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Thermodynamic activation parameters of fish myofibrillar ATPase enzyme and evolutionary adaptations to temperature

INTERSPECIFIC compensatory adaptations to environmental temperature which occur at the molecular level have been demonstrated for several enzyme systems[1]. Most of these studies have been concerned with either kinetic parameters such as K_m (refs 2, 3) or thermodynamic parameters such as activation energy[2,4]. The significance of changes in these parameters in the overall mechanism of evolutionary temperature compensation is controversial[1]. In the case of activation energy (E_a), as calculated from Arrhenius' equation, a correlation exists with habitat temperature for some enzymes[2,5,6] but not others[3]. Studies of activation energy are principally concerned with the enthalpy of activation ($\Delta H^\ddagger$). There have been comparatively few studies of the free energy of activation ($\Delta G^\ddagger$) between homologous enzymes from animals of different thermal environments[7,8]. Low *et al.*[8] showed a correlation between $\Delta G^\ddagger$ for muscle type (M_4) lactate dehydrogenase and body temperature. The relative importance of enthalpic ($\Delta H^\ddagger$) and entropic ($\Delta S^\ddagger$) activation between poikilotherms and homoeotherms was also shown to be different[8]. Similar results have been obtained for skeletal muscle myofibrillar ATPase activity[7]. Since these studies deal with homologous enzymes from animals with very different phylogenetic positions it is difficult to assess directly the adaptive significance of changes in the magnitude of these parameters.

We have determined thermodynamic activation parameters for the Mg^{2+}-activated myofibrillar ATPase of the white muscle of teleost fish inhabiting a wide range of thermal environments from Antarctic to tropical waters.

A plot of $\log_{10}V_{max}$ against the reciprocal of the absolute temperature (K) is called an Arrenhius plot. Arrhenius plots for myofibrillar ATPase activity show a discontinuity at 18.5 °C (ref. 7). This effect has been attributed to the binding of Ca^{2+} to troponin A in systems containing the intact calcium-sensitising system, since it does not seem to be observed with pure myosin in the presence of high levels of Ca^{2+} or with desensitised actomyosin preparations[9]. In the case of some species of fish, myofibrillar ATPase activity also undergoes an initial activation before denaturation at higher temperatures[6,10]. This initial activation effect makes accurate determinations of native specific activity very difficult[11]. Although the occurrence of this initial activation varies considerably between species (occurring at lower temperatures in cold-adapted species[6]) it is not observed in any of the species studied at temperatures below 25 °C. To overcome these complications, we have considered only the temperature range 0–18 °C. The method of calculating the thermodynamic parameters, $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta G^\ddagger$ from the corresponding Arrhenius plots and V_{max} determinations is given in the legend to Table 1.

Values of $\Delta G^\ddagger$, the free energy of activation of the reaction, were broadly similar for all species studied. The values obtained for the Antarctic fish *Notothenia rossii*, however, were some 800 calorie mol^{-1} lower than those of species living at the highest environmental temperature (Table 1). Small differences in $\Delta G^\ddagger$ between homoeotherms and poikilotherms have been noted both for myofibrillar ATPase[7] and M_4 type muscle lactate dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase and muscle glycogen phosphorylase b[8], and may be of some adaptive significance. Table 1 shows that a more important feature of temperature adaptation in this enzyme concerns the relative contributions of enthalpic and entropic activation. While $\Delta G^\ddagger$ was relatively constant between species the proportions of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ varied considerably. A positive relationship between entropy of activation and environmental temperature was demonstrated. Values of $\Delta S^\ddagger$ varied from large negative values for the cold-adapted species to high positive values for the tropical species. Similar differences have been found between the entropy terms of the myofibrillar ATPase from birds and mammals where entropy was high and positive relative to amphibians and reptiles, where the values were low or negative[7]. There was also a strong positive correlation between enthalpy of activation and the mean annual habitat temperature of the species. Values for $\Delta H^\ddagger$ varied from 6,850 calorie mol^{-1} for the Antarctic species (*Notothenia rossii*) (mean water temperature 0–2 °C) to 33,000 calorie mol^{-1} for a species from an equatorial hot springs

Table 1 Thermodynamic activation parameters for fish myofibrillar ATPase activity

