

Further Studies on the Adaptation of Fish Myofibrillar ATPases to Different Cell Temperatures

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Summary. Previous studies on fish $Mg^{2+}Ca^{2+}$ activated myofibrillar ATPases have been extended to species inhabiting diverse thermal environments. Cold adapted ATPases have considerably higher catalytic centred activities at low temperatures than warm adapted ATPases. Differences in cell temperature have also lead to evolutionary modifications in thermodynamic activation parameters. The free energies (ΔG^{2+}), enthalpies (ΔH^{2+}) and entropies (ΔS^{2+}) of activation of the $Mg^{2+}Ca^{2+}$ myofibrillar ATPase are positively correlated with adaptation temperature.

Myofibrils with $CaATP^{-2}$ as substrate in the absence of Mg^{2+} will hydrolyse ATP by a mechanism not associated with fibril shortening. Differences in activation enthalpies (ΔH^H) between cold and warm adapted Ca^{2+} -activated myofibrillar ATPases are less pronounced than for the physiological ATPase. Furthermore the Ca^{2+} -activated ATPase showed no relationship between environmental temperature and substrate turnover number or free energy of activation (ΔG^{2+}).

Key words: Cell temperature – Environmental temperature – Fish – Myofibrillar ATPase – Temperature compensation.

INTRODUCTION

The cell temperature in aquatic poikilotherms closely parallels that of the environment. Representatives of the teleost fishes exploit thermal habitats across almost the entire physiological range of temperatures, $-2^\circ C$ to $40^\circ C$. Evolutionary adaptations to different cell temperatures has resulted in thermal compensation of metabolic rates in many poikilotherms (Bullock, 1955; Hazel and Prosser, 1974). For example, Antarctic fish are capable of similar levels of muscular

performance to their tropical counterparts in spite of a difference in body temperature of some $25-30^\circ C$. Recently, there have been a number of reviews of temperature compensation phenomena at the molecular and cellular levels (Hazel and Prosser, 1974; Somero, 1975; Somero and Low, 1976). Enzymes adapted to low cell temperatures have higher substrate turnover numbers and lower activation enthalpies than their homologues isolated from warm adapted species (Bendall, 1969; Low and Somero, 1976; Johnston and Walesby, 1977). In addition, there is often a good correlation between enzyme thermostability and cell adaptation temperature (Ushakov, 1964; Johnston et al., 1973). It has been suggested that during the course of enzyme evolution a "compromise" has been reached between structural stability and catalytic efficiency (Johnston et al., 1973; Somero, 1975). According to this hypothesis enzymes with more open tertiary structures are thought to be capable of more flexible and energetically efficient conformational changes during the activation process (Somero, 1975; Low and Somero, 1976).

Muscle contraction results from the cyclic interaction of myosin crossbridges with actin filaments. The energy for the detachment and reattachment of the crossbridges is derived from the splitting of ATP by the globular head of the myosin molecule (Subfragment 1). Myosins isolated from cold-adapted fish are unstable compared to mammalian and avian myosins (Connell, 1961, 1969). This instability is characterised by the ready formation of aggregated products and a concomitant decline in ATPase activity to low levels (Connell, 1969). The pronounced instability of myosins from the muscles of Antarctic and North Sea fish makes studies of the effects of temperature on their ATPase activities impracticable. However, myofibrils prepared from the same muscles are much more stable and are suitable for comparative studies (Johnston et al., 1972). There is also evidence that the proteins of the

tropomyosin-troponins complex effect the temperature dependence of rabbit Mg^{2+} -actin activated myosin ATPase (Hartshorne et al., 1972). If this finding is generally applicable to other animals then studies of the temperature dependence of myofibrillar ATPases are likely to more closely parallel the in vivo physiological situation than those involving pure myosins. In vitro myofibrils and glycerinated fibre preparations hydrolyse both $MgATP^{-2}$ and $CaATP^{-2}$ at appreciable rates. However, it is only the splitting of the in vivo substrate $MgATP^{-2}$ which is associated with contraction and mechano-chemical transduction of free energy (Bendall, 1969).

Previous studies in this laboratory (Johnston et al., 1975; Johnston and Walesby, 1977) have shown a marked correlation between cell temperature and the thermostability and catalytic efficiencies of different Mg^{2+} -activated myofibrillar ATPases. It is of interest to extend these studies in comparing the $MgATP^{-2}$ and $CaATP^{-2}$ catalytic centred ATPase activities.

