## Myofibrillar ATPase Activities of Red and White Myotomal Muscles of Marine Fish

Fish myotomal musculature is made up of two main types of muscle fibres which in many species are arranged in separate and distinct anatomical regions. The bulk of the musculature often consists of anaerobic white fibres, while the red fibres (5%–20%) are found in a thin strip lying just beneath the lateral line. These two types of muscle differ not only in colour but in innervation, blood supply, myoglobin content, fibre size and abundance of mitochondria[1,2]. Although there are biochemical and morphological differences between these two types of muscle, their physiological roles are controversial. Some authors[1,3] suggested that the red muscle produces the slow continuous swimming movements for maintaining the fish at a slow cruising speed and the white muscle is used for short bursts of vigorous activity. However, other authors[4,5] have reached the opposite conclusion, namely that white muscle is used for slow activity and red for vigorous activity. BRAEKKAN[6,7] has even suggested that the primary function of the red muscle is to act as a supplementary liver, supplying metabolites for the white musculature, rather than as a contractile organ. In an attempt to clarify the situation regarding the physiological roles of the two types of fibres we have measured the ATPase activity of myofibrils from red and white muscle of marine fish.

Three species of marine teleost were used in these investigations, Coalfish (*Gadus virens*), North Sea Cod (*Gadus morhua*) and Plaice (*Pleuronectes platessa*). The fish were stunned by a blow to the head and killed by decapitation. Red and white myotomal muscles were excised at 0 °C, taking care to minimise cross-contamination of fibre types. White muscle was dissected from the dorsal muscle anterior to the first dorsal fin and red muscle from the whole length of myotomal muscle. Myofibrils were prepared from the muscle immediately after excision.

The method of preparation of myofibrils was based on that of PERRY and GREY[8]. Muscle was minced with scissors and homogenized at 0 °C with a Polytron blender (Kinetica GmbH Luzern, Switzerland) in 0.1 *M* KCl, 5 m*M* *tris*-HCl, pH 7.0, containing 5 m*M* EDTA[9]. The homogenate was centrifuged at 600 *g* for 15 min and myofibrils prepared from the residue in 0.1 *M* KCl, 5 m*M* *tris*-HCl, pH 7.0. The homogenizations were reduced to 25 sec each because of the different texture of fish muscle. All stages of the preparations were monitored by careful microscopic examination. Well-washed myofibrils in 0.1 *M* KCl, pH 7.0, were then treated with an equal volume of glycerol overnight at 0 °C and then washed free of glycerol by repeated applications of 0.1 *M* KCl, 5 m*M* *tris*-HCl, pH 7.0, and finally suspended in this medium at a concentration of approximately 5 mg/ml. Glycerol treatment disrupts membrane organization, consequently reducing contamination by membranous ATPases.

The standard assay for ATPase activity was performed at 25 °C in a volume of 2 ml of 50 m*M* *tris*-HCl, pH 7.5, 6 m*M* ATP, 6 m*M* $Mg^{2+}$, 0.2 m*M* $Ca^{2+}$ at an ionic strength of 0.124 (adjusted with KCl) at a myofibril concentration of 1–5 mg/ml. The reaction was started by addition of ATP and terminated by addition of 1.0 ml of 15% (w/v) trichloroacetic acid. Inorganic phosphate was measured by the method of ALLEN[10]. All incubations were performed in duplicate, and appropriate enzyme and reagent blanks were included in all experiments. Basal $Mg^{2+}$-activated ATPase in the absence of $Ca^{2+}$ (i.e. $< 10^{-7}M$) was measured in the presence of 4 m*M* ethylene glycol *bis*(*β*-aminoethylether) N N′ tetra acetic acid (EGTA). Protein concentrations were determined by the biuret method[11].

The measurements of myofibrillar ATPase activity of red and white muscle are summarized in the Table. There was little variation between the same type of muscle for fish of the same species. However, there was always a considerable difference between the red and white muscle of all fish studied ($P < 0.01$). There was little species variation in the activity of white muscle myofibrils measured under standard conditions (0.2 m*M* $Ca^{2+}$). Also under standard conditions the red muscle had about a quarter of the activity of the white for coalfish and cod. In the presence of 4 m*M*/EGTA ($Ca^{2+} < 10^{-7}$ m*M*) the ATPase activity of the myofibrils was considerably reduced. In the Table the non-calcium-activated ATPase is expressed as a percentage of the calcium-activated ATPase. However, the activity of the red muscle was still lower than the white. The results given in the Table were of the measurements made on fresh myofibril preparations because the $Ca^{2+}$ sensitivity was found to decline quite rapidly. BENDALL[12] reported corresponding changes in myofibrillar preparations of mammalian muscle. However, the rate of loss of