Species	Approximate environmental temperature (°C)	Assay temperature (°C)	Vmax (μmol Pi mg^{-1} min^{-1})	E_a (calorie mol^{-1})	No. assays	$\Delta H^\ddagger$ (calorie mol^{-1})	$\Delta S^\ddagger$ (entropy units)	$\Delta G^\ddagger$ (calorie mol^{-1})
Nothenia	South Georgia, British	0.5	0.24			6,850	−42.2	18,450
rossii	Antarctica (0°–2 °C)	18	0.81	7,400**	21	6,800	−39.4	18,300
Gadus	North Sea	0.5	0.06			10,200	−33.7	19,400
virens	(5°–14 °C)	18	0.82	10,700*	18	10,100	−28.1	18,900
Gadus	North Sea	0.5	0.07			11,350	−26.2	18,500
morhua	(5°–14 °C)	18	0.61	11,900***	24	11,300	−24.6	19,050
Amphiprion	Indian Ocean	0.5	0.013			16,750	− 9.6	19,400
sebea	(about 23°–25 °C)	18	0.57	17,300*	18	16,700	− 6.1	18,500
Carassius	Domestic	0.5	0.03			21,400	8.3	19,050
carassius	(Acclimatised to 26 °C)	18	0.35	21,900*	18	21,300	8.6	18,800
Tipalia	Equatorial African	0.5	0.017			31,000	42.9	19,300
nigra	freshwater lake (23°–31 °C)	18	0.045	31,600**	18	31,500	42.5	18,600
Tipalia	Equatorial hot springs	0.5	0.016			32,800	49.2	19,300
grahami	soda lake (35°–38 °C)	18	0.69	33,300***	18	33,300	49.2	19,000

Myofibrils were prepared from the dorsal epaxial musculature as before[11], care was taken to exclude superficial red muscle as this has a different myofibrillar ATPase activity[12]. The assay for ATPase activity was performed in 1.5 ml of 40 mM Tris-HCl (*p*H 7.5) with 6 mM ATP, 6 mM $MgSO_4$ and 0.2 mM $CaCl_2$ at $I = 0.12$ (adjusted with KCl) and at a myofibrillar concentration of 0.4–0.5 mg ml^{-1}. The reaction was terminated by addition of TCA and the Pi liberated was determined[13,14]. Appropriate controls and reagent blanks were included in all experiments. Determinations of myofibrillar ATPase activity were made in triplicate at a series of temperatures between 0° and 18 °C. Activation energies (E_a) for the reactions over this temperature range were calculated from the corresponding Arrhenius plots. The Arrhenius plots were found to be linear within this temperature range for all species studied (statistical analyses given above). Thermodynamic parameters were calculated according to the following relationships[15]: $\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S$; $\Delta H^\ddagger = E_a - RT$; $\Delta S^\ddagger = 4.576(\log K - 10.753 - \log T + E_a/4.576T)$ and K (s^{-1}) = V_{max} per mg of enzyme × molecular weight × 10^{-3} mmol μmol^{-1} × 1 min per 60 s, where the molecular weight is expressed as mg mmol^{-1} and V_{max} as μmol mg^{-1} min^{-1}. The proportion of myosin in the myofibril was assumed to be 54% (ref. 7), with a molecular weight of 240,000 per enzyme site[16,17]. All protein determinations were carried out using a Biuret method[18].

*$P = 0.01$.
**$P = 0.005$.
***$P = 0.001$.

