

# MYOFIBRILLAR ATPase IN THE VARIOUS RED AND WHITE TRUNK MUSCLES OF THE TUNNY (*THUNNUS THYNNUS* L.) AND THE TUB GURNARD (*TRIGLA LUCERNA* L.)\*

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**Abstract**—1. Measurements of myofibrillar adenosine triphosphatase activities have been made from the various red and white muscles of the tunny (*Thunnus thynnus* L.) and the tub gurnard (*Trigla lucerna* L.).

2. The ratios of the activities of the white to red muscle were found to be 4.4 times in the case of the tub gurnard and 2 times in the case of the tunny.

3. The activity of the superficial red muscle was found to be twice as high in the tunny as in the tub gurnard although the activities in the white muscle were similar.

4. No difference in specific activity was found between the superficial and deep red muscles of the tunny.

5. These results are discussed in terms of the division of labour between fish myotomal muscles.

## INTRODUCTION

THE MYOTOMAL musculature of fish is made up of a large number of different muscles. In most species red and white muscles are recognizable. The red muscle often only constitutes about 5–10 per cent of the muscle bulk and runs in a thin strip just underneath the lateral line. In some species, including the tunny, the red muscle is much more extensive and there is an additional region of deep red muscle adjacent to the vertebral column. Recent studies on two gadoid species have shown that the  $Mg^{2+}$ -activated myofibrillar adenosine triphosphatase (ATPase) of the white muscle is four times higher than that of the superficial red muscle (Johnston *et al.*, 1972). There is very little information in the literature on the comparative physiology and biochemistry of red and white muscles from fish with distinctly different locomotory habits. It has now been firmly established that there is a distinct division of labour between the red and white swimming muscles which is dependent on swimming speed (Bone, 1966; Smit *et al.*, 1971;

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Johnston & Goldspink, 1973a-c; Hudson, 1973; Walker & Pull, 1973). However, it is not known how the division of labour between the myotomal muscles is influenced by the normal range of cruising speeds exhibited by the species. For this reason it seemed of interest to extend the earlier study on myofibrillar ATPase of teleost red and white muscles to two species, the tunny (*Thunnus thynnus*) and the tub gurnard (*Trigla lucerna*) which show very different types of swimming behaviour. The tunny is a fast swimming pelagic fish whereas the tub gurnard is a relatively slow swimming bottom living species. In addition the myofibrillar ATPase activities of the two types of red muscle occurring in the tunny myotome have been compared. The results obtained are discussed in terms of the division of labour between fish red and white muscles.

### MATERIALS AND METHODS

Tunny (*Thunnus thynnus* L.) were obtained from commercial fishermen (Carloforte, Sardinia, Italy) soon after slaughter and samples of superficial and deep red and white muscle were dissected from the middle region of the myotome. Small pieces of muscle were stored in a 50 : 50 (v/v) solution of glycerol-0.02 M phosphate buffer pH 7.0 at  $-20^{\circ}\text{C}$ . All preparations of myofibrils from stored muscle were made within a period of 2 weeks. Tub gurnard (*Trigla lucerna* L.) were obtained from the public aquarium, Zoological Station, Naples. The fish were stunned by a blow to the head and killed by decapitation. Red and white myotomal muscles were excised at  $0^{\circ}\text{C}$ , taking care to minimize cross-contamination of fibre types. White muscle was dissected from the dorsal musculature in the middle region of the myotome and red muscle from the whole length of the trunk. Myofibrils were prepared from the muscle immediately after excision.

#### *Preparation of myofibrils*

The method of preparation of myofibrils was essentially as described previously (Johnston *et al.*, 1972) with some minor modifications. Muscle was minced with scissors and homogenized at  $0^{\circ}\text{C}$  with a blender (Marcelli Ltd., Milan, Italy) in 10 vol. of 0.1 M KCl, 5 mM Tris-HCl pH 7.0 containing 5 mM EDTA to minimize reversal of cross-striation (Perry & Corsi 1958). The homogenizations were 5-25 sec at full speed with cooling between each homogenization. The homogenate was centrifuged at 600 g for 15 min and the supernatant containing soluble enzymes was discarded. The residue was resuspended in 5 vol. of ice-cold 40% (w/v) sucrose, 0.1 M KCl, 5 mM Tris-HCl pH 7.0 and centrifuged at 15,000 g for 10 min. The sucrose retards the sedimentation of mitochondria, membrane fragments, etc. and the preparation is purified by the principle of zonal centrifugation (Muir *et al.*, 1971). The residue was subjected to two further similar centrifugation steps before being resuspended in the original medium of 0.1 M KCl, 5 mM Tris-HCl pH 7.0. This was centrifuged at 400 g for 2 min. The supernatant was then decanted and centrifuged at 600 g for 15 min, the residue being either discarded or subjected to further homogenizations. Centrifugation at this lower speed serves to remove pieces of muscle fibre, connective tissue and large aggregates of myofibrils. Usually three series of centrifugation steps were necessary to obtain intact myofibrils reasonably free from other cellular material. All stages of the preparation were monitored by careful microscopical examination. Myofibrils were stored at  $0^{\circ}\text{C}$  in 0.1 M KCl, 5 mM Tris-HCl pH 7.0 at a concentration of 1-4 mg/ml.

