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*p*H Sensitivity of Myofibrillar-ATPase in the Myotomal Muscles of *Heteropneustes fossilis*

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Three distinct layers of red, pink and white muscle fibres, were identified and classified based on their SDH and myofibrillar-ATPase staining pattern. Both histochemical and biochemical *p*H profile studies showed the *p*H sensitivity of these fibre types with respect to myofibrillar-ATPase. m-ATPase of red fibre region was most sensitive while that of pink fibre region most resistant to alkaline *p*H. Also, red fibre region had low m-ATPase level as compared to white and mixed fibre region. The relationship of m-ATPase activity with the contraction speed of various muscles, is discussed.

Key Words: *Heteropneustes fossilis*, **Myofibrillar-ATPase, Myotomal muscles, Succinic dehydrogenase, Histochemical profile**

Introduction

The muscle fibres in the fish myotome are organized in a very different way as compared to other vertebrate groups. They are arranged in distinct separate regions. The regions of red and white muscle fibres can be distinguished macroscopically by their colour. The red muscle forms a region lying just underneath the lateral line and runs from behind the head to the caudal fin. The white muscle forms the remainder of the myotomal mass.

The red and white muscles of a number of fish species have been shown to correspond to 'slow' and 'fast' muscle types respectively in relation to their myofibrillar-ATPase activities (Johnston et al. 1972 and Davisan et al. 1976). Many workers have modified (Johnston et al. 1974 and Mosse & Hudson 1977) the histochemical method given by Guth and Samaha (1970) for Ca^{++}-activated ATPase in mammalian muscle for use on fish muscle.

In teleosts four or five fibre types have been distinguished (Patterson et al. 1975, Johnston et al. 1975 and Korneliussen et al. 1978) on the basis of the distribution and relative activities of glycogen, lipids, succinic dehydrogenase and myofibrillar-adenosine triphosphatase activity (m-ATPase). However, most of the studies have been restricted to the marine teleosts. Very little attention has been given to the fishes of fresh water. Present histochemical and biochemical study has been carried out on the musculature of a fresh water catfish, *Heteropneustes*

fossilis, of North India. Particular attention has been given to the fibre types and the *p*H-sensitivity of their myofibrillar-ATPases.

Material and Methods

The fishes were collected locally and maintained in the tanks of fresh pond water and regularly fed on a diet of chopped rat liver. The water in the tanks was replaced every week.

(a) Histochemical methods

Preparation of muscles: Heteropneustes fossilis about 12–16 cm long were stunned by a blow to the head and killed by decapitation. Skin was carefully removed, care being taken not to remove the superficial fibre layer along with it. Muscle samples were taken from the region behind the pectoral fin containing the middle red region, substantial amounts of white muscle and the mixed muscle from the deepest part of the myotome adjacent to the vertebral column. Small muscle pieces were mounted on cryostat chucks and quickly frozen by immersion in liquid nitrogen. The blocks were placed in the cryostat (IEC-Model CTD International Harris Cryostat) at −25°C. The frozen sections of 10 μm thickness were cut and used for histochemical staining.

Myofibrillar-ATPase: Frozen sections were air dired for 60–180 min and stained using the routine myofibrillar-ATPase method of Padykula and Herman (1955) after pre-incubating the sections in acid or alkaline medium as described by Guth and Samaha (1970). The pre-incubation time was varied from a dip (of about 5 sec) to over 6 min. The incubation was carried out in every case at 37°C, *p*H 9.4 for about 15 min.

Histochemical pH-profile for m-ATPase: Myofibrillar ATPase staining was performed under the same conditions as described above at a serial range from *p*H 4.35–10.4 with varying pre-incubation timings.

Succinic dehydrogenase: Fresh frozen sections of the muscle were incubated for 15–20 min in the medium described by Nachlas et al. (1957) using Nitro-BT as an electron acceptor.

