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The Effects of Environmental Temperature on the Properties of Myofibrillar Adenosine Triphosphatase from Various Species of Fish

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1. Myofibrillar adenosine triphosphatase (ATPase) activities were measured for white myotomal muscle of 19 species of fish. 2. The activity was measured at different temperatures and after periods of preincubation at 37°C. 3. The inactivation half-life at 37°C depended on environmental temperature, increasing as the temperature increased. 4. Cold-water fish had higher myofibrillar adenosine triphosphatase activity at low temperatures than had warm-water fish. 5. The significance of these results is discussed.

It is well known that the myosins of many species of fish are unstable when compared with those isolated from mammalian muscles (Connell, 1961). This instability is characterized by a ready formation of aggregated products and a concomitant decline in the ATPase† activity to zero (Connell, 1960). Fish known to have unstable kinds of myosin are cod, haddock, plaice (Connell, 1961), carp (Syrovy *et al.*, 1970) and trout (Buttkus, 1966). Other species of fish, striped bass, tuna (Richards *et al.*, 1967) and sea mullet (Hamoir *et al.*, 1960), appear to have myosins of stability comparable with those of rabbit or ox myosins.

It has been suggested that there is a relationship between the stability of the myosin and the environmental temperature at which the fish lives (Connell, 1969); if this were so the myosins isolated from warm- and tropical-water species would belong to the stable type and those from arctic and temperate waters to the less stable type. To investigate this suggestion a study was made of the effect of temperature on the myofibrillar ATPase of 19 species of fish which live at widely different environmental temperatures. Rates of inactivation at 37°C and the effect of temperature on the myofibrillar ATPase activity have been examined both for cold- and tropical-water fishes.

Materials and Methods

Fish

A total of 19 species of teleost fish were used in the experiments on the thermal inactivation of the Mg^{2+}-activated ATPase activity. They consisted of the following: plaice (*Pleuronectes platessa*), bull rout (*Myoxocephalus scorpius*), dab (*Limanda limanda*), coalfish (*Gadus virens*), North Sea cod (*Gadus morhua*), all obtained from Bridlington Bay, Yorks., U.K.; rainbow wrasse (*Coris julis*), striped mullet (*Mullus barbatus*), grey mullet (*Mugil cephalus*), pipe fish (*Syngnathus acus*), striped bream (*Lithognathus morymus*), rock goby (*Gobius pagnellus*), annular bream (*Diplodus annularis*), comber (*Serranus cabrilla*) and blue damsel fish (*Chromis chromis*), all obtained from Naples Bay, Italy; *Halichoeres centriquadris*, *Epinephalus garratophalus* and *Grammistes sexlineatus* (from the Indian Ocean, Mombasa, Kenya); and two species of equatorial lake fish, *Tilapia nigra* from Lake Naivasha and *Tilapia grahami* from Lake Margadi, Kenya.

Preparation of myofibrils

Myofibrils were prepared from the deep epaxial white muscle adjacent to the first dorsal fin. Care was taken to exclude any superficial red muscle, as this has been shown to have a different myofibrillar ATPase activity (Johnston *et al.*, 1972). The fish were stunned by a blow on the head and killed by decapitation. Myofibrils were prepared from the muscle immediately after excision. Muscle was minced with scissors and homogenized at 0°C with a Polytron blender for 2×30s in 0.1M-KCl–5mM-Tris–HCl buffer, pH 7.0, containing 5mM-EDTA. The extent of homogenization was monitored by microscopical examination. The homogenate was centrifuged at 600*g* for 15min and myofibrils were prepared from the residue by the method of Perry & Grey (1956). Well-washed myofibrils were suspended in this medium at a concentration of approx. 5mg/ml.