MATERIALS AND METHODS

Fish. Three species of Antarctic fish were used in these experiments. *Notothenia rossii* were caught by light trap at King Edward Point, South Georgia. *Notothenia neglecta* were caught by trammel net at Signy Island, South Orkney Islands. The single specimen of

Champscephalus gunnari a haemoglobin-less "ice-fish" (*Chaenichthyidae*) was obtained by otter trawl from 250 m depth off South Georgia. Antarctic fish were returned to the U.K. in tanks of filtered recirculated seawater maintained at 1°C. Specimens of the North Sea species *Cottus bubalis* were obtained from local fishermen at Pittenweem, East Fife, Scotland. Brown Trout (*Salmo trutta*) were obtained from a commercial hatchery, Pickering, East Yorkshire. The 3 species of Indo-Pacific fish were obtained from fish dealers in Edinburgh. Specimens of the hot-springs species *Tilapia grahami* were obtained from L. Magardi, Kenya and were used for experiments the same day at the Zoology Department, University of Nairobi. The mean habitat temperatures of each species are given in Figure 1. Fish were maintained at their respective habitat temperatures for several weeks before use.

Preparation of Myofibrils. Fish were stunned by a blow on the head and killed by decapitation. White myotomal muscle was rapidly dissected from the whole length of the trunk and finely minced with scissors. Muscle was homogenised in 5 vols of 0.1 M KCl, 5 mM Tris-HCl pH 7.0 with an Ultra-Turrax or Polytron homogeniser for 2 × 40 s at 0°C. All subsequent operations were carried out at 0–4°C. The homogenate was centrifuged at 10000 × g for 10 min and myofibrils prepared from the residue by differential centrifugation as described previously (Johnston et al., 1972; Johnston and Tota, 1974). All stages of preparation were monitored by microscopical examination. In preliminary experiments following preparation myofibrils were treated with a 1% solution of the anionic detergent Triton X-100 as described by Solaro et al. (1971). Treatment with Triton solubilises the sarcoplasmic reticulum and reduces possible contamination with membranous ATPases without affecting the myofibrillar ATPase activity (Solaro et al., 1971). Detergent was removed by washing 5 times in 50 vols of 0.1 M KCl, 5 mM Tris-

