

TEMPERATURE ADAPTATION AND THE KINETICS OF THE Ca^{2+} -INDEPENDENT AND Ca^{2+} -DEPENDENT ATPases OF FISH SARCOPLASMIC RETICULUM

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Abstract—1. Studies have been made on the effect of temperature on the kinetics of Ca^{2+} -independent and Ca^{2+} -dependent ATPases of fish sarcoplasmic reticulum. Highly purified sarcoplasmic reticulum was isolated from the fast trunk muscles of *Notothenia rossii* (Antarctica -2 to $+2^\circ\text{C}$), *Salmo gairdneri* (temperate rivers 2 to 12°C), *Tilapia mossambica* and *Sarcocherodon niloticus* (African freshwater lakes, 20 to 30°C). Assayed at 30°C the Ca^{2+} -ATPases of the different species are similar. However, at 0°C the Ca^{2+} -ATPase of the cold-adapted species is 7–20 times higher than for the two tropical fish. In contrast, activities of the Ca^{2+} -independent ATPase did not correlate with environmental temperatures at any assay temperature. Values of activation enthalpy (ΔH^*) are positively correlated with environmental temperature for the Ca^{2+} -dependent but not the Ca^{2+} -independent ATPase activity of fish sarcoplasmic reticulum.

2. The Ca^{2+} concentration required to give $\frac{1}{2} V_{\max}$ (K_{Ca}) is dependent on assay temperature. At 0°C , the magnitude of K_{Ca} is positively correlated with environmental temperature varying from 0.7 to $8.6 \mu\text{mol}$ in *N. rossii* and *Sn. niloticus*, respectively. However, when determined at the environmental temperature normal for each species there is a considerable degree of conservation of K_{Ca} s with values falling in the range 0.4 – $0.7 \mu\text{m}$.

3. The ratio of Ca^{2+} -dependent to total ATPases is around 80–90% for the Antarctic fish at assay temperatures between 0 and 30°C . In contrast for the two tropical species, the ratio of Ca^{2+} -dependent to total ATPases rises from 0–15% at 0°C to around 85–90% at 25 – 30°C . Thus over the normal environmental-temperature range for each species a similar proportion of the total ATPase activity is Ca^{2+} -dependent.

INTRODUCTION

FISH have become adapted to a wide range of different thermal environments both in terms of absolute temperature and annual temperature fluctuation. In general, species with similar life styles show comparable levels of physiological activity in spite of wide differences in body temperature (Hazel & Prosser, 1979).

Studies with skeletal-muscle sarcoplasmic reticulum (SR)† have shown that the mechanisms of temperature compensation differ according to the time scale of adaptation. For example, the fraction of muscle-fibre volume occupied by SR is higher in goldfish acclimated to 4°C than 26°C (Penney & Goldspink, 1980). The kinetic properties of the Ca^{2+} -ATPase are not altered by temperature acclimation, and compensation results from increases in the concentration of the pump protein. In contrast, adaptation of V_{\max} and thermodynamic activation parameters are observed between species adapted to different temperatures over evolutionary time periods. For example, it has been shown that sarcoplasmic reticulum isolated from cold-adapted fish assayed at 0°C transports Ca^{2+} at 6 times the rate of warm-adapted species (McArdle & Johnston, 1980). In common with other enzymes

(Low & Somero, 1976; Johnston & Walesby, 1977; Johnston, 1980) activation enthalpy (ΔH^*) of the Ca^{2+} -ATPase is positively correlated with adaptation temperature (McArdle & Johnston, 1979, 1980).

The SR is known to contain both Ca^{2+} -dependent and Ca^{2+} -independent ATPases (Inesi *et al.*, 1976; Tada *et al.*, 1978). In the present study, we have investigated the effects of temperature on the kinetics of the ATPases with respect to Ca^{2+} ions.

This work is an extension of our previous studies on fish SR-ATPases but differs in that imidazole buffer has been used instead of Tris (see McArdle & Johnston 1980).

The results are discussed in relation to the nature of the Ca^{2+} -independent ATPase and to the movement of Ca^{2+} during the contraction–relaxation cycle.

MATERIALS AND METHODS

Four species of fish were used in these experiments: *Notothenia rossii* (~200 g fish from Antarctica -2 to $+2^\circ\text{C}$), rainbow trout, *Salmo gairdneri*, (~300 g from temperate rivers $+2$ to 18°C) and two species from African freshwater lakes (20 to 30°C), *Tilapia mossambica* (~200 g) and *Sarcocherodon niloticus* (~100 g). Specimens were killed by a blow to the head and transection of the spinal cord. Fast trunk muscle was rapidly excised, ensuring that there was no contamination with other fibre types.

