranes which may play a role in the temperature dependence of the activity of certain membrane-bound enzymes. This pool of lipids, observed calorimetrically in both the denatured membranes and extracted lipids, melts over a temperature interval which encompasses the break temperatures of succinate oxidase, ATPase, succinate: cytochrome *c* reductase and β-hydroxybutyrate dehydrogenase [5].

A possible explanation for the latency of this lipid transition is that the small pool of higher-melting lipids might preferentially associate with native proteins but is released following denaturation. The appearance of the higher-temperature phase change in the extracted lipids as well as in the denatured membranes strongly suggests that this latent transition probably arises from lipids rather than from denatured protein or lipid-protein interactions.

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