Inactivation experiments

The inactivation of the Mg^{2+}-activated myofibrillar ATPase activity was accomplished by incubating the myofibrils (0.8–1.1mg/ml) in a water-jacketed glass reaction vessel fitted with a magnetic stirrer in a

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† Abbreviation: ATPase, adenosine triphosphatase.

medium of 0.05M-KCl–40mM-Tris–HCl, pH7.5, at 37°C. After 1min samples were taken at appropriate intervals and pipetted into test tubes cooled in ice to prevent further inactivation. The standard assay for ATPase activity was performed at 25°C in a volume of 0.4 or 0.8ml of 40mM-Tris–HCl buffer, pH7.5, with 6mM-ATP, 6mM-$MgSO_4$ and 0.2mM-$CaCl_2$ at I 0.124 (adjusted with KCl) at a myofibril concentration of 0.4–0.55mg/ml. The reaction was terminated by the addition of 15% (w/v) trichloroacetic acid and P_i released was measured by the method of Allen (1940). Myofibrils were also assayed for ATPase activity under the same standard conditions, but at a series of different temperatures. The Arrhenius plot for myofibrillar ATPase shows a marked transition at about 18.5°C (Bendall, 1964), above which temperature the slope is less. This has been shown to apply to fish myofibrils (I. A. Johnston, unpublished work) and therefore in this study energies of activation were measured in the range of 0–18°C. Protein concentrations were determined by a biuret method (Gornall *et al.*, 1949). Appropriate enzyme and reagent blanks were included in all experiments. At least two and usually three inactivation experiments from different preparations of myofibrils were made for each species. The computed regression lines obtained from these experiments were compared for significance by using the method of analysis of variance.

Results

Thermal inactivation

The inactivation of the Mg^{2+}-activated ATPase activity followed first-order reaction kinetics. The half-lives of inactivation at 37°C for the 19 species of teleosts are given in Table 1. The species with the shortest half-lives of inactivation were those living in the temperate waters of the North Sea. The most stable ATPase under these conditions, that of *Tilapia grahami* from Lake Magardi, Kenya, had a half-life of inactivation 50 times greater than that of *Gadus virens*, a North Sea fish ($P<0.001$). *Tilapia grahami* habitually lives at a temperature of 37–40°C in a hot-spring soda-lake in equatorial Africa. In general the half-life of inactivation was related to the environmental temperature at which the fish was living, the order of stability of the enzyme in the fish studied being: Equatorial Lakes > Indian Ocean > Mediterranean > North Sea. The half-life of inactivation in Mediterranean species was widely varied, ranging from 13.07min for *Serranus cabrilla* to 76.72min for *Mugil cephalus*. These species were significantly different in relation to the inactivation of their myofibrillar ATPase activity ($P<0.001$). There was also some overlap in the rates of inactivation in the Mediterranean species and Indian Ocean fish. The most readily inactivated enzyme from an equatorial-

Table 1. *Relationship between the half-life of inactivation of myofibrillar adenosine triphosphatase activity at 37°C and the environmental region from which the fish originated*

For details of the assay conditions see the text.

Species	Source	No. of preparations	Inactivation half-life (min)	Probability of regression line	Degrees of freedom
Gadus virens	North Sea	3	9.70	$P<0.001$	20
Myoxocephalus scorpius	North Sea	3	10.78	$P<0.001$	14
Gadus morhua	North Sea	3	11.13	$P<0.001$	12
Limanda limanda	North Sea	3	11.35	$P<0.001$	20
Pleuronectes platessa	North Sea	3	12.36	$P<0.001$	12
Serranus cabrilla	Mediterranean	3	13.07	$P<0.001$	22
Gobius pagnellus	Mediterranean	3	20.39	$P<0.001$	16
Diplodus annularis	Mediterranean	3	21.28	$P<0.001$	21
Mullus barbatus	Mediterranean	3	30.56	$P<0.001$	21
Lithognathus morymus	Mediterranean	3	36.49	$P<0.001$	19
Syngnathus acus	Mediterranean	2	38.97	$P<0.001$	12
Coris julius	Mediterranean	3	45.25	$P<0.001$	20
Epinephalus garratophalus	Indian Ocean	2	56.87	$P<0.001$	9
Chromis chromis	Mediterranean	3	62.13	$P<0.001$	18
Grammistes sexlineatus	Indian Ocean	2	72.05	$P<0.02$	9
Mugil cephalus	Mediterranean	3	76.72	$P<0.001$	18
Halichoeres centriquadras	Indian Ocean	3	100.23	$P<0.005$	11
Tilapia nigra	Equatorial Lake	3	263.29	$P<0.001$	17
Tilapia grahami	Equatorial Lake	3	502.63	$P<0.001$	11