(b) Biochemical methods

The method of preparation of myofibrils was based essentially on that of Perry and Grey (1956). The muscles were homogenised in a medium containing 0.1M KCl in 10 mM Tris-HCl at *p*H 7.0 and the myofibril preparation was finally suspended in the same medium. Ca^{++}-ATPase was assayed in the myofibrils from various muscles i.e., red, white and mixed. For obtaining different muscles, the central myotomal region just below the lateral line was taken for red muscle, a piece from the remaining myotomal region for white muscle and the red region along with the white for mixed muscle.

The reaction mixture for Ca^{++}-ATPase consisted 40 mM Tris-HCl (*p*H 7.5), 40 mM KCl, 10mM $CaCl_2$, 0.2 ml of myofibril and 3mM of ATP. The assay was performed at 37°C in a total volume of 1.5 ml of the reaction mixture. The reaction was initiated by the addition of ATP and terminated after an incubation of 3 min by the addition of 1.5 ml of 10% TCA (Trichloroacetic acid). All incubations were performed in duplicate and appropriate enzyme and reagent blanks were included in the experiment. Protein concentration of the myofibril preparation used for ATPase assay was determined by the biuret method (Gornall et al. 1949). ATPase activity was expressed as μ moles Pi liberated/min./mg protein.

pH-profile for m-ATPase: Ca^{++}-activated ATPase enzyme assay was performed

on the myofibrils prepared from various muscles under the same assay conditions as described above at a serial range of *p*H from 4.35 to 11.0 in accordance with the work reported earlier (Talesara & Goldspink 1978).

Results and Discussion

Muscle fibres of *Heteropneustes fossilis* are shown to be of three types (figure 1), on the basis of their m-ATPase and SDH enzyme activity (figures 2–10 and table 1). Considerable differences have been reported in the stability of the m-ATPase of marine teleost muscles (Johnston et al. 1974, 1975, 1977). Also the fish muscle fibres are on the whole much more susceptible to inactivation at low and high *p*H than mammalian fibres (Johnston et al. 1974 and Bone & Chubb 1978).

In the present study no specific staining is observed in all the three types of fibres below *p*H 5.5. From *p*H 5.5 onwards differential staining is observed in the three fibre types (table 1). At *p*H 5.5 some staining was observed with short pre-incubation period (30 sec to 2 min). As the *p*H is increased the intensity of staining also increased simultaneously (figures 2–4). Intensive staining of all the fibres is seen at *p*H 10.0 and 10.1 with pre-incubation period of about 5–10 sec (figure 5) whereas when the pre-incubation period was increased, staining became less intense in the red fibre region (table 1). These observations show that fish muscle fibres cannot be classified as pure acid stable, alkali labile fibres, rather all the fibres are acid as well as alkali stable to some extent.

At *p*H 10.2, some inactivation of red fibres was observed with pre-incubation period below 2 min (figure 6). With pre-incubation time 2–4 min, there was complete inactivation of the red fibre region (figure 7). These observations show that the superficial layer of fibres just underneath

