THERMAL ANALYSIS OF SARCOPLASMIC RETICULUM MEMBRANES

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1. Introduction

The Ca^{2+} transport and ATPase activity of sarcoplasmic reticulum are dependent upon membrane phospholipids [1]. Marked transitions in the rates of these processes occur around 20°C [2] which are also reflected in the temperature dependence of the spectral characteristics of lipophilic spin-labeled probes and protein-bound spin labels [2]. These changes were attributed to cooperative transitions in the alkyl-chain regions of membrane lipids and it was proposed that the activity of the Ca^{2+} transport ATPase is limited by the local viscosity of the membrane below the transition temperature.

We investigated the thermal transition properties of sarcoplasmic reticulum membranes and isolated membrane phospholipids by differential scanning calorimetry [3]. A broad endothermic transition was detected in freeze-dried rabbit sarcoplasmic reticulum membranes which is attributable to membrane lipids. The maximum of the transition was at 14–16°C. Its magnitude increased after denaturation of membrane proteins by heat treatment, but was unaffected by selective extraction of cholesterol. Addition of water to sarcoplasmic membranes or to isolated phospholipids caused a shift of the transition to lower temperatures.

Materials and methods

The measurements were performed with a Du Pont 990 differential scanning calorimeter in the temperature range of –40°C to 100°C. The instrument was operated in the calibrated mode at an effective sensitivity of 0.01–0.2 mcal/sec/inch, using a heating rate of 2°C/min. The samples of sarcoplasmic reticulum membranes (5.0–5.3 mg dry weight) or membrane phospholipids (1–2.5 mg dry weight) were encapsulated in hermetically sealed aluminum sample pans. Fragmented sarcoplasmic reticulum membranes were prepared from rabbit skeletal muscles as described earlier [4,5]. After a final washing with water the preparations were lyophilized and stored at –20°C. Freeze drying of sarcoplasmic reticulum membranes leaves the Ca^{2+} transport and ATPase activity unaffected. Microsomal lipids were extracted according to Folch [6]. For the removal of cholesterol the dry sarcoplasmic reticulum membranes were extracted with ethyl ether at 4°C [7]. ATPase activity and Ca^{2+} transport were measured as described earlier [8].

2. Results and discussion

Upon heating of freeze-dried sarcoplasmic reticulum membranes from –40°C to 100°C, broad endothermic transitions were observed with maxima at 15°, 35°, 52°, 78° and 90°C (fig. 1A). The transition at 15°C may be attributed to lipids as isolated sarcoplasmic reticulum phospholipids used in amounts similar to those present in sarcoplasmic reticulum membranes gave a single broad transition at 15°C (fig. 1C) which had, however, significantly greater transition enthalpy than that obtained with sarcoplasmic reticulum membranes. The approximate value of ΔH for sarcoplasmic reticulum was 0.23 mcal/mg dry weight and for phospholipids, 2.5 mcal/mg. The latter value is reasonably close to data in the literature [9,10].

After cooling the samples to –40°C, the scan was repeated (fig. 1B). The magnitude of the endothermic heat flow obtained with sarcoplasmic reticulum around 15°C was significantly greater in the second scan

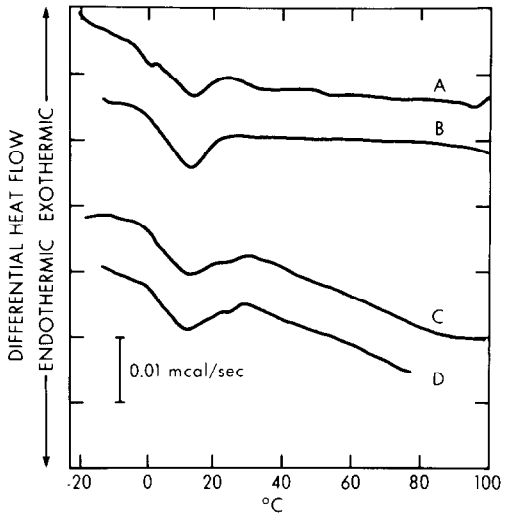

Fig. 1. Thermal transitions in sarcoplasmic reticulum membranes and isolated phospholipids. (A) Freeze-dried membranes (5.01 mg), first scan. (B) Freeze-dried membranes (5.01 mg), second scan. (C) Microsomal phospholipid extract (1.01 mg), first scan. (D) Microsomal phospholipid extract (1.01 mg), second scan.

($\Delta H \simeq 0.43$ mcal/mg) than in the first scan (0.23 mcal/mg), and did not change during repeated measurements. Considering that sarcoplasmic reticulum membranes contain about 0.4 mg phospholipid per mg protein, the value of ΔH obtained in the second scan corresponds to 1.8–2.0 mcal/mg phospholipid, i.e., it is close to the value of ΔH=2.5 mcal/mg obtained with isolated phospholipids. The transitions observed in sarcoplasmic membranes during the first scan at 35°, 52°, 78° and 90°C (fig. 1A) were usually absent in the second and subsequent scans (fig. 1B). They are tentatively attributed to endothermic changes connected with the heat denaturation of membrane proteins.

With several sarcoplasmic reticulum preparations the endothermic peak at 15°C was much less noticeable in the first scan than in fig. 1A, but even in these preparations it reached its full magnitude, as compared with isolated membrane phospholipids, in the second or third scans. Exposure of the samples to 60–100°C is necessary to induce this change; heating to 40°C is ineffective.