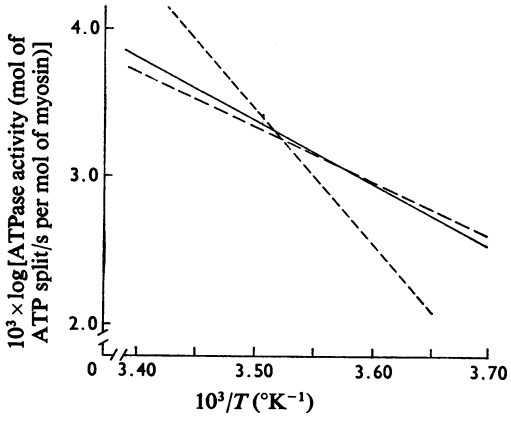

Fig. 1. *Arrhenius plots for Mg^{2+}-activated myofibrillar ATPase activity from cold- and warm-water fishes*

Assay details are given in the text. All regressions are more significant than $P = 0.005$. ----, *Tilapia grahami*; — —, *Gadus morhua*; ——, *Pleuronectes platessa*.

lake fish was, however, significantly more stable than the most stable one from an Indian Ocean fish ($P<0.01$).

In all species with a half-life of inactivation greater than about 20 min an initial apparent activation of the ATPase activity occurred when the myofibrils were adjusted to 37°C. The reason for this apparent activation of the myofibrils from warm-water fish is unknown. However, it may reflect differences in molecular structure between these and cold-water fish.

Effect of temperature on enzyme activity

The effect of temperature on the Mg^{2+}-activated myofibrillar ATPase activity of two species of North Sea fish, *Gadus morhua* and *Pleuronectes*, was different when compared with that in an equatorial hot-springs lake fish from East Africa, *Tilapia grahami* (Fig. 1). The apparent energies of activation for the Mg^{2+}-activated myofibrillar ATPase under standard conditions were determined from Arrhenius plots of the reactions over the temperature range 0–18°C. They were 5208 and 4587 J/mol for *Gadus morhua* and *Pleurenectes platessa* respectively and 8505 J/mol for *Tilapia grahami*. The gradients of the Arrhenius plots for the cold-water species were significantly different from that of the warm-adapted species ($P<0.001$).

Discussion

Since the original suggestion (Connell, 1969) of a possible relationship between the stability of myosins and the environmental temperatures of the fish, there has been no systematic investigation on this subject, probably because of the difficulty of obtaining reliable results for the enzyme activity of myosins from cold-adapted species. We have previously noted that whereas myosins of such fish species are very unstable when isolated (Connell, 1961), myofibril preparations from the same muscles are much more stable (Johnston *et al.*, 1972). The results presented here for myofibrillar preparations show that there is a strong relationship between the temperatures at which the fish live and the stability of their myofibrillar ATPase. A similar relationship between the stability of the myosin ATPase of different species of lizards and the temperatures at which they live has been shown by Licht (1964).

The results reported here also show that the myofibrillar ATPase activity of the cold-water fishes is relatively higher at the lower temperatures than the myofibrillar ATPase of warm-water fish. Bendall (1964, 1969) showed that in general a graph of myofibrillar ATPase activity against temperature is much steeper for animals with higher body temperatures, homotherms in his study, than for heterothermic animals of lower body temperatures. Similar differences in activation energies between homotherms and poikilotherms have been shown for other enzymes. The activation energy of muscle glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) for *Dissostichus mawsoni*, an antarctic fish, has been shown to be significantly lower than that of rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (Greene & Feeney, 1970). It seems that the price paid for the possession of a myofibrillar ATPase that is more active at low temperatures is the relative instability of the enzyme (Table 1). The differences in the properties of myofibrillar ATPase in different fish are interesting because this is a good example of physiological adaptation to the environment seen at the molecular level. Also the properties of the myofibrillar ATPase of the various species of fish might well be one of the important factors influencing their contemporary geographical distribution.

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