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Phase transitions of *Acholeplasma laidlawii* membranes

The involvement of Mg^{2+}-ATPase in the C transition

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Highly sensitive differential scanning calorimetry has been employed to study the phase transitions of *A. laidlawii* membrane. The DSC curves obtained show five distinct transitions between 20 and 80°C which contain a reversible lipid thermotropic transition at about 37°C and four irreversible denaturation transitions of the membrane proteins occurred at about 44°C, 52°C, 62°C and 67°C, respectively. Total enthalpy of the thermal denaturation of membrane proteins is 3.4 ± 0.5 cal/g. Further study of *A. laidlawii* membrane preparations by means of thermal gel analysis and enzyme acitvity measurements at various temperatures provided information that the third peak (C transition) of the DSC curve involved primarity with Mg^{2+}-ATPase on *A. laidlawii* membranes.

A. laidlawii membrane; Mg^{2+}-ATPase; DSC

1. INTRODUCTION

Mycoplasma laidlawii membrane as a convenient model for biomembrane has been studied by calorimetry. It has been confirmed that the membrane lipids in living *Mycoplasma laidlawii* exhibit a phase transition characteristic of that from crystal to liquid crystal within the bilayer conformation [1]. Recently, with the advent of highly sensitive differential scanning calorimetry, it has been possible to monitor directly the thermotropic properties of individual components within heterogeneous biological membrane [2], DSC was also used to study ligand–protein interaction [3], local anesthetic effects on F_1-ATPase and submitochondrial particles [4] and conformation shifts of proteins [5,6]. However, calorimetry in itself is not extremely useful in identifying particular membrane components that are involved in structure transition. A method, thermal gel analysis (TGA), has been developed and the application of this technique to the transition of erythrocyte ghosts gives further information on the participation of specific proteins in these transitions [7]. In other words, the temperature which corresponds to the loss of the gel area ($T_{1/2}$) for a particular protein band should be identical with the midpoint (T_m) of the calorimetric transition of the proteins which are actually a participant in the transition.

Although lipid–protein interactions of *A. laidlawii* membrane have been studied [8,9], a calorimetric study of *A. laidlawii* membrane with sufficient resolution to identify thermotropic transition of proteins has not been made. In the present paper, measurements of the endothermic transition of *A. laidlawii* membrane are presented by highly sensitive DSC. The results suggest that the Mg^{2+}-ATPase of *A. laidlawii* membrane is participant in the C transition.

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2. MATERIALS AND METHODS

2.1. *Materials*

A. laidlawii A1H089 was obtained from the Institute of Husbandry and Veterinary Medicine, Jiangsu Academy of Agricultural Science. The growth, harvest and lysis of cells and isolation of membrane were referred to the previous paper [10]. All reagents were of analytical grade.

2.2. *Calorimetry*

Experiments were performed with a microccal MC-2 differential scanning calorimeter operating at a scanning rate 0.5°C/min. The buffer used in scanning calorimetry experiments was 50 mM Tris/0.15 M NaCl/10 mM β-mercaptoethanol (pH 7.4). A 1.21 ml aliquot of the suspension of *A. laidlawii* membranes at a concentration of 4 mg/ml was added to the sample cell, and an equal volume of buffer was added to the reference cell. The samples were carefully degassed before the calorimeter was loaded.

2.3. *Thermal gel analysis*

Individual aliquots of membrane sample in above buffer (2 mg/ml) were heated at designing temperature for 10 min. The samples were solubilized by the addition of solubilization buffer: 2% Triton X-100, 0.1% deoxycholate and 10% glycerol (final concentration). After incubation at 37°C for 15 min, the samples were electrophoresed on 0.1% Triton X-100 3–8% polyacrylamide gradient gels [10]. The gels were stained by Coomassie blue and scanned by Hitachi CS-910 at 590 nm. The peak areas for each band were determined with a planimeter, and the areas were normalized with respect to the 38°C sample. The normalized area was then plotted vs. temperature to obtain the curves from which the thermal midpoint ($T_{1/2}$) was determined [7].

2.4. *Determination of enzyme activity*

The suspension of *A. laidlawii* membrane (2 mg/ml) was incubated at a rate close to that of calorimeter in water bath. 50 μl of the sample was removed at various temperatures and cooled to room temperature. The Mg^{2+}-ATPase activity was measured according to the procedure of Pollack et al. [11].

The protein concentration was estimated by the method of Lowry et al. [12], using bovine serum albumin as the standard.