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† Abbreviations used: SR, sarcoplasmic reticulum; EGTA, ethylene glycol-bis (β -aminoethyl ether)-*N,N'*-tetraacetic acid; ET, environment temperature.

Highly purified sarcoplasmic reticulum (SR) was prepared as previously described (McArdle & Johnston, 1979). Briefly, a partially-purified microsomal pellet was isolated from the homogenized muscle by differential centrifugation, resuspended in isolation buffer (0.3 M sucrose, 10 mM imidazole, pH 7.2) and layered onto a sucrose density gradient. The fraction sedimenting between 30–35% sucrose has been shown to be free of other membrane components (McArdle & Johnston, 1979, 1981) and was used in all experiments.

ATPase activity was assayed in a medium containing 60 mM KCl, 5 mM MgCl₂, 40 mM imidazole, pH 7.2 (10°C) and a Ca²⁺-EGTA buffer which was varied to give different free-Ca²⁺ concentrations. Free-Ca²⁺ concentrations were estimated using an iterative computer programme.

Prior to initiating the reaction, SR (0.2–0.5 mg/ml) was preincubated in the incubation medium 3 min at the required temperature ($\pm 0.2^\circ\text{C}$). The reaction was started by adding ATP (final concentration 2 mM), and terminated with the addition of an equal volume of 10% trichloroacetic acid (TCA). The denatured protein was precipitated by centrifugation, and the phosphate released was measured using the method of Rockstein & Herron (1951).

An apparent rate constant $K(\text{s}^{-1})$ was obtained assuming that the ATPase accounted for 70% of the total protein and that its relative molecular mass was 100,000 (Inesi *et al.*, 1976). Activation energies ($E_a = \Delta H^* - RT$) were calculated from Arrhenius plots of $\log K$ against $1/T$ ($^\circ\text{K}$). Best-fit lines were computed using linear regression analysis. The Ca²⁺ concentration which gave half maximal activation of the ATPase (K_{Ca}) was determined from the Hill plot of

$$\log v/(V_{\max} - v) = h \log \text{Ca}^{2+} - \log K_{Ca}$$

where h is Hill's coefficient, v is observed ATPase activity and V_{\max} is the ATPase activity at saturating Ca²⁺ levels.

Protein concentration was estimated by the Maddy & Spooner (1970) modification of the Lowry method (Lowry *et al.*, 1951).

Statistical analyses were performed using the Student's t -test.

RESULTS

The effects of temperature on the Ca²⁺-dependent and the Ca²⁺-independent ATPase activities of fish

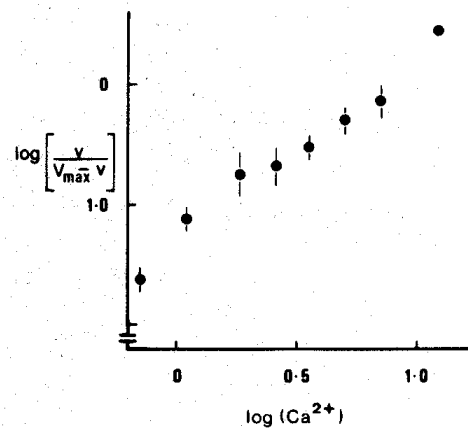


Fig. 1. A Hill plot of the Ca²⁺-dependent ATPase activity of SR isolated from *N. rossii* at 5°C. Values represent the mean \pm SEM of 4 preparations. ATPase activities are expressed in units pmol P_i released, mg SR protein⁻¹ min⁻¹.

SR are quite distinct. Table 1 shows the ATPase activities of fast-muscle SR isolated from four species of fish, estimated at a free-Ca²⁺ concentration of 1.6 μM and at two temperatures. At 30°C the Ca²⁺-ATPases of the different species are similar. At 0°C, however, the Ca²⁺-ATPases of the cold-adapted species is 7–20 times higher than for tropical species. In contrast, there is no obvious relationship between the activities of the Ca²⁺-independent ATPase (the basal ATPase) at 0°C and environmental temperature (Table 1).

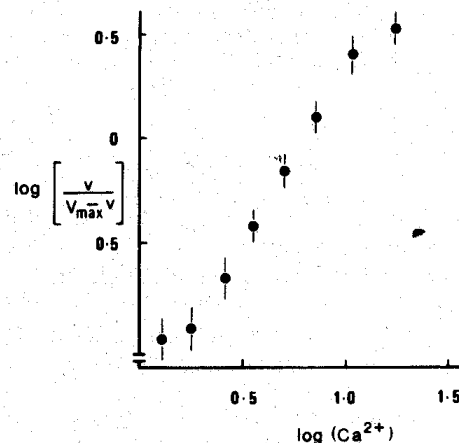


Fig. 2. A Hill plot of the Ca²⁺-dependent ATPase activity of SR isolated from *S. gairdneri* measured at 25°C. Values represent the mean \pm SEM of 6 observations. ATPase activities are expressed in units pmol P_i released, mg SR protein⁻¹ min⁻¹.

