ARRHENIUS DISCONTINUITIES OF Ca^{2+}-ATPase ACTIVITY ARE UNRELATED TO CHANGES IN MEMBRANE LIPID FLUIDITY OF SARCOPLASMIC RETICULUM

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1. Introduction

The Ca^{2+}-ATPase of sarcoplasmic reticulum is an intrinsic protein which is dependent upon its membrane lipids [1] or suitable detergents [2] for activity. The physical properties of the lipid matrix in which the protein is situated markedly affects enzymic activity [3]. We have shown that modulation of the fluidity of native sarcoplasmic reticulum by incorporating cholesterol into the membrane causes a reduction of enzyme activity in proportion to the amount of cholesterol present [4]. We discounted an irreversible loss of enzyme activity by demonstrating that re-extraction of the cholesterol from the membrane resulted in a corresponding recovery of Ca^{2+}-ATPase activity.

When the non-linear Arrhenius behaviour of Ca^{2+}-ATPase was first reported [5] the discontinuity observed was attributed to a thermotrophic order–disorder phase transition of the membrane lipid. While later workers offered a variety of alternative explanations for this behaviour they were unanimous in attributing it to the lipid component [6–8]. However, essentially the same Arrhenius plot has been obtained for the delipidated, detergent-solubilized enzyme [2]. We have attempted to clarify the role of the membrane lipids, particularly that of membrane fluidity, by observing the effect of assay temperature on Ca^{2+}-ATPase activity for native and cholesterol-enriched sarcoplasmic reticulum membranes.

2. Experimental

Sarcoplasmic reticulum was prepared from rabbit hind leg muscle by the method in [9] and further purified by sucrose density gradient centrifugation [10]. Cholesterol-rich liposomes (molar ratio dipalmitoyllecithin : cholesterol, 1:2) were prepared as in [4] and were incubated with sarcoplasmic reticulum membranes (1.5 mg protein/ml) at 2 mg/ml. The incubation medium consisted of 0.1 M KCl, 5 mM histidine buffer (pH 7.4), 100 U/ml penicillin G and 100 μg/ml streptomycin sulphate. Incubations were carried out in siliconised flasks gassed with nitrogen and gently shaken for 5 h in a waterbath maintained at 20°C. The sarcoplasmic reticulum vesicles and cholesterol-rich liposomes were then separated as in [4]. Calcium-dependent ATPase activities were measured in the presence of an ATP regenerating enzyme system as in [4]. Assay temperatures were determined using a thermocouple inserted into the spectrophotometer cuvette. Diphenylhexatriene was used as a fluorescent probe to report on the properties of the hydrocarbon region of the membrane. The probe was introduced into the sarcoplasmic reticulum vesicles as in [11]. The fluorescence polarisation of diphenylhexatriene in sarcoplasmic reticulum vesicles was determined as a function of temperature using a Perkin-Elmer MPF-44A spectrofluorimeter in the front-face fluorescence mode. Appropriate corrections were made for light scattering and for the polarisation produced by the emission monochromator. Membrane 'microviscosities' were determined from the anisotropy and estimated fluorescence lifetime of the probe at each temperature [12].

3. Results and discussion

The Ca^{2+}-ATPase of rabbit muscle sarcoplasmic reticulum is an example of a membrane-bound enzyme

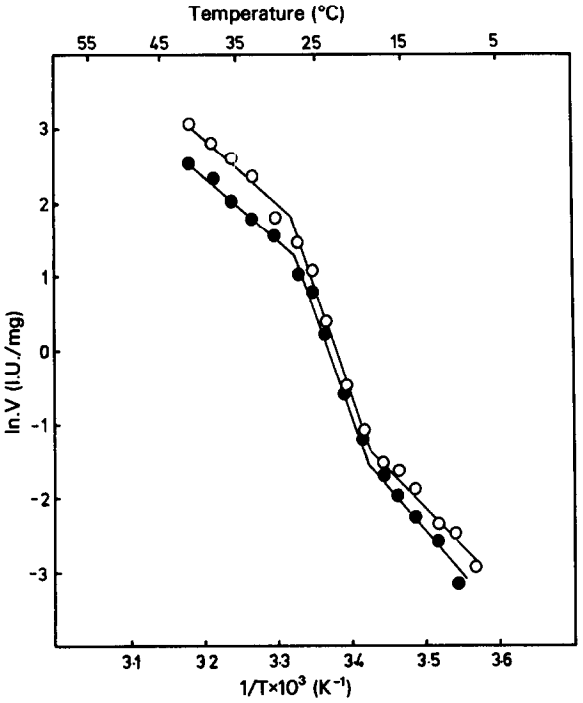

Fig.1. Arrhenius plots of Ca^{2+}-ATPase activities for native sarcoplasmic reticulum (phospholipid:cholesterol molar ratio 20:1) (○); and cholesterol-rich sarcoplasmic reticulum (phospholipid:cholesterol molar ratio 3:1) (●).

