PRESSURE EFFECT ON THE ARRHENIUS DISCONTINUITY IN Ca^{2+}-ATPase FROM SARCOPLASMIC RETICULUM

Evidence for lipid involvement

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1. Introduction

Ca^{2+}-ATPase from sarcoplasmic reticulum is an intrinsic membrane protein. It is one of the most intensively studied proteins in the field of lipid–protein interactions.

The enzyme shows a nonlinear Arrhenius plot which has been attributed to phenomena related to thermotropic transitions in membrane lipid [1–5].

However, the break at 20°C was also observed for a nearly lipid-free detergent-solubilized Ca^{2+}-ATPase [6]. From physical–chemical studies, the changes in conformation of the protein were concluded to be responsible for the breaks. These studies include: hydrogen exchange [7]; saturation transfer ESR spectroscopy [8,9]; flash photolysis techniques [10].

We have shown that the effect of pressure on the temperature at which the break occurs, presents a critical test for the involvement of lipids in discontinuities of Arrhenius plots [11,12].

Here we show that the discontinuity in the Arrhenius plot of Ca^{2+}-ATPase from sarcoplasmic reticulum is due to a thermotropic transition of lipids.

2. Experimental

Sarcoplasmic reticulum was prepared from rabbit hind leg muscle as in [13]. Ca^{2+}-dependent ATPase activity was measured with the coupled enzyme system as for NaK-ATPase [12]. The reaction mixture contains: 100 mM KCl; 36 IU/ml lactate dehydrogenase; 40 IU/ml pyruvate kinase; 1.5 mM phosphoenolpyruvate; 0.26 mM NADH; 5 mM Na_2ATP; 5.7 mM $MgCl_2$; 5 mM NaN_3; 30 mM imidazole (pH 6.8); 0.1 mM ouabain; 0.87 mM $CaCl_2$; 1 mM EGTA.

Activity under pressure was measured as in [12]. The A_{340} was followed at a preset temperature and pressure as a function of time. The activity of the enzyme is not irreversibly affected by pressure. Infact activities at 1 atm. were always higher after a series of measurements under pressure. The phenomena had no effect on the position of the break in the activity versus pressure profiles.

3. Results and discussion

The activity of Ca^{2+}-ATPase decreases with increasing pressure. A logaritmic plot of the relative change in activity (referred to 1 atm) against pressure is shown in fig.1. The plot is biphasic. The activation volume ($\Delta V^{\neq}$) is obtained from the relation:

$$\frac{d\ln k}{dP} = -\frac{\Delta V^{\neq}}{RT}$$

$\Delta V^{\neq}$ is small (+3.5 ml) below the break and increases above the break. A molecular interpretation of the activation volume cannot be given at present since it is not clear how this would be related to a conformational transition of the enzyme with dephosphorylation [14]. If the pressure-dependent fluidity change of the membrane would be responsible for the activ-

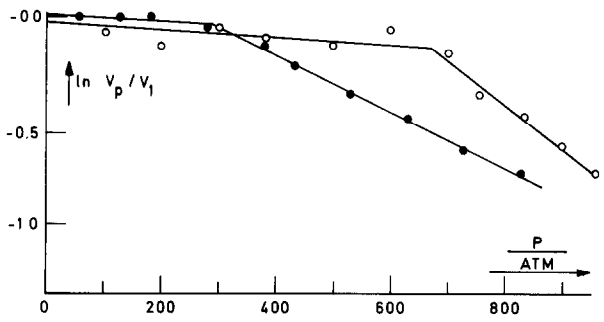

Fig.1. Pressure dependence of the activity of Ca^{2+}-ATPase from sarcoplasmic reticulum. V_p and V_1 are the enzyme activities at pressure P and 1 atm, respectively. Reaction temperature 24.7°C (•) and 35°C (○).

ity change of the enzyme with pressure, one would expect a $\Delta V^{\neq} = +7$ ml from the pressure dependence of the viscosity of *n*-octane [15].

If the experiments are done at lower temperatures (25°C) the pressure at which the break occurs is lowered. If the temperature is increased, the pressure increases. A plot of the transition temperature against the pressure is given in fig.2. The slope $dT/dP = 27$ K/1000 atm. Extrapolation of the break to 1 atm gives 18°C which corresponds to the break point in the Arrhenius plot observed in [1–5]. Similar effects of pressure on the Arrhenius plots have been observed for other systems where phospholipids have been shown to be involved [11,12,16]. This is a first argument in favor of the involvement of lipids in the break of Ca^{2+}-ATPase.

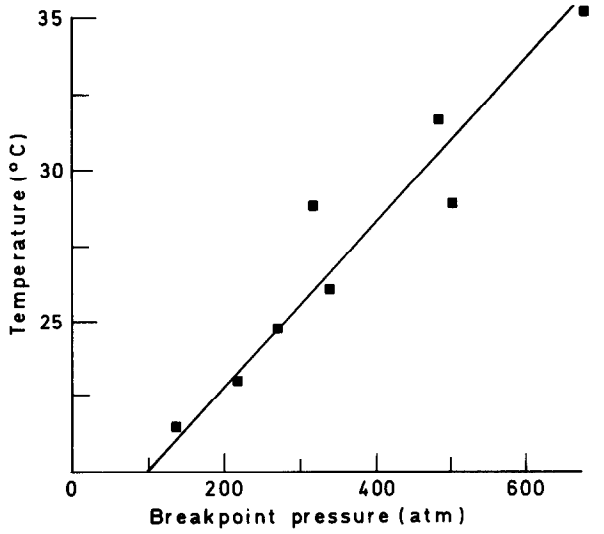

Fig.2. Temperature dependence of the breakpoint pressure as obtained from plots as in fig.1. $dT/dP = 27$ K/1000 atm.

A second argument comes from the pressure dependence of lipid phase transitions and phase separations as measured by several techniques [17–19]. dT/dP for phospholipids range from 17–22 K/1000 atm depending on the chainlength of the fatty acids. This is the range observed for normal hydrocarbons [20]. We can assume that other thermotropic transitions such as the melting of annular lipids or trapped lipids have a similar pressure dependence.

The pressure-dependent conformation changes of proteins, although less documented, are much smaller (2–5 K/1000 atm) [21].

Thus, the pressure effect on the break in the Arrhenius plot, or as reported here, the temperature effect on the break in the ln activity vs pressure plot, provides evidence for a mechanism whereby changes in the physical state of the lipids, trigger changes in the conformation and/or changes in the association of the protein as observed with saturation transfer ESR [8,9] or flash photolysis techniques [10]. Since Arrhenius plots are not related to changes in fluidity of the bulk membrane lipid [22], the simplest interpretation of the observed effect is the existence of a lipid annulus [3].

The fact that breaks are also observed after substitution experiments with detergents [6] can be explained by the observation that a correlation is observed between the melting point of detergents and the break in Arrhenius plots of adenylate cyclase [23].

A more general conclusion from this work is that temperature together with pressure are important parameters for the study of membrane phenomena. More specifically because thermotropic lipid transitions are more sensitive to pressure changes than protein conformational changes are, pressure changes present a useful tool for discrimination between both phenomena in more complex systems such as repetitive generation in crustacean axons [24] and heat activation of fungal spores [25].

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