These observations indicate that only a fraction (perhaps less than half) of the phospholipid content of native sarcoplasmic reticulum membranes participates in the endothermic transition at 15°C (fig. 1A). The remaining phospholipids are in a specially organized state, presumably bound to proteins. We suggest that after heat denaturation of membrane proteins (fig. 1B) these phospholipids are released, as the magnitude of the endothermic transition now indicates the participation of most of the membrane phospholipids.

The transition enthalpy of dimyristoyl L-α-lecithin and dipalmitoyl L-α-lecithin decreases with increasing cholesterol content [11,12] and vanishes at a cholesterol to phospholipid ratio of 1:2 [11]. As sarcoplasmic reticulum membrane preparations contain a small amount of cholesterol [13], its contribution to the magnitude of the observed transitions was investigated. Extraction of sarcoplasmic reticulum membranes with ethylether selectively removes 75–80% of the cholesterol without major change in phospholipid content or ATPase activity [7]. Ether extraction caused no significant change or only a slight decrease in the magnitude or location of the principal transitions. This is not surprising since the cholesterol to phospholipid mole ratio in sarcoplasmic reticulum membrane preparations is only about 1:10 and most of the cholesterol may be present in contaminating surface membrane fragments.

Effect of water. Addition of water to sarcoplasmic reticulum membranes (fig. 2) or to isolated phospholipids (not shown) causes a shift of the phospholipid transition to lower temperatures. Even with water content as high as 10–15% the endothermic peak at 0°C corresponding to free water was not observed (fig. 2B,C) indicating that the sarcoplasmic reticulum membranes are able to bind this amount of water. A major peak due to the melting of ice appeared in samples containing 20% water (fig. 2D).

The endothermic transition in membrane phospholipids is shifted from 15°C to about 4°C in the presence of 10–15% water and becomes partly obscured by the melting of ice when the water content is raised to 20%. Similar data were obtained with isolated microsomal phospholipids.

The thermal transition temperatures obtained with sarcoplasmic reticulum membranes in the presence of water do not correspond to the temperatures at which discontinuities occur in the Arrhenius plots of enzymatic activity or in the empirical motion parameters of lipophilic spin labels [2]. While the conditions of these experiments are sufficiently different to

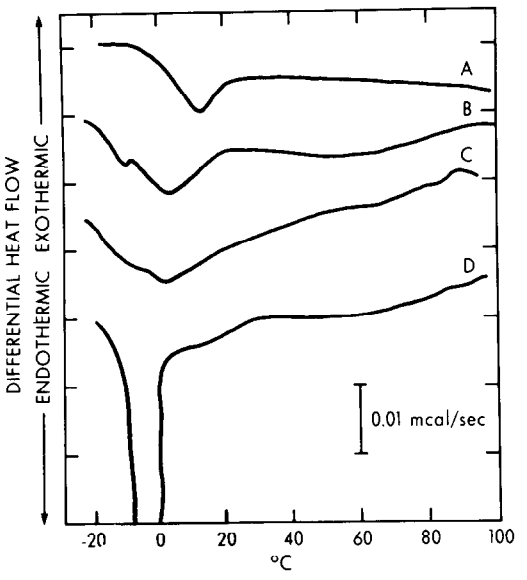

Fig. 2. Effect of water upon the thermal transitions in freeze-dried sarcoplasmic reticulum membranes. (A) Membranes (5.01 mg). (B) Membranes (4.54 mg) and 0.5 μl water. (C) Membranes (4.21 mg) and 0.7 μl water. (D) Membranes (4.02 mg) and 1.0 μl water. After addition of water, the pans were sealed and the samples kept overnight at 4°C. Second scans are represented.

prevent detailed comparisons, the thermal data suggest that above 10°C in the presence of water the phospholipids of sarcoplasmic reticulum are in a liquid-crystalline state. Therefore the observed discontinuities in the temperature dependence of enzymatic activity around 20°C probably do not reflect the thermotropic transition of membrane lipids from the gel phase to the liquid-crystalline phase.

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References

[1] Martonosi, A. (1972) Current Topics in Membranes and Transport (F. Bronner and A. Kleinzeller, eds.) Vol. 3, pp. 83–197, Academic Press, New York.
[2] Inesi, G., Millman, M. and Eletr, S. (1973) J. Mol. Biol. 81, 483–504.
[3] Sturtevant, J. M. (1974) Ann. Rev. Biophys. Bioeng. 3, 35–51.
[4] Martonosi, A., Donley, J. R. and Halpin, R. A. (1968) J. Biol. Chem. 243, 61–70.
[5] Boland, R., Martonosi, A. and Tillack, T. (1974) J. Biol. Chem. 249, 612–623.
[6] Folch, J., Lees, M. and Sloane Stanley, G. H., (1957) J. Biol. Chem. 226, 497–509.
[7] Drabikowski, W., Sarzala, M. G., Wroniszewska, A., Lagwinska, E. and Drzewiecka, B. (1972) Biochim. Biophys. Acta 274, 158–170.
[8] Martonosi, A., Donley, J. R., Pucell, A. G. and Halpin, R. A. (1971) Arch. Biochem. Biophys. 144, 529–540.
[9] Chapman, D. and Urbina, J. (1971) FEBS Letters 12, 169–172.
[10] Reinert, J. C. and Steim, J. M. (1970) Science 168, 1580–1582.
[11] Hinz, H. J. and Sturtevant, J. M. (1972) J. Biol. Chem. 247, 3697–3700.
[12] Ladbrook, B. D., Williams, R. M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340.
[13] Martonosi, A. (1968) Biochim. Biophys. Acta 150, 694–704.