3. RESULTS AND DISCUSSION

Highly sensitive differential scanning calorimetry has been used as a useful tool in the study of the structure and phase behavior of biological membrane [13,14]. Earlier studies have shown that for the plasma membranes of *Mycoplasma laidlawii*, two thermal transitions were observed, a reversible transition attributed to membrane lipids and an irreversible transition at higher temperature attributed to denaturation of the membrane protein [1]. But, the heat capacity curves were dominated by a reversible lipid phase transition making it difficult to study the contribution from the protein components. Under our experimental conditions, five distinct thermal transitions of *A. laidlawii* membrane are observed (Fig. 1a). A transition with T_m of 37 ± 1°C is reversible, checked by reheating the sample after it had been cooled to room temperature in the calorimeter and is known as crystalline to liquid-crystalline phase transition of the membrane lipids (Fig. 1b). This T_m is similar to the value reported for rat intestinal plasma membrane [13]. The broad transition of membrane lipid may be caused by complex mixtures of lipids with different transition temperature. In contrast to the endothermic transition of membrane lipids, the higher temperature transitions are irreversible and occur at 44 ± 1°C (B), 52 ± 1°C (C), 62 ± 1°C (D) and 67 ± 1°C (E), respectively (Fig. 1). The protein denaturations gave broad transitions with native membrane. It seems likely that each of the calorimetrically observed transitions represents the denaturation of more than one type of protein molecule. C transition in calorimetry involved primarily with the Mg^{2+}-ATPase of *A. laidlawii* membranes (see below). The total enthalpy of the denaturation endotherm, 3.4 ± 0.5 cal/g of membrane protein, is lower than the value reported for submitochondrial particles, 5 ± 1 cal/g of SMP protein [4], is similar to 3–4 cal/g of membrane protein of erythrocyte membrane [15].

It is of interest to determine whether the calorimetric transitions can be associated with particular protein. The thermal gel analysis method which is capable of providing detailed information on the participation of proteins in various cooperative structural changes occurring in the membranes has been employed [7]. In a prior study, the *A. laidlawii* membranes were solubilized in 2% Triton X-100 followed the samples were electrophoresed on 0.1% Triton X-100 polyacrylamide gradient gels [10]. Shown in Fig. 2 are gel scans taken from the identical sample which had been heated to the designated temperature. The band 1 remains unchanged up to a temperature of 48°C. After that it decreases quickly from the gel and disappears near 65°C. A plot of the relative area of band 1 against temperature is given in Fig. 3. It shows the temperature which corresponds to the 50% loss of the gel area ($T_{1/2}$) due to denaturation of Mg^{2+}-ATPase is 51 ± 1°C. Obviously, it is very close to the T_m of C transition obtained by DSC experiments. We have confirmed by Coomassie blue and ATPase activity staining method that the band 1 in the gel pattern attributed to Mg^{2+}-ATPase and there were no other proteins in the vicinity of the enzyme activity band [10]. From these results, one might predict that the C transition of DSC curve contributed mainly to Mg^{2+}-ATPase of *A. laidlawii* membranes. The thermal denaturation

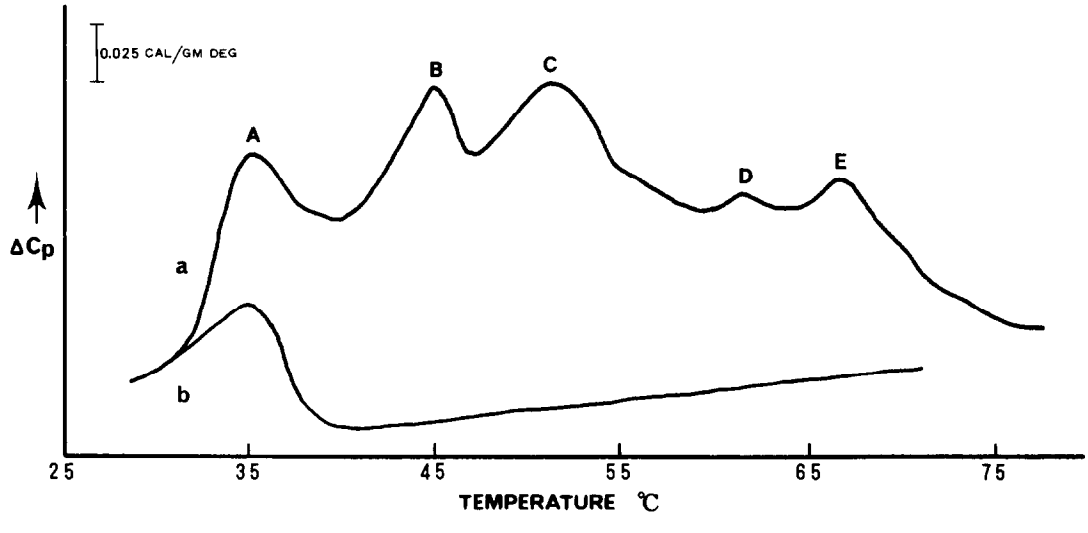

Fig. 1. Differential scanning calorimetry traces of *A. laidlawii* membranes in 50 mM Tris-HCl/0.15 M NaCl/10 mM β-mercaptoethanol, pH 7.4 (a), and second scan of the same membrane preparation after denaturation of protein (b).