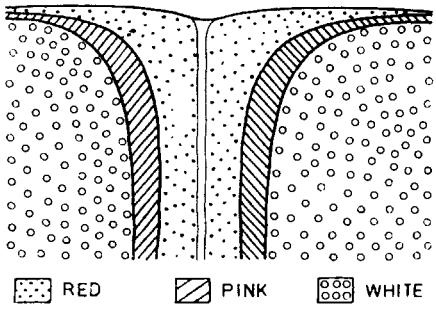
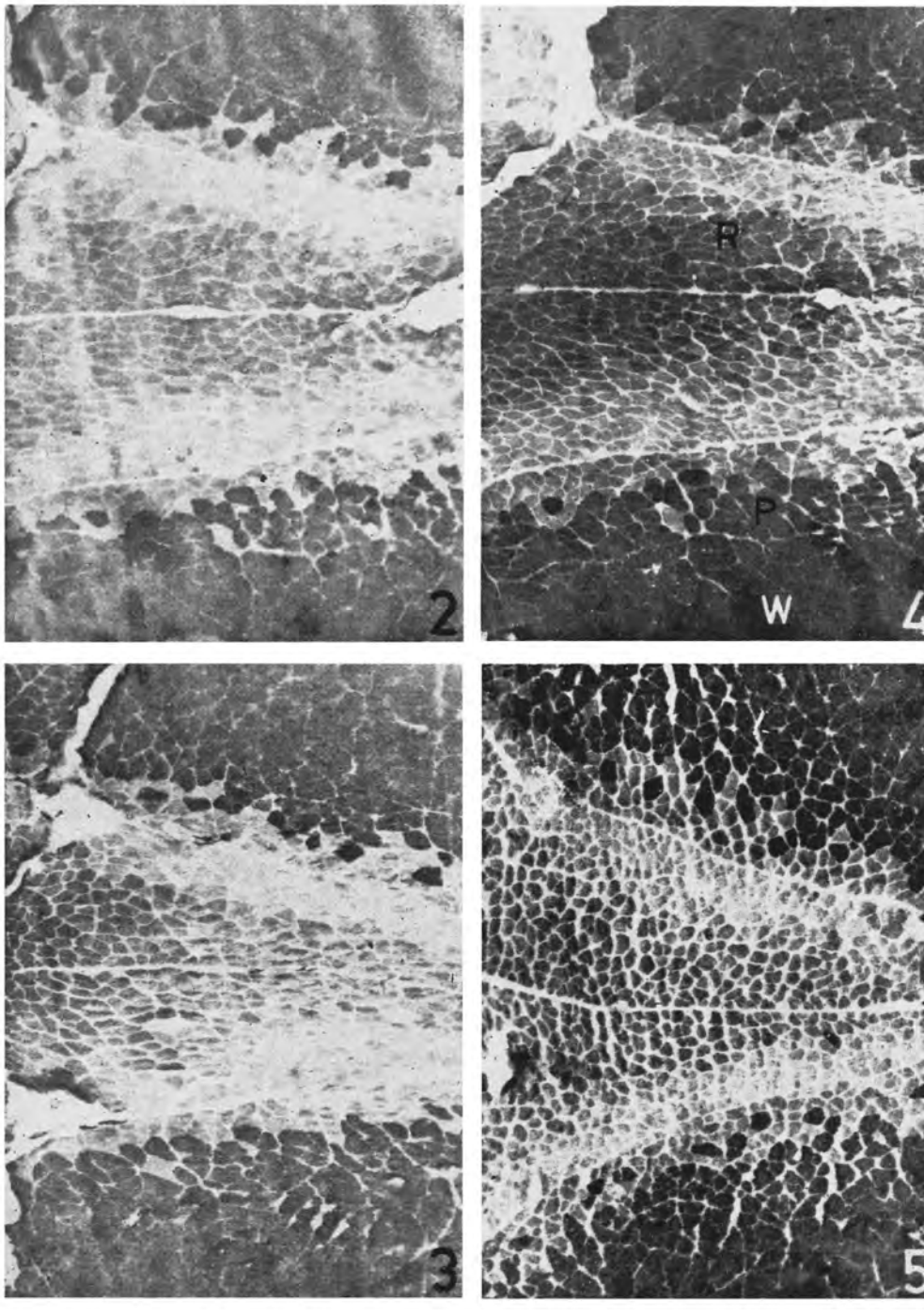


Figure 1 A diagram showing the distribution and relative abundance of red, pink and white muscle fibres in the musculature of a fresh water teleost *Heteropneustes fossilis*

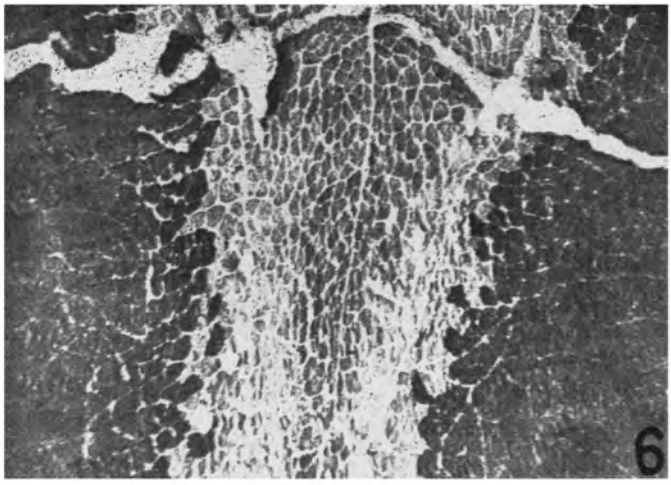
Table 1 *Histochemical pH-profile for m-ATPase in fish muscle*