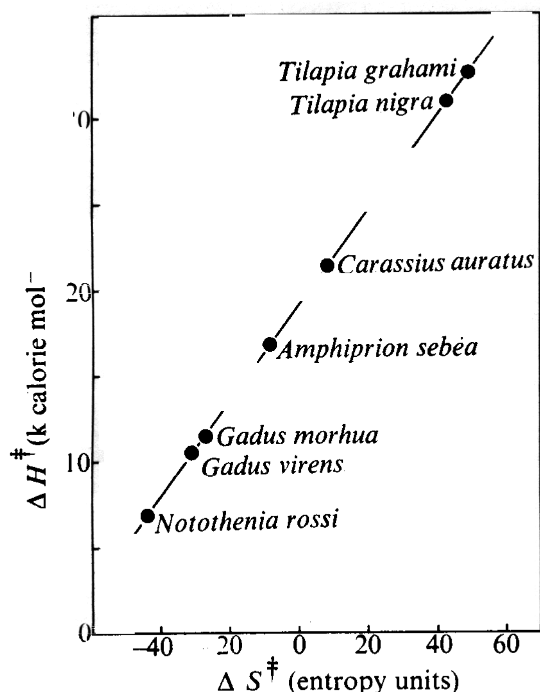

Fig. 1 The relationship between the enthalpy of activation and the entropy of activation for a range of species of fish living at different environmental temperatures.

soda lake, *Tilapia grahami* (35–38 °C). Both enthalpies and entropies of activation were fairly independent of assay temperature.

A compensation plot[19] of entropy change ($\Delta S^{\ddagger}$) against enthalpy change ($\Delta H^{\ddagger}$) for the different species was highly linear ($P < 0.001$) (Fig. 1). The slope of this plot has the dimensions of K and is called a proportionality constant or compensation temperature[19]. Our values of 280 and 300 K for assay temperatures 0.5 and 18 °C respectively are in the range reported for similar plots for a wide range of protein reactions[19-22]. Examples include the binding of oxygen to vertebrate haemoglobin[20] and the formation of methaemoglobins for several mammalian species[21,22]. The existence of a similar relationship between entropy change and enthalpy change, yielding similar compensation temperatures, for various processes of small solutes in water solution, has led some authors to implicate the participation of liquid water in these protein reactions[19,20]. At present it is not clear whether the linear compensation patterns of these and other protein reactions are attributable to some general feature of protein–water interactions or arise entirely from some properties of the protein molecules themselves. If such compensation behaviour was found to indicate a general water-based phenomenon in protein reactions, this would have considerable biological importance in providing a theoretical framework both for the study of specific physiological processes and in the elucidation of mechanisms of molecular evolution.

Certainly, compensation behaviour of this type seems to provide biologically useful adjustments in the activation entropies and enthalpies for poikilothermic enzymes. The observed changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for myofibrillar ATPase, both of which increase from cold water to tropical habitats, are reflected in corresponding changes in the heat and entropy content of the muscle cells. Fish living at the higher environmental temperatures, where enthalpic activation is presumably facilitated, show a larger contribution of the enthalpic activation component. At low habitat temperatures, however, where enthalpic activation is likely to be energetically more unfavourable, the enthalpic component is partially replaced by a larger entropic contribution to the free energy of activation (Table 1). This has the advantage of greatly reducing the temperature sensitivity of the rate-limiting step in forming the activated enzyme–substrate complex. It might be expected that this would give significant adaptive advantage both to species which experience large fluctuations in environmental temperature and to those living in cold and temperate waters, since the limitations of the low heat content of the cellular environment have been partly offset.

It is known that myosins isolated from cold water species are characterised by a ready formation of aggregated products, within a few hours of preparation, and a concomitant decline of ATPase activity to zero[23]. Such preparations are also more easily denatured by urea and heat than the corresponding myosins from homoeotherms[24]. It seems therefore that the concomitant structural adaptations in the tertiary structure of fish myosins consistent with their evolutionary modification for a low enthalpy environment necessarily make the molecule unstable at higher temperatures. Indeed a striking relationship has been shown between the thermostability of fish myofibrillar ATPase activity and the environmental temperature at which the species lives[25]. The general order of thermal denaturation for this enzyme at 37 °C from 22 species of fish has been shown to be African equatorial lakes > Indian Ocean > Mediterranean > North Sea > Antarctic[6,25]. The approximate half life of inactivation of the enzyme in comparable conditions varied some 350 times between these two temperature extremes. Species adapted to tropical environments presumably need a more rigid molecular structure to confer thermal stability at the higher environmental temperatures. Somewhat less marked correlations between thermostability and thermal environment have been shown for several mitochondrial enzymes in teleosts[26].

In view of the relevance of studies of myofibrillar ATPase to an understanding of muscle contraction, the molecular mechanisms of temperature compensation among this group of proteins seem to be of particular interest. A more detailed account of these phenomena will be presented later.

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