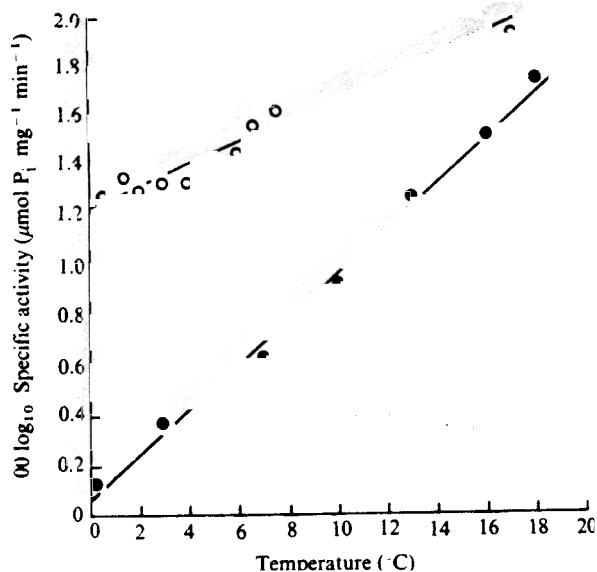


## Temperature adaptation in myosin of Antarctic fish

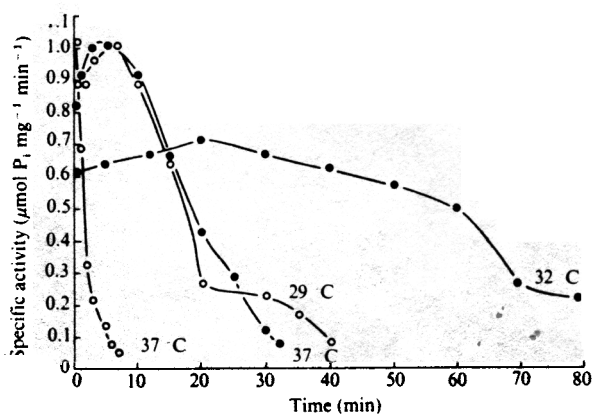
A RELATIONSHIP exists between the thermostability of fish skeletal muscle myosin, actomyosin and myofibril preparations and the environmental temperature at which the fish lives<sup>1-3</sup>. Compared with those isolated from mammals and warm sea fishes, the myosins isolated from cold water species readily aggregate on storage, are more sensitive to denaturation by heat and urea, and quickly lose all ATPase activity following preparation<sup>4,5</sup>. We have compared the properties of myofibrils and myosin prepared from the white muscle of an Antarctic fish, *Notothenia rossii*, South Georgia, British Antarctica, with homologous preparations from a tropical species, *Amphiprion sebae* (Indian Ocean; 23-27 °C). We suggest that the thermal lability of cold-adapted fish myosins arises from differences in the higher order in the structure of the molecule; this is probably an evolutionary response to attain high catalytic efficiency at low temperatures.

The myofibrillar ATPase enzyme from the Antarctic fish (Fig. 1) has a much higher specific activity at low temperatures (0-10 °C) than does the tropical fish. The apparent energies of activation for the reactions between 0 and 18 °C were



**Fig. 1** The effect of temperature on the specific activity of white muscle myofibrils of *Notothenia* (○) and *Amphiprion* (●). Myofibril preparations were used for ATPase activity studies because the ATPase of myosin isolated from cold water fish is extremely unstable. Myofibrils were prepared from fish expaxial white muscle<sup>6</sup>, excluding superficial red muscle which has a different myofibrillar ATPase activity<sup>7</sup>. Studies on the thermal denaturation of myofibrillar ATPase activity were carried out between 25 and 37 °C by incubating myofibrils (1 mg ml<sup>-1</sup>) in 0.05 M KCl, 40 mM Tris-HCl (pH 7.5). Myofibrils were added to an 18-fold excess of continuously stirred medium and an initial sample taken 0.5 min later. Subsequent samples were taken at appropriate intervals and pipetted into tubes at 0 °C to prevent further inactivation. Myofibrils partially inactivated by exposure to high temperatures were assayed for ATPase activity at 18 °C. The assay for ATPase activity was performed in 1.5 ml 40 mM Tris-HCl (pH 7.5) with 6 mM ATP, 6 mM MgSO<sub>4</sub> and 0.2 mM CaCl<sub>2</sub> at *I* = 0.124 (adjusted with KCl), and at a myofibril concentration of 0.4-0.5 mg ml<sup>-1</sup>. The reaction was terminated with 1.5 ml TCA and the liberated phosphate was determined<sup>8</sup>. Determinations of myofibrillar ATPase activity were made at temperatures between 0 and 18 °C in the same assay conditions. Appropriate controls and reagent blanks were included in all experiments.