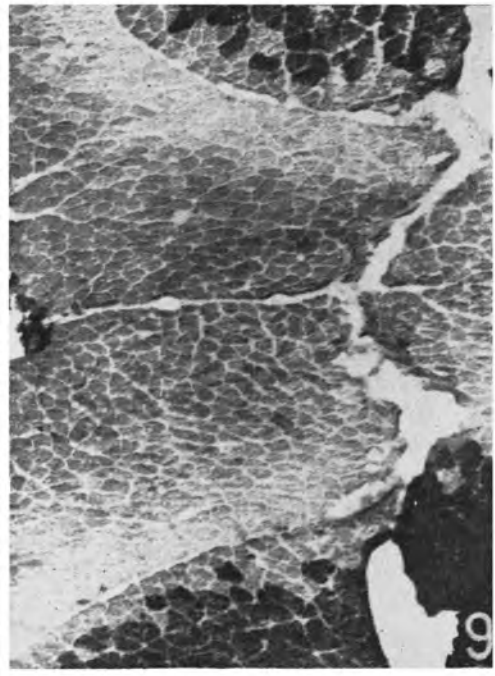
Pre-incubation		Fibre types		
*p*H	Time	Pink	Red	White
4.35–5.45	30 sec–2 min	±	±	±
5.5– 7.5	30 sec–2 min	1+	±	1+
8.0– 9.0	30 sec–2 min	3+	1+	3+
9.4– 9.8	30 sec–2 min	4+	2+	4+
10.0	5 sec	4+	4+	4+
10.0	30 sec–3 min	4+	3+	4+
10.1	5 sec	5+	4+	4+
10.1	30 sec–3 min	4+	3+	4+
10.2	5 sec	4+	3+	4+
10.2	30 sec	4+	2+	4+
10.2	2–4 min	4+	—	4+
10.2	Over 6 min	4+	—	—
10.3	5 sec	4+	1+	3+
10.3	2–4 min	3+	—	2+
10.4	5 sec	4+	—	3+
10.4	30 sec–2 min	±	±	±
10.4	3–5 min	—	—	—
No pre-incubation	—	4+	4+	4+
SDH	—	2+	4+	±

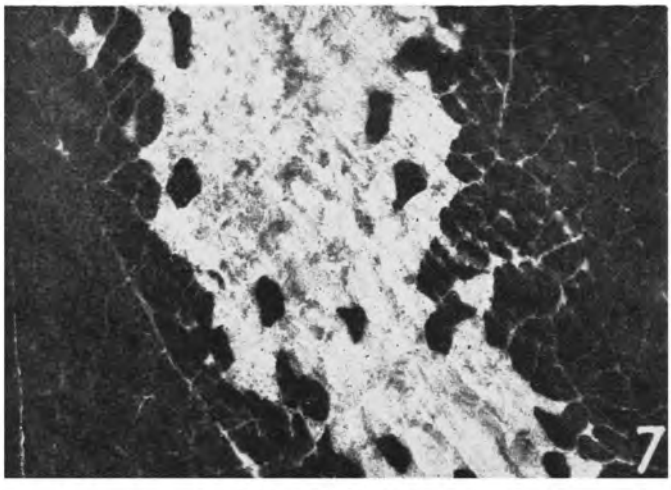
Note: Marks (−) to (5+) represent the staining intensity for enzyme activity.