Table 1. Ca²⁺-ATPase activities of SR from fish white muscle at 1.07 μM free Ca²⁺

Species	Environmental temperature	Assay temperature	Basal	ATPase activity nmol P _i , mg SR protein ⁻¹ min ⁻¹	Total
<i>N. rossii</i>	Antarctica (-2 to +2°C)	0	9 \pm 2	128 \pm 11	137 \pm 12
		30	179 \pm 13	1485 \pm 165	1674 \pm 60
<i>S. gairdneri</i>	Temperate river (2 to 18°C)	0	39 \pm 5	71 \pm 14	110 \pm 16
		30	219 \pm 60	3370 \pm 324	3590 \pm 332
<i>T. mossambica</i>	African lake (20 to 30°C)	0	20 \pm 4	16 \pm 2	35 \pm 3
		30	404 \pm 27	2676 \pm 444	2878 \pm 627
<i>Sn. niloticus</i>	African lake (20 to 30°C)	0	38 \pm 5	2 \pm 2	40 \pm 5
		30	45 \pm 5	1219 \pm 90	1237 \pm 168

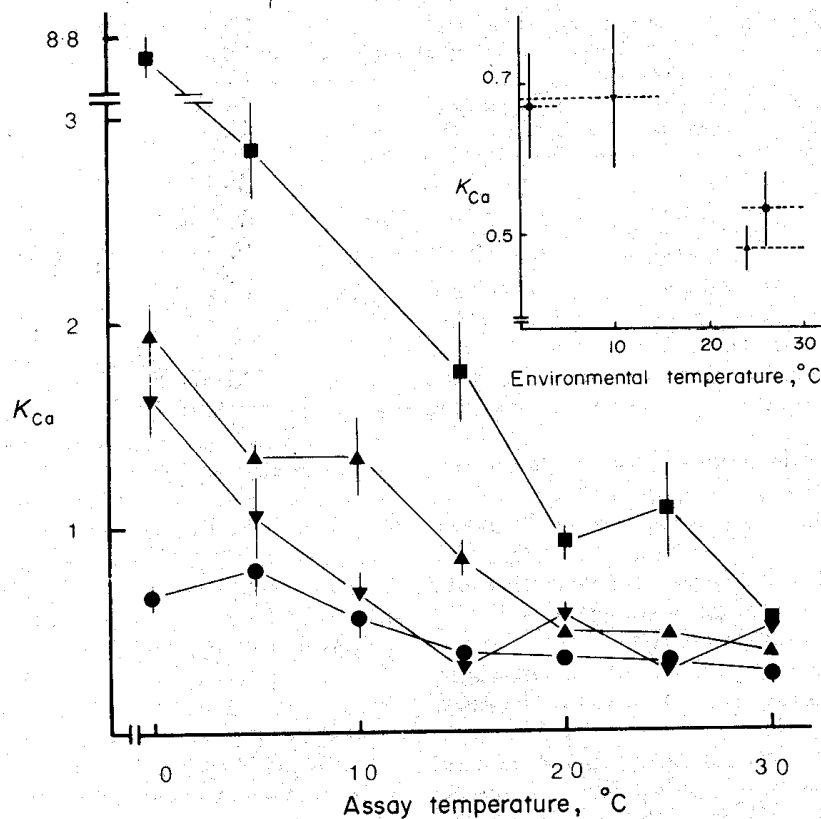


Fig. 3. The K_{Ca} (μM) of *N. rossii* (●), *S. gairdneri* (▼), *T. mossambicca* (▲) and *Sn. niloticus* (■) has been measured at different temperatures. The inset shows the K_{Ca} at the animals' environment temperature. Values represent the mean \pm SEM of at least 4–6 observations.

The Ca^{2+} -dependent ATPase shows sigmoidal saturation kinetics for Ca^{2+} , which transform according to Hill's equation (Figs 1 & 2). The Ca^{2+} concentration required to give 50% V_{\max} (K_{Ca}) has been estimated for seven temperatures (Fig. 3). K_{Ca} is temperature dependent. At 0°C, K_{Ca} is positively correlated with environmental temperature. However, when determined at the normal environmental temperature of

each species there is a considerable degree of conservation of K_{Ca} s with values falling in the range 0.4–0.7 μM (see inset to Fig. 3).