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Myofibrillar ATPase activity of red and white myotomal trunk muscles of marine fish

| Species | Muscle | In the presence of $Ca^{2+}$ No. of fish | Activity μMoles Pi, $mg^{-1}$, $min^{-1}$ Mean ± S.E. | In the presence of EGTA No. of fish | Activity μMoles Pi, $mg^{-1}$, $min^{-1}$ Mean ± S.E. | non $Ca^{2+}$ ATPase (%) |
|---|---|---|---|---|---|---|
| Coalfish (*Cadus virens*) | Red | 23 | 0.26 ± 0.024 | 5 | 0.06 ± 0.01 | 21.2 |
| | White | 22 | 0.94 ± 0.024 | 5 | 0.15 ± 0.03 | 16.2 |
| North Sea Cod (*Gadus morhua*) | Red | 8 | 0.23 ± 0.01 | 5 | 0.04 ± 0.01 | 17.5 |
| | White | 15 | 1.1 ± 0.04 | 5 | 0.13 ± 0.03 | 12.0 |
| Plaice (*Pleuronectes platessa*) | White | 9 | 1.01 ± 0.05 | 6 | 0.11 ± 0.02 | 11.3 |

calcium sensitivity in this case was lower at 20 °C than for fish myofibrils at 0 °C.

It is now clear that the speed of contraction of a muscle is not necessarily related to redness of the fibres, though there does appear to be a good correlation between the speed of contraction and myosin ATPase activity[13]. Preparations of myosins from either red or white muscles of different species of fish have given widely differing values for the $Ca^{2+}$-activated ATPase[14–18].

In many cases low activities have been attributed to the apparent instability of fish myosins[14,16,18–20]. Variation in the stability of myosin preparations could obscure any intrinsic differences in enzymic activity. However, the results obtained in this study using myofibril preparations clearly show that the ATPase activity under these conditions is stable and differs considerably between red and white muscle fibres. It therefore seems from this work that the white muscle has the biochemical properties of fast muscle whereas the red muscle has the biochemical properties of slow muscle.

It has been shown that the slow twitch muscles of mammals are efficient in maintaining tension, while the fast muscles are efficient when contracting isotonically and doing external work[21–23]. It is, however, difficult to understand what advantage it is to the fish, from the efficiency point of view, to have slow muscles for swimming, as swimming requires isotonic contractions rather than the maintenance of tension. If the red muscle is used for slow cruising it may be that this is best achieved by a slow muscle, rather than a heavily loaded fast muscle or antagonistic fast muscles. The division of labour between these different types of muscle at different swimming speeds requires further study.

*Zusammenfassung.* Die $Ca^{++}$- und nicht-$Ca^{++}$-abhängige ATPase-Aktivität von isolierten Myofibrillen der roten und weissen Muskeln von drei Meerfischen wurde gemessen und festgestellt, dass die weissen Muskeln eine höhere Aktivität besitzen.

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## Initiation and Spreading of Coxsackievirus $A_1$ Infection in Muscles of Newborn Mice[1]

Histopathological changes induced by coxsackievirus in muscles of the newborn mouse are characterized by the so-called 'segmental involvement' of individual muscle fibers[2,3]. The term 'segmental involvement' is used to describe the observation that in coxsackievirus infected muscles one or several distinct parts of the muscle cell seem to be altered and other parts remain intact. This is due to an asynchrone cytopathic effect[4] within one muscle cell. The present paper deals with this phenomenon of asynchrony and elucidates the mechanisms by which the infection spreads in vivo from one cell to another. Moreover, the results reported earlier on mechanisms of virus release from an infected cell[5] and on the localization of viral RNA synthesis[6] are confirmed.

*Materials and methods.* Newborn mice between 12 and 24 h after birth received an injection of 0.1 ml ($10^6$ to $10^7$ $LD_{50}$) coxsackievirus $A_1$ suspension in one foreleg. To demonstrate viral RNA synthesis the animals were injected with 2.5 $\gamma$ actinomycin D (Merck, Sharp and Dohme) to stop cellular RNA synthesis before administration of 100 $\mu$Ci uridine-$^3$H (The Radiochemical Centre, Amersham, England). Uninfected control animals were treated in exactly the same manner. After fixation, the muscles were embedded in the usual way in Epon 812. Light microscopic (LM) and electron microscopic (EM) autoradiographs were made from the same blocks. We used essentially the autoradiographic techniques given by STEVENS[7]. For technical details of our autoradiographic methods see[6].

*Results and conclusions.* Up to 8 to 10 h p.i., viral RNA is synthesized to a very small extent within or in close proximity to the nuclei of infected muscle cells[6]. As soon as typical small bodies[8] are formed, uridine is also incorporated in these regions. Figure 1 shows an EM autoradiograph of an actinomycin and uridine-$^3$H treated, coxsackievirus infected muscle about 5 h p.i. The nucleus is lobed and its chromatin is condensed. These are the first signs of a coxackievirus infection[9]. In the zone around the nucleus, the first small bodies are seen. As indicated by the silver grains, viral RNA synthesis takes place in the

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