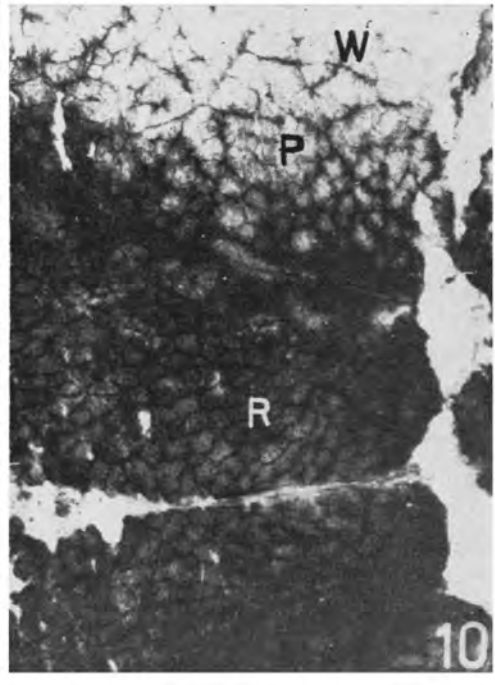


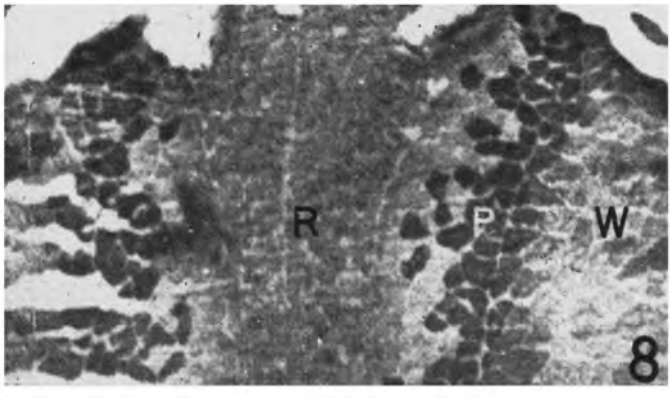
Figures 2–5 Transverse sections of *Heteropneustes fossilis* myotomal muscles showing red (R), pink (P) and white (W) fibres stained for m-ATPase activity after variable pre-incubation *p*H. Incubation at usual *p*H 9.4 (× 120); *2*, Pre-incubation (PI) at *p*H 6.5 (30 sec), some staining is seen in all the three fibre types; *3-4*, PI at *p*H 8.0 and 9.4 (30 sec) respectively. Note progressive increase in staining; *5*, PI at *p*H 10.1 (5 sec), sharp and good staining is seen in all the three fibre types











Figures 6–10 Same as in figures 2–5 but with varying PI period and figure 10 for SDH (×120); *6*, PI at *p*H 10.2 (30 sec)—Red fibre region is partially inactivated; *7*, PI at *p*H 10.2 (2 min)—Red fibre region is completely inactivated while rest of the fibres show usual staining; *8*, PI at *p*H 10.2 (over 6 min)—only pink fibre region is stained; *9*, PI at *p*H 10.3 (30 sec)—Red fibre region is again partially inactivated; *10*, Muscle stained for SDH activity. Red fibres are intensely stained, pink fibres moderately and white fibre region is least stained

skin as well as the central myotomal below lateral line show similar staining in acid and alkaline m-ATPase. These are the most readily inactivated fibres under alkaline pre-incubation conditions. At the same *p*H if the pre-incubation time is increased to over 6 min (figure 8), the whole white myotomal region also gets inactivated and only 3–4 cell thick region at the transition of central red and the remaining white region, is seen to be stained. These are the pink fibres, which do not constitute a distinct separate region but gradually merge into the white fibre region. These pink fibres are the most resistant kind under alkaline pre-incubation conditions. The remaining myotomal region constitutes the white fibres. Their inactivation at alkaline *p*H is between red and pink fibres.

At *p*H 10.3 much less staining was seen in red fibres with pre-incubation time of 5–30 sec (figure 9). The red fibre region was again completely inactivated when the pre-incubation time was increased whereas the pink and white fibres remained stained.

With further increase in *p*H, the staining in all the fibres becomes less intense. At *p*H 10.4 (pre-incubation time below 2 min) only traceable amounts of staining is observed. With increased pre-incubation period (above 2 min) all the fibres are completely inactivated (table 1). No staining is observed with increased *p*H. Thus in the fish *H. fossilis* the fibres become alkali labile at *p*H 10.4.