The ratio of Ca^{2+} -dependent ATPase to total ATPase (Ca^{2+} -dependent + Ca^{2+} -independent) is temperature dependent. At 0°C, the ratio Ca^{2+} -dependent/total ATPase is highest in the Antarctic fish, intermediate in the rainbow trout and lowest in the tropical species at all Ca^{2+} -concentrations (Fig. 4). Assayed at 25°C, the ratio of Ca^{2+} /total ATPase is around 85–90% for all species.

Arrhenius plots of fish SR Ca^{2+} -ATPase activity are linear over the temperature range 0–30°C and do not show a discontinuity in slope at around 12–15°C as has been reported for the rabbit enzyme (Inesi *et al.*, 1976). Values of activation enthalpy are shown in Table 2. ΔH^* is positively correlated with environmental temperature for the Ca^{2+} -dependent, but not the Ca^{2+} -independent, ATPase (Table 2).

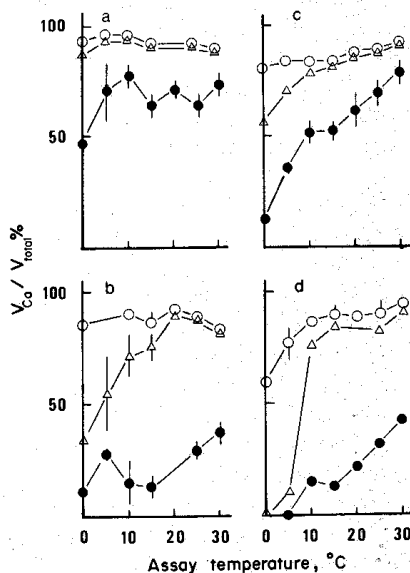


Fig. 4. The effect of temperature on the ratio of Ca^{2+} /total ATPase activity at (●) 0.11 μM , (Δ) 1.06 μM and (○) 8.78 μM free Ca^{2+} for 4 species of fish: (a) *N. rossii*, (b) *S. Gairdneri*, (c) *T. mossambicca* and (d) *Sn. niloticus*. Values represent the mean \pm SEM of at least 4 observations.

DISCUSSION

Studies of enzymes of intermediary metabolism have shown that enzyme K_m or $S_{0.5}$ is highly temperature dependent. However, when measured at the normal cell temperature, many enzymes show similar and highly conserved K_m values. For example, Yancey & Somero (1978) have shown that M_4 -LDHs for a variety of homeotherms and ectotherms have comparable K_m s when assayed at physiological temperature and pH (a range of 0.15–0.35 mM for pyruvate). This result is not unexpected, since the substrate concentrations for many enzymes are often similar

Table 2. ΔH^* (kJ/mol) values of the total- and Ca^{2+} -ATPases of SR from fish fast muscle measured at a free Ca^{2+} of $1.06 \mu\text{M}$

Species	Environmental temperature	n	ΔH^*	
			Ca^{2+} -independent	Ca^{2+} -ATPase
<i>N. rossii</i>	2 to $+2^\circ\text{C}$	4	77 ± 6	59 ± 4
<i>S. gairdneri</i>	2 to 8°C	6	43 ± 3	93 ± 4
<i>T. mossambica</i>	20 to 30°C	3	66 ± 7	100 ± 7
<i>Sn. niloticus</i>	20 to 30°C	3	60 ± 7	162 ± 20

between different species, and are generally about half that required to saturate the enzyme (Atkinson, 1976).

Compensatory modifications in the magnitude and temperature dependence of K_{Ca} are also observed for the SR-ATPase (Fig. 3). K_{Ca} values generally increase with assay temperature. However, K_{Ca} s for cold-adapted species are less temperature dependent and comparable with that for warm-adapted fish (Fig. 3). This is likely to be of adaptive significance since the SR-ATPase probably functions at subsaturating Ca^{2+} -concentrations during a major part of the relaxation cycle.

