ADAPTATIONS IN Mg^{2+}-ACTIVATED MYOFIBRILLAR ATPase ACTIVITY INDUCED BY TEMPERATURE ACCLIMATION

I. A. JOHNSTON

Research Unit for Comparative Animal Respiration, University of Bristol, Woodland Road, Bristol BS8 1UG, England

W. DAVISON and G. GOLDSPINK

Muscle Research Laboratory, Department of Zoology, University of Hull, Hull HU6 7RX, N. Humberside, England

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1. Introduction

Early work on myosin isolated from North Sea fish showed the molecule to be peculiarly unstable relative to corresponding preparations from homoiothermic animals [1,2]. The tendency for fish myosin preparations to form aggregated products together with its thermal lability and rapid loss of activity on storage have made studies of the ATPase activity difficult [3]. In contrast to myosin preparations myofibrils isolated from cold water fish have been shown to be relatively more stable allowing accurate and meaningful studies of the ATPase to be carried out [4]. Studies of temperature adaptation of the Mg^{2+}-activated myofibrillar ATPase between different species have shown there to be a strong positive correlation between thermostability and environmental temperature [5]. The myofibrillar ATPase activity of fish adapted to low environmental temperatures has been found to be relatively higher at low temperatures than homologous preparations from warm adapted species [5,6]. As with certain other enzyme systems [7,8] the apparent energy of activation was found to be positively correlated with the adaptation temperature of the species [5,6].

While most species are restricted to quite a narrow thermal tolerance range a few fish species, such as the goldfish (*Carassius auratus* L.), are able to survive almost the whole range of physiological temperatures. In this present paper the properties of white muscle myofibrils from goldfish acclimated to 1°C and 26°C has been studied. Evidence has been obtained for adaptive changes of the myofibrillar ATPase in response to temperature acclimation. While similar induced changes in kinetic properties are common in many enzymes of intermediate metabolism [7,8] to the authors knowledge this is the first reported example of this phenomenon in a contractile protein.

2. Experimental procedure

Goldfish (*Carassius auratus* L.) were acclimated to 1°C and 26°C for a period of 2 months. Both groups of fish were fed a propriety brand of fish flake daily, although feeding was not observed in the fish held at the lower temperature. Fish were stunned by a blow to the head and killed by decapitation. White muscle was dissected from the dorsal epaxial musculature. Care was taken to exclude any contamination with the superficial red muscle as this is known to have a different Mg^{2+}-activated myofibrillar ATPase activity [4]. A total of 7 myofibril preparations was made from each group of fish in a similar manner to that described by Johnston et al. [4]. Following preparation myofibrils were treated with a 1% solution of Triton X-100 at 0°C as described by Solaro et al. [9] in order to solubilise the sarcoplasmic reticulum. Treatment with Triton reduces any possible contamination of the myofibril preparation with S.R. ATPases without effecting the myofibrillar ATPase [9]. The Triton was removed by washing 5 times in 20 vols of 0.1M KCl, 5mM Tris-HCl pH 7.0. Myofibrils were finally suspended in the above medium at a concentration of 10 mg/ml. Protein

concentrations were determined using a biuret method [10].

Studies on the thermal denaturation of the Mg^{2+}-activated myofibrillar ATPase were carried out at 37°C in a water-jacketed glass reaction vessel fitted with a magnetic stirrer. Myofibrils were added, at a final concentration of 1 mg/ml, to an 18-fold excess of continuously stirred preincubated medium consisting of 0.05 M KCl, 40 mM Tris–HCl pH 7.5. An initial sample was taken 30 sec later, and at appropriate intervals, by pipetting incubated myofibrils into ice cooled tubes to prevent further inactivation. Following exposure to 37°C for varying periods of time myofibrils were assayed for Mg^{2+}-activated ATPase activity at 25°C. No significant activation or inactivation of the ATPase activity occurred over the incubation/preincubation period employed (5 + 3 min). The assay for Mg^{2+}-activated ATPase activity was performed in 1.5 ml of 40 mM Tris–HCl, pH 7.5 with 6 mM ATP, 6 mM $MgSO_4$, 5 mM sodium azide and 0.2 mM $CaCl_2$ at I = 0.124 (adjusted with KCl) and at a myofibril concentration of 0.2 mg/ml. Although the mitochondrial content of carp white muscle is very low [11], 5 mM azide was incorporated into the incubation medium in order to inhibit any contaminating mitochondrial ATPases which might have contributed to the total ATPase activity [12]. The reaction was terminated by addition of 1.5 ml of 10% (w/v) trichloroacetic acid and the liberated phosphate assayed by the method of Rockstein and Herron [13]. Determinations of myofibrillar ATPase activity under the same assay conditions were also made at a series of temperatures between 0°C and 18°C. Appropriate controls and reagent blanks were included throughout. Statistical analysis was carried out by the method of analysis of variance.