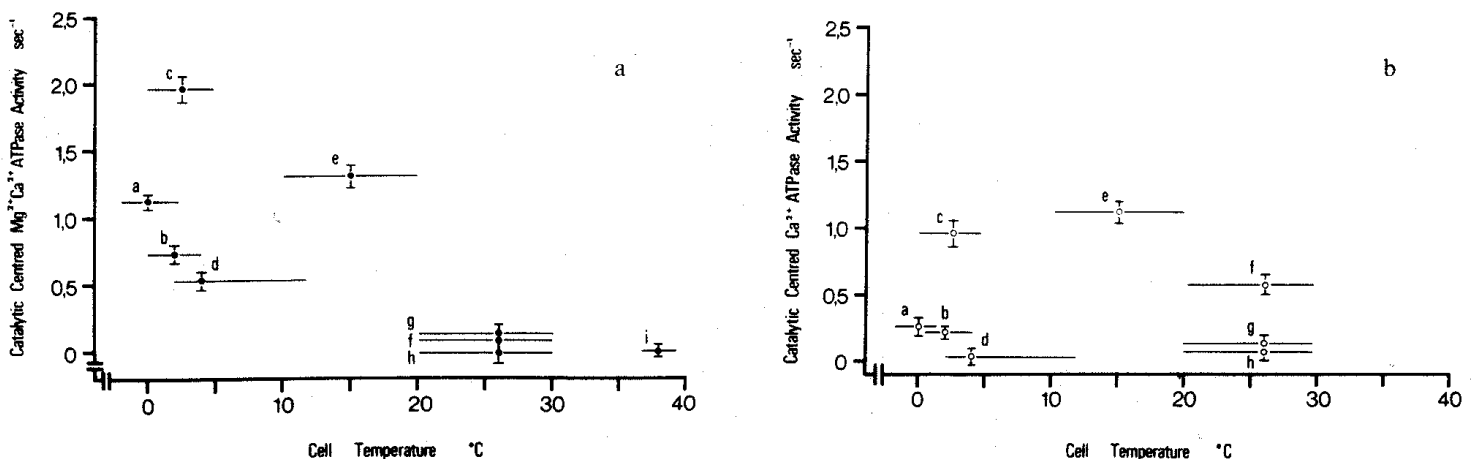


Fig. 1. (a) Relationship between catalytic centred $Mg^{2+}Ca^{2+}$ -activated myofibrillar ATPase activity (moles ATP split, mole myosin active site $^{-1} \cdot s^{-1}$) and cell temperature (environmental temperature) from the fast twitch muscles of the following species of fish: a *Champscephalus gunnari* (Antarctic species) (data from Johnston and Walesby, 1977); b *Notothenia neglecta* (Antarctic species) (data from Johnston and Walesby, 1977); c *Notothenia rossii* (Antarctic species) (this study); d *Cottus bubalis* (North Sea) (data from Johnston and Walesby, 1977); e *Salmo trutta* (Northern Europe) (this study); f *Abudefduf oxydon* (Indian Ocean) (this study); g *Pomacentrus uniozellatus* (Indo-Pacific) (this study); h *Dascyllus carneus* (Indo-Pacific) (data from Johnston and Walesby, 1977); i *Tilapia grahami* (Hot springs equatorial soda-lake, East Africa) (this study). Vertical bars represent mean \pm S.E. of 9 fish except for *Cottus bubalis* and *Tilapia grahami* where 6 and 3 fish were used respectively. Horizontal lines correspond to temperature variation experienced by each species and the position of the point to the environmental temperature of the fish prior to capture. Values of catalytic centred activities and activation parameters are calculated for 273° K. $Mg^{2+}Ca^{2+}$ -activated myofibrillar ATPase activities were assayed in a medium of 40 mM Tris-HCl pH 7.5, 5 mM disodium ATP, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$ at a myofibril concentration of 0.4–0.5 mg ml $^{-1}$ and at an ionic strength of 0.10 (adjusted with KCl). (b) Relationship between catalytic centred activity of Ca^{2+} myofibrillar ATPase and cell temperature for the species shown in (a). All data from the present study. Vertical bars represent mean \pm S.E. of 9 fish except for *Cottus bubalis* where 6 fish were used. Horizontal lines correspond to temperature variation experienced by each species and the position of the point to the environmental temperature of the fish prior to capture. Values of catalytic centred ATPase activities and activation parameters are calculated for 273° K. Ca^{2+} myofibrillar ATPase activities were assayed in a medium of 40 mM Tris-HCl, pH 7.5, 5 mM disodium ATP, 5 mM $CaCl_2$ at a myofibril concentration of 0.4–0.5 mg ml $^{-1}$ and an ionic strength of 0.10 (adjusted with KCl)

HCl pH 7.0. The contribution of the sarcoplasmic reticulum ATPases to the total activity in the original preparation was found to be negligible. ATPase assays were also carried out in the presence of 5 mM sodium azide or 0.5 mM oligomycin to inhibit mitochondrial ATPases. Preparations were found to be essentially free of non-myofibrillar ATPases under the assay conditions employed. Myofibrils were finally suspended in the preparation medium at a concentration of approximately $10 \text{ mg} \cdot \text{ml}^{-1}$. Protein concentrations were determined by a standardised biuret method (Gornall et al., 1949).