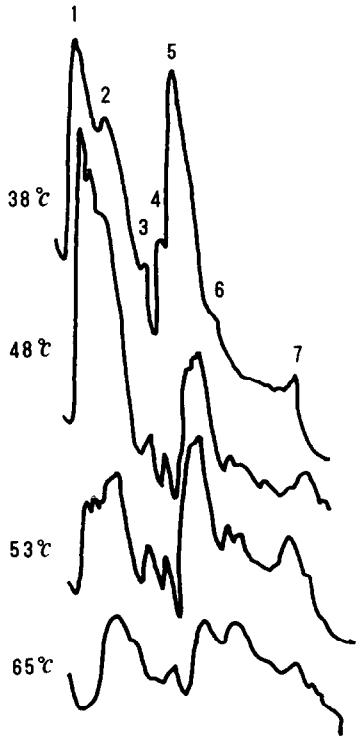

Fig. 2. Polyacrylamide gradient gel electrophoresis of *A. laidlawii* membrane heated to different maximum temperature. The gels were stained by Coomassie blue and scanned at 590 nm. (a) 38°C, (b) 48°C, (c) 53°C, (d) 65°C.

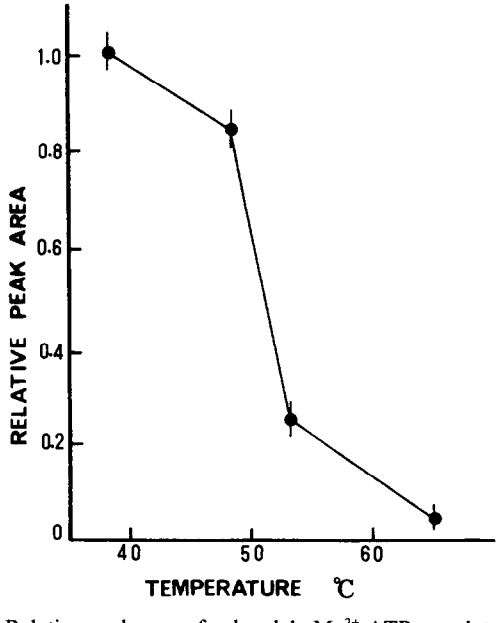

Fig. 3. Relative peak areas for band 1, Mg^{2+}-ATPase, plotted as a function of the maximum temperature to which the membranes were heated. The area of sample at 38°C was taken as 100%.

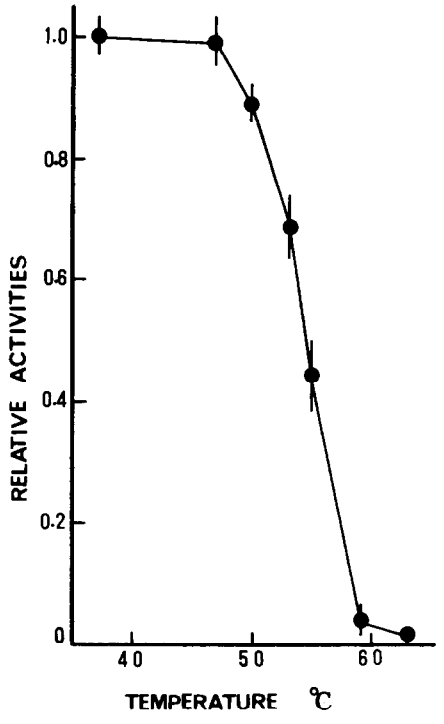

Fig. 4. Relative activities of Mg^{2+}-ATPase of *A. laidlawii* membranes after thermal denaturation at different temperature. The value of enzyme activity at 37°C was taken as 100%.

temperature of Mg^{2+}-ATPase of *A. laidlawii* is lower than that of ATPase of chloroplast membranes and F_1-ATPase prepared from beef heart submitochondrial particles the T_m being 66°C and 80.5°C, respectively [14,16].

Another method called thermal denaturation experiments also gave similar information about the Mg^{2+}-ATPase of *A. laidlawii* membrane might be responsible for the C transition. Fig. 4 showed that temperature (T_D) which lost 50% of the activity of Mg^{2+}-ATPase was 53 ± 1°C, fairly close to $T_{1/2}$ and T_m. The Mg^{2+}-ATPase of *A. laidlawii* membrane has been shown to be tightly associated with the cell membrane [17]. It is an integral protein and depends on membrane lipids for its activities [18]. So, the changes of physical state of the membrane lipids, from an ordered crystalline to a liquid crystalline phase, may have an influence on the activities of membrane-associated Mg^{2+}-ATPase. However, the lipid transition of the native membrane in the present work occurred at lower temperature, between 25–39°C, which is suitable for activities of Mg^{2+}-ATPase of the native membrane. We conclude that the inactivity of the enzyme at higher temperature (T_D) is caused by denaturation of membrane-associated Mg^{2+}-ATPase rather than lipid transition.

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