3. Results and discussion

The effects of temperature on Mg-myofibrillar ATPase activity in goldfish acclimated to 1°C and 26°C is shown in fig.1. At 1°C the myofibrillar ATPase activity of the cold acclimated fish was 2.8 times higher than that of the warm acclimated fish assayed at the same temperature ($P < 0.01$). Plots of myofibrillar ATPase activity versus temperature have been shown to have different slopes between 0–18°C and 18.5–37°C in fish [14]. In the present study the log plots of activity versus temperature were found to be significantly different ($P < 0.01$) fig.1. The apparent energies of activation for the reaction over this temperature range calculated from Arrhenius

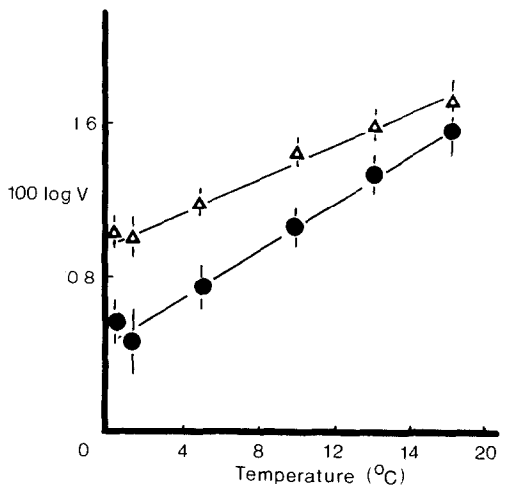

Fig.1. A plot of 100 $\log_{10}$ Mg^{2+}-activated myofibrillar ATPase activity (mol Pi · mg^{-1} · min) (V) versus temperature for goldfish white muscle myofibrils from fish acclimated to 1°C (△–△–△) and 26°C (●–●–●). Assay conditions are given in the text.

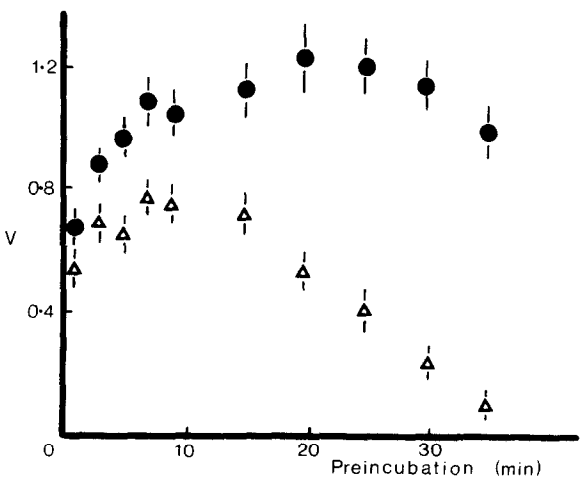

Fig.2. A plot of myofibrillar ATPase activity (V) (moles Pi · mg^{-1} · min^{-1}) versus time preincubated at 37°C in 0.05 M KCl, 40 mM Tris–HCl pH 7.5. Assay conditions are given in the text.

plots were found to be 14.3 Kcal/mol and 21.9 Kcal/mol for cold and warm acclimated goldfish respectively. The lower activation energy of the cold acclimated fish corresponds to values obtained for species habitually adapted to similar low temperatures [5]. In addition to differences in enzymic activity myofibrils from cold and warm acclimated fish were found to have different thermostabilities (fig.2). The kinetics of thermal inactivation of the Mg^{2+}-activated myofibrillar ATPase in goldfish was found to be complex. Incubation of myofibrils at 37°C produced an initial activation of the myofibrillar ATPase in both warm and cold acclimated fish (fig.2). This activation effect was found to be most marked and of longer duration in the case of fish acclimated to the higher temperature. Similar activation effects have been noted in myofibrils from fish habitually adapted to high temperatures [5,6] and in fish myosin preparations during the initial stages of denaturation by low concentrations of urea [15]. Cold acclimated goldfish myofibrils showed a rapid loss of enzymic activity after about 10 min incubation and a 75% loss of activity following 40 min exposure to 37°C (fig.2). This is in marked contrast to the myofibrils form warm acclimated fish which were still showing a relative activation of their ATPase activity following a similar period of incubation ($P < 0.001$).

The thermal denaturation of the Mg^{2+}-activated myofibrillar ATPase is presumably the result of critical changes in the higher orders of structure of the contractile complex in the region of the active site. The results in figs.1 and 2 provide strong evidence for either a change in structure or accessibility of the active site in response to temperature acclimation. These changes appear to have positive adaptive advantages in terms of a higher catalytic efficiency at the range of environmental temperatures experienced by the fish (fig.1). Molecular adaptations in the contractile apparatus in response to environmental temperature changes such as those described are obviously an interesting subject for further study.

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