ATPase Assays. Conditions for the $\text{Mg}^{2+}\text{Ca}^{2+}$ -activated ATPase were as follows: 40 mM Tris-HCl pH 7.5, 5 mM disodium ATP, 5 mM MgCl_2 , 0.1 mM CaCl_2 at a myofibril concentration of $0.4\text{--}0.5 \text{ mg} \cdot \text{ml}^{-1}$ and an ionic strength of 0.10 (adjusted with KCl). Trace concentrations of calcium are required in the fibrillar system to overcome the inhibitory effect of the tropomyosin-troponins regulatory complex. All preparations of myofibrils were calcium sensitive. Assay conditions for the Ca^{2+} -activated ATPase were 40 mM Tris-HCl pH 7.5, 5 mM disodium ATP, 5 mM CaCl_2 at a myofibrillar concentration of $0.4\text{--}0.5 \text{ mg} \cdot \text{ml}^{-1}$ and ionic strength of 0.10 (adjusted with KCl). The reaction was performed in a volume of 1.0 ml and started by addition of ATP to preincubated myofibrils and terminated with 1.0 ml of 10% (w/v) trichloroacetic acid. Precipitated protein was removed by centrifugation and P_i measured in an aliquot of the supernatant by the method of Rockstein and Herron (1951). Appropriate enzyme and reagent blanks were included in all experiments. ATPase activity was measured usually in duplicate at a series of eight temperatures between 0°C and 18°C . Arrhenius plots of fish $\text{Mg}^{2+}\text{Ca}^{2+}$ -activated myofibrillar ATPase activity often show a transition break between 15°C and 18°C (Bendall, 1969; Johnston and Walesby, 1977). In addition the ATPase activities of myofibrils from cold-adapted species are extremely thermolabile at temperatures in excess of $29\text{--}30^\circ \text{C}$. For this reason, for comparative purposes, activation parameters were compared over the range 0 to 15°C . Apparent activation energies (E_a) over this range were calculated from the slopes of the corresponding Arrhenius plots. Thermodynamic activation parameters were derived from the following relationships as described by Lehrer and Baker (1970) and Low et al. (1973):

$$\Delta H^{2+} = E_a - R \cdot T$$

$$\Delta S^{2+} = 4.576 \left(\log_{10} k - 10.753 - \log_{10} T + \frac{E_a}{4.576 \cdot T} \right)$$

$$\Delta G^{2+} = \Delta H^{2+} - T \cdot \Delta S^{2+}$$

The rate constant k (s^{-1}) corresponded to the substrate turnover number derived from the measurements of V_{max} expressed as moles ATP hydrolysed $\cdot \text{mole} \text{ myosin}^{-1} \cdot \text{s}^{-1}$. The proportion of myosin in the myofibril wet weight was assumed to be 54% (Bendall, 1969) with a molecular weight of 240000 daltons per active site (Lowey et al., 1969).

RESULTS AND DISCUSSION

It is known that the speed of shortening of vertebrate muscles is related to the steady state actomyosin ATPase (Bárány, 1967). It would appear from the present study that there is also a relationship between cell temperature and catalytic centred ATPase activity (Fig. 1a).

The steady-state $\text{Mg}^{2+}\text{Ca}^{2+}$ myofibrillar ATPase of cold adapted species is considerably higher at low temperatures than for warm adapted species (Fig. 1a).

Increased turnover number is associated with a decrease in the free energy of activation (ΔG^{2+}) of the ATPase (Fig. 2a). There is also a marked positive correlation between activation enthalpy and cell adaptation temperature (Fig. 3a). Reduction in the enthalpic contribution (ΔH^{2+}) to the free energy of activation presumably has the advantage of conferring a degree of temperature independence to the activation process. Corresponding reductions in activation entropy (ΔS^{2+}) of the cold adapted ATPase, although energetically unfavourable, compensate for the biologically advantageous reductions in activation enthalpy (Fig. 4a). A good correlation exists between values for the free energy of activation of different species and activation enthalpy (Fig. 5). Cold adapted species are associated with lower $\Delta G^{2+}/\Delta H^{2+}$ ratios. Similar relationships between adaptation temperature and substrate turnover number and thermodynamic activation parameters

