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. Most of these studies have been concerned with either kinetic parameters such as $K_m$ (refs 2, 3) or thermodynamic parameters such as $AH^*$ and $AS^*$. The significance of changes in these parameters in the overall mechanism of evolutionary temperature compensation is controversial in the case of activation energy ($E_a$), as calculated from Arrhenius' equation, a correlation exists with habitat temperature for some enzymes but not others. Studies of activation energy are principally concerned with the enthalpy of activation ($AH^*$). There have been comparatively few studies of the free energy of activation ($AG^*$) between homologous enzymes from animals of different thermal environments. Low et al. showed a correlation between $AG^*$ for muscle type ($M_s$) lactate dehydrogenase and body temperature. The relative importance of enthalpic ($AH^*$) and entropic ($AS^*$) activation between poikilotherms and homeotherms was also shown to be different. Similar results have been obtained for skeletal muscle myofibrillar ATPase activity. Studies of temperature have been comparatively few for myofibrillar ATPase activity showing a discontinuity at 18.5°C (ref. 7). This effect has been attributed to the binding of Ca$^{2+}$ to troponin 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. In the case of some species of fish, myofibrillar ATPase activity also undergoes an initial activation before denaturation at higher temperatures. This initial activation effect makes accurate determinations of native specific activity very difficult. Although the occurrence of this initial activation varies considerably between species (occurring at lower temperatures in cold-adapted species) it is not observed in any of the species studied at temperatures below 18°C. To overcome these complications, we have considered only the temperature range 0–18°C. The method of calculating the thermodynamic parameters, $AH^*$, $AS^*$ and $AG^*$ from the corresponding Arrhenius plots and $V_{\text{max}}$ determinations is given in the legend to Table 1.

Values of $AG^*$, the free energy of activation of the reaction, were broadly similar for all species studied. The values obtained for the Antarctic fish Nototenia rossii, however, were some 800 calorie mol$^{-1}$ lower than those of species living at the highest environmental temperature (Table 1). Small differences in $AG^*$ between homeotheomers and poikilotherms have been noted both for myofibrillar ATPase and $M_s$ type muscle lactate dehydrogenase, $\beta$-glycerdehyde-3-phosphate dehydrogenase and muscle glycogen phosphorylase, 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 $AG^*$ was relatively constant between species the proportions of $AH^*$ and $AS^*$ varied considerably. A positive relationship between entropy of activation and environmental temperature was demonstrated. Values of $AS^*$ 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. There was also a strong positive correlation between enthalpy of activation and the mean annual habitat temperature of the species. Values for $AH^*$ varied from 6,850 calorie mol$^{-1}$ for the Antarctic species (Nototenia rossii) (mean water temperature 0–2°C) to 33,000 calorie mol$^{-1}$ for a species from an equatorial hot springs

<table>
<thead>
<tr>
<th>Species</th>
<th>Approximate environmental temperature</th>
<th>Assay temperature (°C)</th>
<th>$V_{\text{max}}$ (μmol Pi mg$^{-1}$ min$^{-1}$)</th>
<th>$E_a$ (calorie mol$^{-1}$)</th>
<th>No. assays</th>
<th>$AH^*$ (calorie mol$^{-1}$)</th>
<th>$AS^*$ (entropy units)</th>
<th>$AG^*$ (calorie mol$^{-1}$)</th>
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<tbody>
<tr>
<td>Notothenia</td>
<td>South Georgia, British 0.5 18 0.24</td>
<td>7,400** 21</td>
<td>6,850</td>
<td>42.2</td>
<td>18,450</td>
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<tr>
<td>rossii</td>
<td>Antarctica (0–2 °C) 18 0.81</td>
<td>10,000</td>
<td>10,200</td>
<td>33.7</td>
<td>19,000</td>
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<td>North Sea 0.06</td>
<td>10,700* 18</td>
<td>10,100</td>
<td>28.1</td>
<td>18,900</td>
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<tr>
<td>virens</td>
<td>5–14°C 18 0.82</td>
<td>11,900*** 24</td>
<td>11,300</td>
<td>26.2</td>
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<tr>
<td>Gadus</td>
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<td>16,700</td>
<td>9.6</td>
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<td>morhua</td>
<td>5°C 18 0.61</td>
<td>17,300* 18</td>
<td>16,600</td>
<td>6.1</td>
<td>18,500</td>
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<tr>
<td>Amphiprion</td>
<td>Indian Ocean 0.05</td>
<td>21,900 18</td>
<td>21,300</td>
<td>8.6</td>
<td>18,800</td>
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<td>sebea</td>
<td>(about 23–25°C) 18 0.57</td>
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<td>42.9</td>
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<tr>
<td>Carassius</td>
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<tr>
<td>carassius</td>
<td>(Acclimatised to 26°C) 18 0.45</td>
<td>31,600** 18</td>
<td>31,500</td>
<td>42.5</td>
<td>18,600</td>
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<tr>
<td>Tipula</td>
<td>Equatorial hot springs 0.5 0.046</td>
<td>33,300*** 18</td>
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<td>49.2</td>
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<td>nigra</td>
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<td>Tipula</td>
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<td>33,300</td>
<td>49.2</td>
<td>19,000</td>
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Myofibrils were prepared from the dorsal epaxial musculature as before, care was taken to exclude superficial red muscle as this has a different myofibrillar ATPase activity. The assay for ATPase activity was performed in 1.5 ml of 40 mM Tris-HCl (pH 7.5) with 6 mM ATP, 6 mM MgSO$\text{\textsubscript{4}}$, and 0.2 mM CaCl$\text{\textsubscript{2}}$ at $T = 0.12$ (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. 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: $\Delta G^* = \Delta H^* - T\Delta S^*$; $\Delta H^* = E_a - RT$; $\Delta S^* = 4.576(\log K - 10.753 - \log T + E_a/4.576T)$ and $K$ ($s^{-1}$) is the rate of change of enzyme $\times$ molecular weight $\times 10^{-4}$ mmol μmol$^{-1}$ min$^{-1}$, $1 \text{ min}$ mmol$^{-1}$, where the molecular weight is expressed as mg mmol$^{-1}$ and $V_{\text{max}}$ 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. All protein determinations were carried out using a Biuret method.

* $P = 0.01$

** $P = 0.005$

*** $P = 0.001$. 


(Monovalent cations were added to the assay as KCl at the final concentration of 100 mM.)
soda lake. *Tilapia grahami* (35–38 °C). Both enthalpies and entropies of activation were fairly independent of assay temperature.

A compensation plot of entropy change (ΔS*) against enthalpy change (ΔH*) 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 (ΔK). 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 species. The ob-

...menon in protein reactions, this would have considerable implications for enzyme function and for 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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