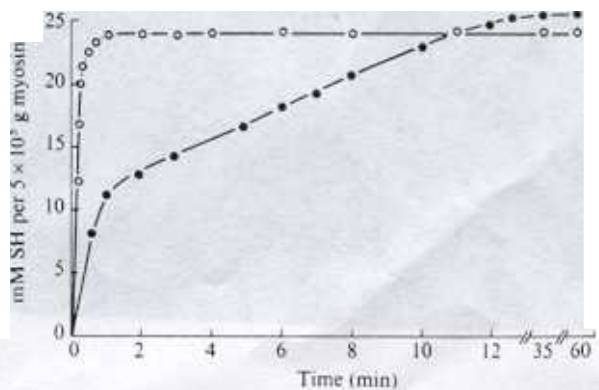
lower in the case of the Antarctic fish, 7.4 kcalorie mol<sup>-1</sup> compared with 17.3 kcalorie mol<sup>-1</sup>. *Notothenia* myofibrils lost 95% of their activity after 7 min incubation at 37 °C, whereas those of *Amphiprion* still retained 45% of their original activity after 20 min incubation. The denaturation reaction (Fig. 2) followed first order reaction kinetics only in the case of the Antarctic fish. In the tropical fish there was an initial activation of the ATPase activity lasting approximately 12 min; the activation in this species occurred between 37-32 °C. A similar activation effect of much shorter duration was noticed in *Notothenia* at somewhat lower temperatures (32-27 °C).



**Fig. 2** The thermal inactivation of myofibrillar ATPase of *Notothenia* (○) at 37 °C and 32 °C and of *Amphiprion* (●) at 37 °C and 29 °C.

Myosins were prepared from the white muscle of the two species by a rapid method<sup>9</sup>. The time-dependent exposure of SH groups of myosin (Fig. 3) was determined by measuring the increasing optical density ( $\lambda = 412$  nm) of the thiophenol anion with 5,5'-dithio bis-2-nitrobenzoic acid (DTNB) following reaction in 0.4 M KCl, 0.1 M phosphate buffer pH 8.0 (refs 10 and 11). The SH groups of *Notothenia* myosin became available for interaction with DTNB very much faster than those of *Amphiprion*, the reaction time being less than 1 min for the Antarctic fish compared with approximately 15 min for the tropical species.

Thus, differences in activation energy ( $E_a$ ), positively correlated with adaptation temperature, may play an important role in the evolutionary temperature adaptation of the enzyme.



**Fig. 3** The time-dependent exposure of myosin SH groups from *Notothenia* (○) and *Amphiprion* (●) measured as the increasing absorbance (412 nm) of the thiophenol anion of DTNB at pH 7.0.

Similar differences in  $E_a$  are important factors in evolutionary rate compensation in other enzyme systems<sup>12,13</sup>. It has also been suggested that the higher catalytic efficiencies between homologous enzyme systems from cold adapted poikilotherms compared with homoiotherms may be explained in terms of an increase in weak bond formation of the activated enzyme-substrate complex in poikilotherms<sup>14</sup>. On this basis the numerous weak bonds involved in stabilising the higher orders of structure of cold adapted poikilotherms necessarily make the molecule more susceptible to thermal inactivation at higher temperatures. This inactivation is probably associated with the disruption of certain hydrogen bonds and hydrophobic interactions between intraprotein amino acid residues, resulting in critical changes in the tertiary structure of the protein, causing loss of enzymic activity. We suggest that the higher activity of the myofibrillar ATPase of Antarctic fish at low environmental temperatures is associated with weaker bonding; in other words, with a more open molecular structure. In the case of the tropical fish a more compact and rigid structure seems to be necessary to give the molecule thermal stability at higher environmental temperatures. Evolutionary temperature adaptation of myosins from different fish may well have important implications in limiting the present day geographical distribution of the different species.

We thank the British Antarctic Survey for supplying the specimens of *Notothenia* and NERC for financial support.

We also acknowledge the technical assistance of Mr S. E. Waterson.

I. A. JOHNSTON

*Research Unit for Comparative Animal Respiration,  
University of Bristol, Bristol BS8 1UG*

N. J. WALESBY

*British Antarctic Survey,  
Monks Wood Experimental Station,  
Abbots Ripton, Huntingdon*

W. DAVISON

G. GOLDSPIK

*Muscle Research Laboratory, Department of Zoology,  
University of Hull, Hull HU6 7RX. UK*

Received November 25, 1974.

- 1 Johnston, I. A., Frearson, N., and Goldspink, G., *Biochem. J.*, **133**, 735 (1973).
- 2 Connell, J. J., *Biochem. J.*, **80**, 503 (1961).
- 3 Chung, C. S., Richards, E. G., and Olcott, H. S., *Biochemistry*, **6**, 3154 (1967).
- 4 Connell, J. J., *Biochem. J.*, **75**, 530 (1966).
- 5 Connell, J. J., in *Proteins as Human Food* (edit. by Lawrie, R. A.), 207-209 (Butterworths, London, 1969).
- 6 Perry, S. V., and Grey, J. C., *Biochem. J.*, **64**, 184 (1956).
- 7 Johnston, I. A., Frearson, N., and Goldspink, G., *Experientia*, **28**, 713 (1972).
- 8 Rockstein, M., and Herron, P. W., *Analyt. Chem.*, **23**, 1500 (1951).
- 9 Syrový, I., Gaspar-Godroid, A., and Hamoir, G., *Archs int. Physiol. Biochem.*, **75**, 299 (1970).
- 10 Ellman, G. L., *Archs Biochem. Biophys.*, **82**, 70 (1959).
- 11 Buttkeus, H., *Can. J. Biochem.*, **49**, 97 (1971).
- 12 Somero, G. N., *Am. Nat.*, **103**, 517 (1969).
- 13 Somero, G. N., and Hochachka, P. W., *Biochem. J.*, **110**, 395 (1968).
- 14 Low, P. S., and Somero, G. N., *Comp. Biochem. Physiol.*, **49(2B)**, 307 (1974).