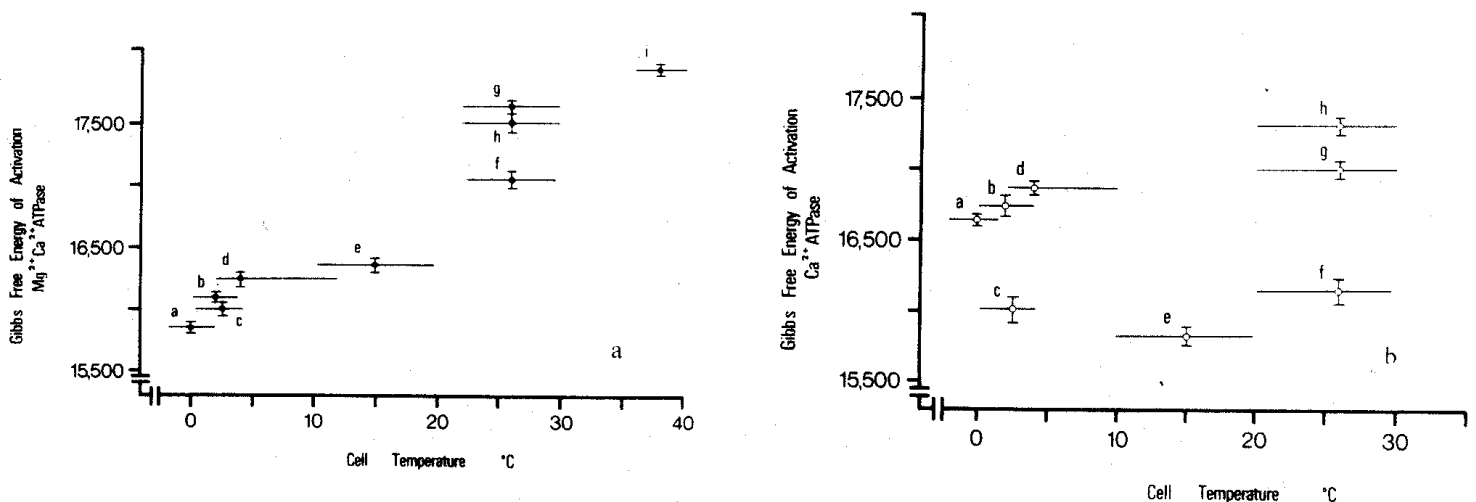


Fig. 2. (a) Relationship between Gibbs Free Energy of activation (ΔG^{2+}) of $\text{Mg}^{2+}\text{Ca}^{2+}$ -activated myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See legend to Figure 1a for species names and other conditions. (b) Relationship between Gibbs Free Energy of activation (ΔG^{2+}) of Ca^{2+} myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See Figure 1a for species names and Figure 1b for other conditions

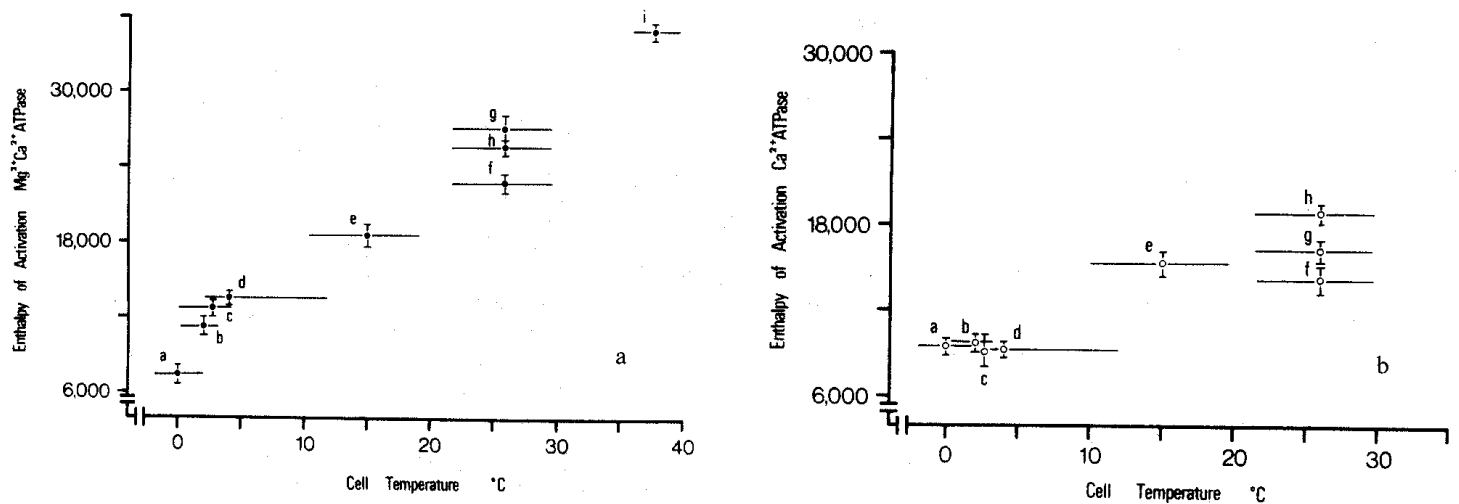


Fig. 3. (a) Relationship between enthalpy of activation (ΔH^{2+}) of $Mg^{2+}Ca^{2+}$ -activated myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See legend to Figure 1a for species names and other conditions. (b) Relationship between enthalpy of activation (ΔH^{2+}) of Ca^{2+} myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See Figure 1a for species names and Figure 1b for other conditions