Using Ca^{2+} -sensitive dyes and luminescent proteins, several groups have studied Ca^{2+} transients during twitches in isolated frog (Blinks, 1973; Miledi *et al.*, 1977) and barnacle (Ashley, 1970; Ashley & Ridgeway, 1970) muscle fibres. Contraction is preceded by a rapid rise in Ca^{2+} concentration, which decays almost to the resting-level prior to the development of tension. Ca^{2+} released from the SR binds very rapidly to Troponin-C (TnC) which has a very high affinity for Ca^{2+} ions ($K_d = 10^{-7} \text{M}$). Ashley & Moiescu (1977) have suggested that the release from TnC is very slow compared to uptake of Ca^{2+} by the SR, so that the free- Ca^{2+} concentration is actually maintained at a comparatively low level (approx $0.5 \mu\text{M}$). In fish muscle, the situation is further complicated by a class of Ca^{2+} -binding proteins known as the parvalbumins ($K_d = 10^5 \text{M}$) (Gillis & Gerday, 1977). These are low molecular weight acidic proteins (11,000–12,000 daltons) occurring in molar excess over TnC, which are distributed throughout the muscle myoplasm (Pécherre *et al.*, 1973; Gillis & Gerday, 1977). It is thought that the Ca^{2+} released from the SR during excitation is rapidly bound to the parvalbumins further reducing the free- Ca^{2+} concentration (Pécherre *et al.*, 1977).

The effects of temperature on the kinetics of the Ca^{2+} -dependent and Ca^{2+} -independent ATPases of fish SR are quite distinct. For example, whereas V_{max} and thermodynamic activation parameters are clearly correlated with environmental temperature for the Ca^{2+} -dependent ATPase this is not the case for the Ca^{2+} -independent ATPase (Tables 1 & 2). The relationship between the Ca^{2+} -transporting properties of SR and the Ca^{2+} -ATPase is clearly established (see Tada *et al.*, 1978). In contrast, the nature and function of the Ca^{2+} -independent ATPase has been the subject of some controversy (Headon *et al.*, 1977; Inesi *et al.*, 1976; Froehlich & Taylor 1975, 1976). There is good evidence that the Ca^{2+} -independent or basal ATPase of sarcoplasmic reticulum represents more than one enzyme system. For example fractionation of the

microsomal pellet by sucrose density fractionation yields: (a) fractions with a low level of Ca^{2+} -dependent ATPase and high levels of Ca^{2+} -independent ATPases (probably of plasmalemmal and T-system origin, Headon *et al.* (1977)); and (b) fractions highly enriched with Ca^{2+} -dependent ATPases which are demonstrably free of other marker enzymes (e.g. cytochrome oxidase, Na^+ - K^+ -ATPase) and yet still retain low levels of Ca^{2+} -independent ATPase activity (McArdle & Johnston, 1979, 1980). Ca^{2+} -independent ATPases in the latter fractions differ from the former in being converted to Ca^{2+} -dependence by treatment with the non-anionic detergent Triton X-100 (Inesi *et al.*, 1976; McArdle & Johnston, 1980).

Inesi and coworkers have suggested that the sarcoplasmic-reticulum ATPase enzyme exists in two interconvertible forms, in thermal equilibrium, only one of which is coupled to Ca^{2+} transport. Increasing the temperature or treating the membrane with detergents can confer Ca^{2+} -dependence on the Ca^{2+} -independent form of the enzyme (Inesi *et al.*, 1976). Thus at 37°C , the Ca^{2+} -ATPase accounts for 89% of the total ATPase activity of rabbit SR compared to only 38% at 0°C (Inesi *et al.*, 1976).

The present study is solely concerned with highly purified SR prepared from the microsomal fraction sedimenting between 30–35% sucrose following 2 h centrifugation at 95,000 g. Previously, we have demonstrated this fraction to be free of significant contamination with other membrane systems (McArdle & Johnston, 1979, 1980). If the Ca^{2+} -ATPase can exist in both Ca^{2+} -dependent and independent forms then it would be predicted that the ratio of Ca^{2+} -dependent/total ATPase would be higher at low-assay temperatures in cold- rather than warm-adapted species.

The results shown in Fig. 4 for the effects of temperature on ratio of Ca^{2+} -dependent to total-ATPase activity are at least consistent with this idea. In the cold-adapted Antarctic species, *N. rossii*, the ratio of Ca^{2+} -dependent ATPase to total ATPase is in the range 85–95% at saturating Ca^{2+} -levels ($1 \mu\text{M}$) compared to only 0 and 34% for the two tropical species. At 20°C , all species have ratios in the range 80–95%. Thus, in the cold-adapted species, all the ATPase protein is already in the form coupled to Ca^{2+} transport whereas in the tropical species higher temperatures favour the transition from the Ca^{2+} -independent (E_1) to Ca^{2+} -dependent forms (E_2). Evolutionary modifications in the ATPase protein and its associated lipid may therefore serve to alter the equilibrium between E_1 and E_2 thus providing a major mechanism in the adaptation of SR to different environmental temperatures.

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Key Word Index—Fish; skeletal muscle; temperature adaptation; sarcoplasmic reticulum.