#### *Assay of adenosine triphosphatase activity*

Measurements of adenosine triphosphatase activity (ATPase activity) of myofibrils were made on the same day as the preparation. The standard assay for ATPase activity was

performed at 25°C in a volume of 2 ml of 40 mM Tris-HCl pH 7.5, 6 mM disodium adenosine triphosphate (ATP), 6 mM MgSO<sub>4</sub>, 0.2 mM CaCl<sub>2</sub> at an ionic strength of 0.124 (adjusted with KCl) and at a myofibril concentration of 0.1–0.4 mg/ml. The reaction was started by the addition of ATP to preincubated incubation medium containing myofibrils, and terminated by the addition of 1.0 ml of 15% (w/v) trichloroacetic acid. Precipitated protein material was removed by centrifugation and an aliquot of the clear supernatant solution was assayed for Pi by the method of Allen (1940). In addition some preparations were assayed in the presence of 5 mM sodium azide with the ionic strength adjusted accordingly. Sodium azide at this concentration is known to inhibit mitochondrial and membranous ATPases (Brierley *et al.*, 1964). All incubations were performed in duplicate and appropriate enzyme and reagent blanks were included in all experiments. Sufficient experiments were done to ensure that the rate of reaction was linear with respect to time for the assay conditions used.

### Statistical analyses

The data obtained for the Mg<sup>2+</sup>-activated myofibrillar ATPase activities of the red and white muscles of the two species was tested for significance using a method for two-factor analyses of variance for unequal sample numbers (Steel & Torrie, 1960). Further comparisons between means were made using the method of least significant difference.

## RESULTS

The results obtained for the Mg<sup>2+</sup>-activated ATPase activity of myofibrils from the various red and white muscles of the tunny and tub gurnard are given in Table 1. The ATPase activity of the myofibrils was found to remain constant for at least 7 days when stored in 0.1 M KCl, 5 mM Tris-HCl pH 7.0 at 0°C. Contamination with non-myofibrillar ATPases was reduced to a low level in most preparations

TABLE 1—MYOFIBRILLAR ADENOSINE TRIPHOSPHATASE ACTIVITIES OF THE VARIOUS RED AND WHITE MYOTOMAL MUSCLES OF THE TUNNY (*Thunnus thynnus* L.) AND THE TUB GURNARD (*Trigla lucerna* L.)

Muscle	No. of preparations	ATPase activity, no azide (mean ± S.E.)	No. of preparations	ATPase activity, 5 mM azide (mean ± S.E.)	% Non-myofibrillar ATPases
Tunny ( <i>T. thynnus</i> L.)					
Superficial red	11	0.54 ± 0.03	12	0.51 ± 0.03	7
Deep red	13	0.59 ± 0.03	19	0.50 ± 0.03	18
White	10	0.97 ± 0.07	11	0.99 ± 0.08	—
Tub gurnard ( <i>T. lucerna</i> L.)					
Superficial red	8	0.27 ± 0.04	9	0.22 ± 0.02	22
White	9	0.95 ± 0.06	10	0.98 ± 0.05	—

Assay conditions are given in the text.

(Table 1) and only in the case of the deep red muscle from the tunny was this found to be significant ( $P < 0.05$ ). The myofibrillar ATPase activity, measured in the presence of azide, of the white muscle of the tub gurnard was about 4.4 times that of the superficial red muscle ( $P < 0.001$ ). A much lower ratio of the activities of the corresponding muscles of the tunny of about two times was obtained under comparable conditions ( $P < 0.01$ ). The myofibrillar ATPase activities of the superficial and deep red muscles of the tunny were found to be similar (Table 1). However, a significant difference was found in the ATPase activities of the superficial red muscles of the tub gurnard and tunny ( $P < 0.01$ ).