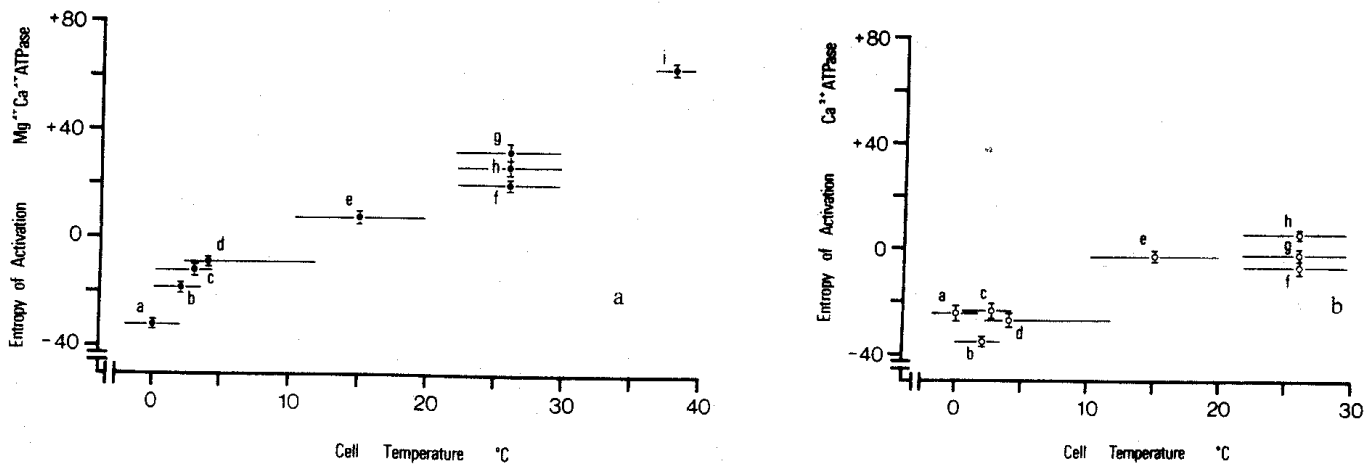


Fig. 4. (a) Relationship between activation entropy (ΔS^{2+}) of the $Mg^{2+}Ca^{2+}$ -activated myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See legend to Figure 1a for species names and other conditions. (b) Relationship between activation entropy (ΔS^{2+}) of the Ca^{2+} myofibrillar ATPase and cell temperature for different species of fish. Assay conditions are given in the text. See Figure 1a for species names and Figure 1b for other conditions

have been described for muscle pyruvate kinases, *m*₄ lactate dehydrogenases and D-glyceraldehyde 3-phosphate dehydrogenases (Low et al., 1973; Somero and Low, 1976; Low and Somero, 1976). Low and Somero (1976) have attributed differences in the activation parameters of cold and warm adapted enzymes to differences in the number of weak bonds formed during the activation process. Lower values of ΔH^{2+} and ΔS^{2+} can result from the making of more bonds during the formation of the activated intermediate or the breaking of fewer bonds during its dissociation. Differences in weak bond formation during the activation process might include bond formations between amino acid side chains and peptide linkages within the enzyme or between the enzyme and the solvent system of the cell.

These adaptive changes in the tertiary structure of the molecule for function at different cell tempera-

tures are presumably mediated ultimately through natural selection of protein primary structure. Interestingly, these evolutionary changes in poikilothermic enzymes are not correlated with phylogenetic status and have occurred subsequently to the divergence of the different vertebrate groups (Somero and Low, 1976). Correlated with changes in catalytic efficiency of enzymes for different cell temperatures are differences in protein thermostability. This is particularly marked in the case of the $Mg^{2+}Ca^{2+}$ myofibrillar ATPase of fish skeletal muscle where the half-life of activation of the enzyme at 37 °C under comparable condition varies some 500 times between Antarctic and hot-spring species (Johnston et al., 1973; Johnston and Walesby, 1977). Similar correlations between protein thermostability and cell adaptation temperature have been shown for a number of poikilotherms (Ushakov, 1964; Hazel and Prosser, 1974). Somero (1975) has