possessing non-linear Arrhenius behaviour. A number of researchers have observed a single discontinuity at ~18°C [2,5–8]. The energy of activation above the discontinuity is less than that observed at lower temperatures. Arrhenius plots of Ca^{2+}-ATPase activity in our assay system show two discontinuities (fig.1), one at ~21°C and another at ~15°C. In terms of apparent activation energy the discontinuity we observe at 21°C may correspond to that observed at ~18°C by other workers. The origin of this break has previously been attributed to thermotropic phase transitions of the membrane lipid [5], the formation of lipid clusters [6], a melting of the annular lipids surrounding the enzyme protein [7] or a critical lipid fluidity [8]. When we examined the activity of Ca^{2+}-ATPase in membranes supplemented with cholesterol the temperatures at which discontinuities occur are the same as those observed for the native membranes.

Cholesterol is known to restrict the motion of disordered fatty acyl residues of pure phospholipid dispersions or phospholipids in biomembranes [13,14] resulting in a decrease in bilayer lipid fluidity at temperatures above the phase transition temperature. To check the effects of cholesterol on the fluidity of the sarcoplasmic reticulum membranes we calculated 'microviscosities' for native and cholesterol-supplemented membranes using the polarization characteristics of diphenylhexatriene. Although microviscosity values obtained by fluorescence polarisation measurements cannot be regarded as absolute [15] they are useful for comparative purposes. The results (fig.2) confirm the report [11] that the hydrocarbon region of the membrane sensed by the probe in native sarcoplasmic reticulum vesicles is in a disordered, fluid condition over 5–40°C. Incorporation of cholesterol into the membrane considerably restricts the probe mobility indicating a marked decrease in membrane lipid fluidity. The changes in 'microviscosity' caused by the presence of cholesterol in the membrane appear to be more marked than the effect on Ca^{2+}-ATPase activity would seem to indicate. The

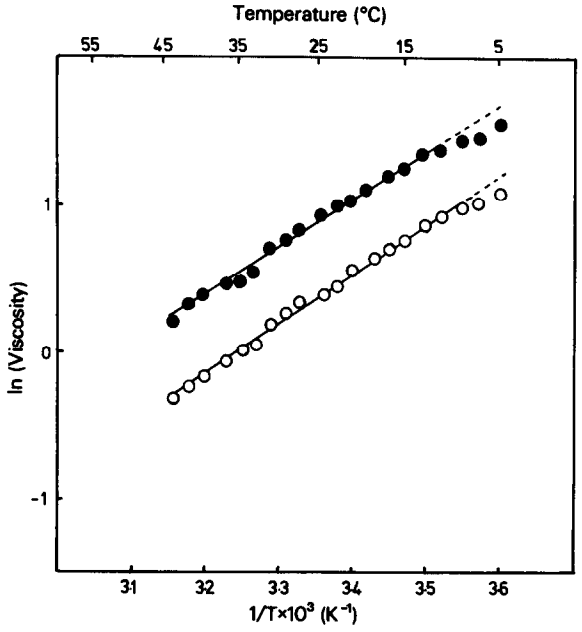

Fig.2. Arrhenius plots of 'microviscosity' for native sarcoplasmic reticulum (○); and cholesterol-rich membranes (●). The molar ratios of phospholipid:cholesterol for these two membranes are given in fig.1.

reason for this may be related to the apparent differences in 'microviscosity' reported by diphenylhexatriene in cholesterol-containing membranes. These effects are believed to reflect changes in the degree of orientation constraint rather than changes in diffusion rates of the probe [16]. Clearly, however, the discontinuities in Arrhenius plots of Ca^{2+}-ATPase are unaffected by changes in the fluidity of the membrane lipid achieved by the incorporation of substantial amounts of cholesterol.

The triphasic form of the Arrhenius plot of Ca^{2+}-ATPase activity (fig.1) is reminiscent of the behaviour of various transport proteins located in the membranes of *Escherichia coli* [17]. In this system, changes in apparent activation energies of these processes were shown to be related to an order–disorder lipid phase transition in the membrane. In sarcoplasmic reticulum, however, the lipid phase transition occurs at temperatures well below those at which discontinuities in Arrhenius plots are observed.

Studies of protein rotation of Ca^{2+}-ATPase in native sarcoplasmic reticulum using saturation transfer ESR [18,19] and flash photolysis techniques [20] have been reported recently. These studies have shown that the discontinuity in the Arrhenius plot of enzyme activity is associated with a marked decrease in rotational motion of the protein, suggesting that a change in conformation and/or state of aggregation of the protein occurs at this temperature. The question as to whether this change in protein rotation is triggered by changes in the physical state of the membrane lipids or is an exclusive property of the protein and independent of lipid fluidity remains unresolved. The results reported here provide support for the idea that thermotropic changes in protein arrangement are not markedly affected by decreases in fluidity resulting from the presence of cholesterol in the membrane.

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