

FUNCTIONAL ADAPTATION OF MUSCLE MEMBRANES  
TO LOW TEMPERATURE

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Summary:

Cold adaptation of Antarctic and Arctic fish muscle sarcoplasmic reticulum has been investigated. Temperature dependent changes in the thermodynamics and kinetics of the ATPases are described. The results are discussed in relation to changes in the saturation of membrane fatty acids described in the literature.

Key words:

Sarcoplasmic reticulum temperature muscle  $\text{Ca}^{2+}$ -ATPase

Introduction

Transport systems are dependent on the associated phospholipids of the membrane for activity, and hence changes in the fluidity of the bilayer can markedly influence the measured enzymic properties. Restriction of the movement of the phospholipid moiety of the sarcoplasmic reticulum (SR) membrane, as measured by electron spin resonance, results in an inhibition of the ATPase enzyme<sup>1</sup>. If the lipids are replaced by more unsaturated analogues, the low temperature inhibition is reversed. For example, substituting SR phospholipids with dioleoyl lecithin, which contains unsaturated fatty acids, eliminates the transition temperature normally seen in Arrhenius plots of rabbit  $\text{Ca}^{2+}$ -ATPase<sup>2</sup>. In cold adapted organisms, a good correlation between the cell temperature and the degree of unsaturation of the membrane fatty acids is generally seen. Tetrahymena pyroformis grown at 39°C showed higher levels of saturated fatty acids than their counterparts grown at 15°C.

Sinensky<sup>4</sup> has suggested that this adaptation occurs in order to maintain a constant membrane viscosity. Although there appears to be good supporting evidence in prokaryotic cells, the correlation between membrane viscosity and cell temperatures in eukaryotes is not as clear. Cossins and co-workers<sup>5</sup> have investigated the effect of temperature on the degree of unsaturation of SR isolated from fish fast muscle, and have found that there is only a poor correlation between environment temperature and viscosity. This result was considered somewhat surprising, especially in view of the known importance of phospholipids in calcium transport. In this report, the kinetic and thermodynamic properties of sarcoplasmic reticulum from fish adapted to Antarctic (-2 to +2°C) and Arctic (0 to 8°C) conditions are compared to those of some temperate and tropical species.

#### Materials and methods

The following species were used:- (1) Notothenia rossii, (Antarctic, -2 to +2°C), (2) Salvelinus alpinus, (Arctic, 0 to 8°C), (3) Salmo gairdneri, (temperate, 3 to 15°C), (4) Tilapia mossambica, (tropical, 24 to 30°C) and (5) Sarotherodon niloticus, (tropical, 24 to 30°C).

Sarcoplasmic reticulum was isolated as previously described<sup>6</sup>. Briefly, white muscle was rapidly dissected, homogenised in 0.3M sucrose, 10mM imidazole, and a microsomal pellet obtained by differential centrifugation. The pellet was resuspended in incubation medium, and further purified by sucrose density centrifugation. The fraction sedimenting between the isolation medium and 35% sucrose has previously been demonstrated to be free of other membrane contaminants, and was used in the studies described here<sup>6</sup>.

Measurement of ATPase activity was carried out in an incubation medium of 60mM KCl, 5mM MgCl<sub>2</sub>, 40mM imidazole, pH 7.2 (10°C), with free Ca<sup>2+</sup> concentration varied by using a Ca<sup>2+</sup>-EGTA buffer. Free Ca<sup>2+</sup> concentration was determined using a computer programme.

SR was preincubated at the desired temperature for 3 minutes, and the reaction started by the addition of ATP (final concentration 2mM). The reaction was terminated by the addition of an equal volume of 10% TCA, the denatured

protein was precipitated by centrifugation, and phosphate released was measured by the method of Rockstein and Herron<sup>7</sup>.

Thermodynamic parameters were determined by using transition state theory, assuming the ATPase to have a molecular weight of 100,000 daltons, and to constitute 70% of the membrane protein<sup>8</sup>, using the following equations:-

$$k = (\bar{k}T/h) e^{-\Delta G^\ddagger / RT}$$

$$\Delta S^\ddagger = (\Delta H^\ddagger - \Delta G^\ddagger) / T$$

$$\Delta H^\ddagger = E_a - RT$$

where  $\bar{k}$  = Boltzmann's constant,  $h$  = Planck's constant and  $E_a$  = the energy of activation derived from the slope of the Arrhenius plot.