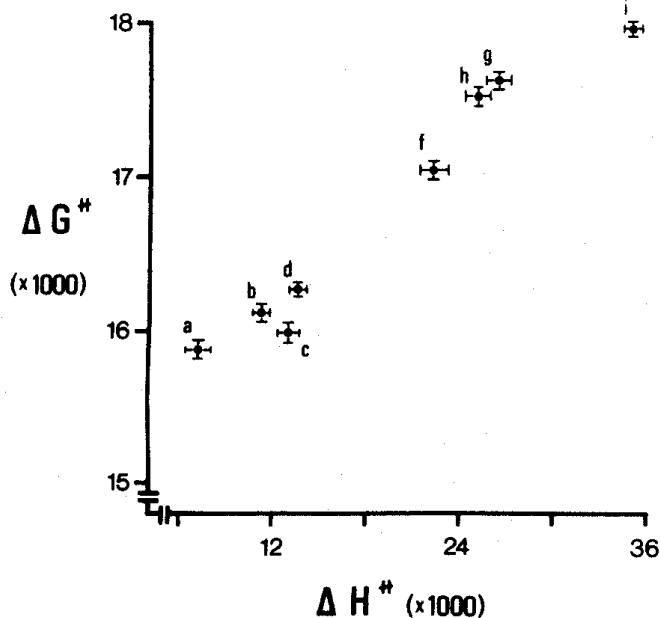


Fig. 5. Relationship between free energy and enthalpies of activation of $Mg^{2+}Ca^{2+}$ -activated myofibrillar ATPases from fish inhabiting different environmental temperatures. Activation parameters are calculated for $273^{\circ}K$.

suggested that a "compromise" has been reached during the course of evolution between the structural flexibility of enzyme tertiary structure and catalytic efficiency. According to this hypothesis a more flexible tertiary structure allows more energetically efficient conformational changes during catalysis (Somero, 1975; Somero and Low, 1976). Animals adapted to higher cell temperatures require a more rigid enzyme structure in the face of the increased kinetic energy in the cell. Thus some reduction in catalytic efficiency is achieved for the sake of increased thermal stability. However, warm-adapted enzymes benefit from a thermal enhancement of reaction rate relative to cold adapted enzymes when compared at their respective physiological adaptation temperatures. Molecular compensations in enzyme function for low cell temperatures are reflected in metabolic compensation at the level of the whole organism (Hazel and Prosser, 1974).

Interestingly, catalytic centered activities of steady state ATP hydrolysis of myofibrils with $CaATP^{-2}$ as substrate were not so clearly correlated with cell adaptation temperature (Figs. 1b, 2b, 3b, 4b), although the enthalpies of activation of 3 tropical species were significantly higher than for the Antarctic species (Fig. 2b, $P < 0.01$). However, there was no relationship between substrate turnover number and Gibbs Free Energy of activation and cell temperature (Figs. 1b, 2b) as was the case for the $Mg^{2+}Ca^{2+}$ -activated ATPase. It is of particular interest that, although myofibrils will split ATP at significant rates, this is not associated with the contraction of fibre bundles

(Bendall, 1969). It would appear that the mechanism of the Ca^{2+} -activated ATPase does not involve actin and it is this ATPase which is associated with mechanochemical transduction of energy and generation of contractile force. Similarly, in the case of subfragment-1 preparations isolated from muscles with different contraction speeds, it has been shown differences in substrate turnover number are most pronounced for the actin-activated ATPase (Weeds and Taylor, 1975; Taylor and Weeds, 1976). In recent experiments involving the hybridization of light and heavy chains from different types of myosin it was found that the light chains had a role in modifying the actin-activated subfragment 1 ATPase (Wagner and Weeds, 1977). Fish white muscle myosins like other vertebrate myosins have two alkali and one DTNB light chain which is capable of being phosphorylated (Focant et al., 1974, 1976). It would appear to be of interest to investigate the role of the alkali light chains in adaptation of myosins to different cell temperatures. Further work along these lines is currently in progress in this laboratory.

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