### DISCUSSION

The properties of red and white myotomal muscles in fish have been extensively reviewed (Bone, 1966; Love, 1970; Patterson *et al.*, 1973). Red muscle is a highly vascularized aerobic tissue rich in mitochondria, myoglobin, cytochromes and the enzymes of oxidative metabolism. In contrast the white muscle is a highly anaerobic tissue and is known to have a primarily glycolytic type of metabolism. Biochemical differences have also been shown to occur between the various types of red myotomal muscle. For example, the deep red muscle of tuna has been found to have higher concentrations of haemoglobin, myoglobin and cytochrome *c* than the superficial red muscle (Matsuura & Hashimoto, 1954). The results of the present study, however, indicate that the two types of red muscle of *T. thynnus* have similar myofibrillar ATPase activities and therefore similar speeds of shortening (Bárány, 1967). In contrast, considerable differences were found between the activities of the myofibrillar ATPases between the red and white muscles of both the species studied. The ratio of myofibrillar ATPase activity in the white compared to the red muscle of the tub gurnard of 4.4 times is similar to that reported for the cod (*Gadus morhua* L.) (Johnston *et al.*, 1972). It is likely, therefore, that in these species at least the white and red muscles correspond to fast and slow muscles respectively as was suggested by Baretts (1961) from a series of histological and physiological investigations. It is known from electromyographical recordings that the red muscle is primarily responsible for low speed swimming (Bone, 1966; Rayner & Keenan, 1967; Hudson, 1973). Indeed the red muscle of fish possess all the metabolic features usually associated with muscles adapted for prolonged periods of continuous activity (George & Bokadawala, 1964). The relative advantage of each muscle type for various kinds of swimming activity must ultimately be explained in terms of differences in the thermodynamic efficiencies of the respective contractile systems. It would appear, however, that the types of movement required by slow speed swimming are best achieved by means of a relatively slow muscle. An interesting finding in this present work is that the myofibrillar ATPase activity of the tunny red muscle is about twice as high as that of the tub gurnard and previously investigated species (Johnston *et al.*, 1972) although the white muscle had similar activities in all the species. It may well be that the division of labour between the red and white muscles of the tunny during swimming

is somewhat different in detail than in these other species. This would not be surprising in view of the specialized locomotory behaviour of the tunny, which has a number of features adapting it to high-speed cruising. In addition to a streamlined fusiform shape and double-jointed lunated tail of high aspect ratio (Marshall, 1971) the tuna has a counter current retial blood supply to the red muscle enabling it to maintain muscle temperatures considerably above ambient (Carey & Teal, 1969; Carey *et al.*, 1971). The resulting elevated muscle temperatures are presumably advantageous in enabling the fish to sustain a high level of activity. Tuna species also show interesting adaptations in their respiratory physiology associated with their active mode of swimming. Unlike most other teleosts in which external respiration is achieved by a series of muscular pumps, tuna species ventilate their gills entirely by means of their movement through the water (Muir, 1969—cited by Shelton, 1970). Thus tuna must cruise continuously at quite high speed in order to ventilate their gills. Indeed it has been shown that tuna quickly die at reduced gill flow rates (Stevens, 1972). The higher myofibrillar ATPase activity and hence the higher speed of shortening of tuna red muscle compared with the red muscle of the gurnard may reflect the habitually higher swimming speed of the former species. It may well be that the red muscle of the tunny is adapted for use at a wider range of cruising speeds than that of slower swimming species. There is some experimental evidence for this suggestion. Recent studies have shown that a certain proportion of the white muscle is used at quite low sustainable swimming speeds in the coalfish (1–2 L/S) (Johnston & Goldspink, 1973a; Walker & Pull, 1973) and in the carp (3–3.4 L/S) (Smit *et al.*, 1971; Johnston & Goldspink, 1973b, c). In contrast no electrical activity was recorded from the white muscle of the skipjack tuna (*Katsuwonus pelamis*) during cruising activity (Rayner & Keenan, 1967). Walker & Pull (1973) have suggested that the threshold of activation of the white fibres may be related to the typical swimming speed of a given species. Electromyographical recordings taken from the red and white muscles at a series of known swimming speeds for species of widely different cruising speeds would be required to resolve this question. If indeed a slow red muscle is more energetically efficient at producing the types of movement required for slow speed cruising then it may not be without significance that the tuna with its relatively high cruising speed has a somewhat faster as well as more extensive red muscle system.

The results presented in this paper underline the importance of investigating the metabolic adaptations and functional roles assigned to red and white muscles in fish in relation to the locomotory physiology and mode of life of each species. It would be interesting, therefore, to examine the myofibrillar ATPase activities of the myotomal muscles of a wide range of fish species with different swimming speeds. Although swimming speed is obviously dependent on numerous hydrodynamic, and mechanical factors, it may be that the speed of contraction of the myotomal fibres is also important in determining swimming speed. Since measurements of the speed of contraction of fish myotomal fibres is technically difficult due to the lack of discrete muscles, myoseptal insertion and complex orientation of the fibres, studies on the relative ATPase activities would appear to be of particular value.

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