Sections which were put directly for incubation without any pre-incubation also showed an overall good degree of staining, however, the staining of the individual fibres was neither sharp nor very specific. This shows that pre-incubation at acid or alkaline *p*H, is essential for the actual differential staining of the individual fibres in a muscle for m-ATPase.

Biochemical methods also showed somewhat similar results. Red muscle m-ATPase is the first to show inactivation during exposure to alkali *p*H and also its ATPase activity is minimum (figure 11). White muscle m-ATPase was more resistant to alkali *p*H. The mixed muscle m-ATPase activity is more than that of the pure red and white muscles individually, indicating thereby that the pink muscle situated between red and white, and taken along with white for biochemical preparations, has a higher m-ATPase activity as that of white muscle. Mixed muscle ATPase is shown in the *p*H-profile curve (figure 11) to be the most resistant to alkali *p*H. The pink muscle region appeares to be the most resistant to alkaline *p*H (figure 8) and also stained maximally histochemically and thus shows

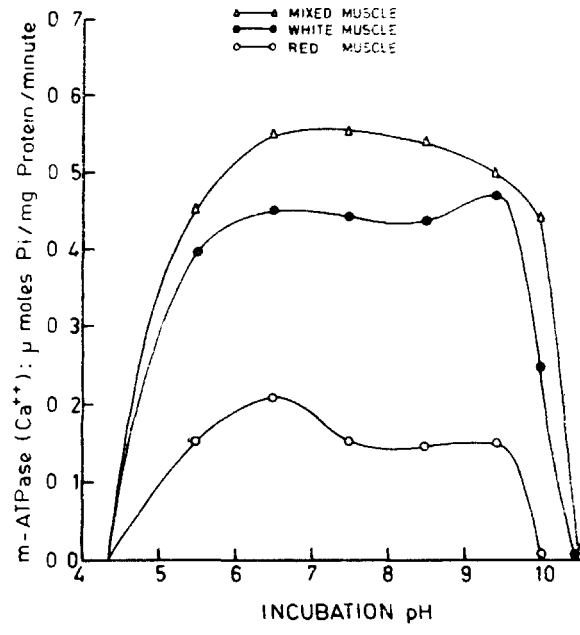


Figure 11 Biochemical *p*H profile of m-ATPase in the red, white and mixed muscles of *Heteropneustes fossilis*

higher m-ATPase as compared to pure red and white muscle population.

For the enzyme SDH also (figure 10), three distinct regions can be observed i.e. red, pink and white. The region just below the lateral line and also a thin sheet just underneath skin (figure 1), show high degree of staining (figure 10). This is the region which gets most readily inactivated and also with minimum m-ATPase activity and constitutes the red fibres. Some staining is also observed just along the inner boundary of red fibres, constituting the pink fibres which are the most resistant kind under alkaline pre-incubation conditions and also have high m-ATPase activity. The remaining myotomal region remains almost unstained for SDH and constitutes the white fibres. These also have high m-ATPase activity and the fibres show inactivation under alkaline conditions between red and pink.

Earlier studies have shown that 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 the m-ATPase activity (Barany 1967). Present histochemical and biochemical observations indicate that the pink muscle may be a fast contracting as that of white, having high m-ATPase activity. Red muscle having low m-ATPase activity may be a slow contracting muscle. This finding is of particular relevance to earlier studies (Lowey & Risby 1971) which reported that the light chain pattern of pink and white muscle myosin of a teleost fish was identical and characteristic of vertebrate fast twitch fibres. It has also been found (Lowey & Risby 1971, Weeds & Pope 1971 and Johnston et al. 1977) that the teleost red muscle shows only two light chains with different mobilities to the components found in the pink and white muscle myosin. This electrophoretic pattern is characteristic of the light chain phenotype of slow twitch vertebrate myosins. The actual co-ordination and contraction pattern of these different muscles during swimming is a matter of conjecture only at present. Further investigations with a coupled histochemical and biochemical studies on a variety of fresh water teleost fishes may throw some light on the role of m-ATPase and light chains in fish muscles.

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