$K_{Ca}$ , the  $Ca^{2+}$  concentration which gives half maximal activation of the ATPase, was determined from the Hill plot of

$$\log v / (V_{max} - v) = n \log S - \log K$$

Protein concentration was determined using the Maddy and Spooner modification of the Lowry method<sup>9</sup>.

## Results

$K_{Ca}$  values are highly temperature dependent. Cold adapted animals have considerably lower  $K_{Ca}$  values at 0°C than warm adapted species, but when compared at their adaptation temperature, there is a considerable degree of conservation, with values falling in the range of 0.5 to 0.7 μM (Table 1). Cold adapted species show much higher  $Ca^{2+}$ -ATPase activities at 0°C than tropical species (Table 1). Activation enthalpy of the  $Ca^{2+}$ -ATPase is positively correlated with environment temperature, as previously described<sup>6</sup>, with Arctic and Antarctic enzymes having the lowest values (Table 1).

Table 1.

Species No.	E.T.	Ca <sup>2+</sup> ATPase at 0°C	$\Delta H^{\neq}$ Total ATPase	K <sub>Ca</sub> ( $\mu$ M)	
				0°C	E.T.
1	-2-4°C	182±29	51±5	0.67±0.07	0.67±0.07
2	0-6	63± 6	64±1	n.d.	n.d.
3	0-15	164±19	65±2	1.61±0.16	0.68±0.10
4	24-30	33± 2	81±4	1.94±0.12	0.48±0.03
5	24-30	32± 7	78±3	<8 $\mu$ M	0.53±0.05

The effects of environment temperature on kinetic and thermodynamic parameters of the ATPases of fish sarcoplasmic reticulum. Species No. refers to the list in the materials and methods. E.T. is environment temperature (°C). ATPase activity is in nmol Ca<sup>2+</sup>/mg/min.  $\Delta H^{\neq}$  is in kJ/mol. n.d. means not determined.

### Discussion

From the evidence presented in this report, it would seem that SR isolated from fish fast muscle has undergone functional adaptation to temperature. The higher catalytic rates of the cold adapted enzymes parallels the observations made by workers on other muscle enzymes<sup>10</sup>.

Adaptation in the Arrhenius plots have also been noted in other membrane bound enzyme systems. For example, Wodtke<sup>11</sup> has noted that, in carp, lowering the acclimation temperature decreases the transition temperature of the Arrhenius plot of succinate dehydrogenase. The involvement of the membrane lipids in this process was demonstrated

by Hazel<sup>12</sup> who showed that reactivation of the enzyme was greater when lipids from goldfish adapted to 5°C were used, irrespective of the origin of the enzyme protein.

In the sarcoplasmic reticulum, Madeira and co-workers<sup>13</sup> found that SR isolated from lobster has higher levels of unsaturated fatty acid than that from rabbit, and that these differences are reflected in the temperature characteristics of the enzymes. In contrast, Cossins<sup>5</sup> did not find any compensatory viscosity changes in SR from three different species of animal, and found only a poor correlation between cell temperature and fatty acid unsaturation.

This report clearly demonstrates a close relationship between adaptation temperature and function properties of the sarcoplasmic reticulum. However, these do not seem to be related to changes in the unsaturated fatty acid components of the membrane. This would suggest that homeoviscous adaptation, as has been described in prokaryotic cells<sup>4</sup>, may be less important in higher organisms. Recent evidence has suggested that, even in homeotherms, the SR phospholipids are in a fluid array throughout the temperature range 0 to 40°C, with only 3% being crystallised at 1°C, and only 1% by 5°C.<sup>14</sup> These workers have suggested that the temperature induced perturbations observed in rabbit SR are not due to a crystalline to liquid-crystalline transition, but rather to dipole orientation on the surface of the bilayer. Another possibility has been suggested by Lee and co-workers, who propose that the low temperature inhibition is a result of an increase in the formation of lipid clusters<sup>15</sup>. Interpretation may also be complicated by the postulated existence of an immobilised annulus of about 30 phospholipid molecules around each transport protein<sup>16</sup>.

In the case of the sarcoplasmic reticulum, modifications in the transport proteins may be more important than adaptations in the bulk lipid phase.

References/

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