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Characterization of Fish Myotomal Muscle Types Based on SDH, m-ATPase and Myosin Light Chain Studies

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Correlative histochemical and biochemical studies have been carried out in the myotomal muscle of five teleost fishes with special reference to SDH and m-ATPase. The myosin light chains of red, white and pink muscles of these fishes have also been studied electrophoretically using polyacrylamide gels. The red muscles show a more uniform pattern of myosin light chains, generally two. In contrast, the pink and white muscles reveal three bands. The light chain pattern seems to be independent of the species and characteristics of a muscle type. Fish muscles reveal the same myosin light chain pattern as mammalian muscles but with more variability of molecular weights.

Key Words: Myosin light chains, Fibre types, Fish myotomal muscles, Myofibrillar-ATPase

Introduction

Vertebrate skeletal muscles are generally classified by a number of inter-related histochemical, biochemical and physiological parameters. Among the various vertebrate groups, the teleost myotomal muscles exhibit a very good experimental model for correlative studies because of the zonal segregation of the fibre types. The different muscle layers (red, white and pink) can be easily separated and analyzed.

It is well documented that the capacity of skeletal muscle adaptation to different functional requirements is invariably reflected in changes of polymorphic forms of myofibrillar proteins (Syrovy 1973, Syrovy & Gutmann 1975, Holt & Lowey 1977, Albis et al. 1979, 1982; Billeter et al. 1981, Johntson 1982). The most important of these is the myosin, being involved in the regulation of ATPase activity.

The subunit composition of skeletal myosins in fishes and other groups of vertebrates consists of two heavy chains and four light chains. The light chains (low molecular weight proteins), indicate the myosin nature as well as contractile capacity of the muscle (Focant et al. 1976, Perzanowska 1979, Syrovy 1979, Pinter et al. 1981). The myosin light chains can be classified according to their relative electrophoretic mobilities on polyacrylamide gels in the presence of SDS showing characteristic band pattern. The fastest and slowest moving components in these gels are LC_3 and LC_1 respectively, corresponding to the alkali light chains. The middle component LC_2 corresponds to DTNB-light chain. Removal of LC_1 and LC_3 in alkaline conditions results in complete loss of ATPase activity whereas treatment of myosin with DTNB results in the removal of LC_2 without loss of ATPase activity.

The present investigation has been undertaken on five freshwater teleost fishes in an attempt to characterize the myotomal muscle types from homogenous populations by correlative histochemical and biochemical studies with respect to SDH, m-ATPase and myosin light chains.

Materials and Methods

Five freshwater teleost used in the investigation were Stinging catfish, *Heteropneustes fossilis*; catfish, *Clarias batrachus*; murrel, *Channa punctatus*; Spiny eel, *Mastacembelus pancalus*; and Rohu carp, *Labeo rohita*. Muscle samples were taken from the region behind the pectoral fin—containing the middle red region with substantial amounts of white muscle. In

Labeo rohita pink region was quite well developed and could be separated for individual type analysis.

Small muscle pieces were mounted on chucks and quickly frozen in liquid nitrogen and cut in a cryostat. They were stained for enzymes SDH (Nachlas et al. 1957) and m-ATPase (Padykula & Herman 1955 modified by Guth & Samaha 1970). Ca^{++}-activated ATPase reaction, was stindict incubation *p*H 9.4 after preincubating for 5–10 sec in *p*H 10.3 (Shashi Kiran & Talesara 1984a)

Preparation of myofibrils: Myofibrils were prepared basically by the method of Perry and Grey (1956) and incubated overnight at 37°C in the buffer containing Tris-SDS-β-mercaptoethanol (*p*H 6.8) and centrifuged at 2000 rpm for 10 min. The supernatant was used for loading on top of the gel. The sample before loading was dissolved in 40% sucrose.

SDS-polyacrylamide gel electrophoresis: Myosin light chains were separated by SDS-PAGE. Electrophoresis was carried out in a system described by Laemmli (1970). Equal quantity of protein samples was loaded in each lane and the different bands were identified by running standard proteins separately. Slab gels were stained in Coomassiee brilliant blue and after destaining relative amount of different isozymes of myosin light chains, in a particular sample was evaluated densitometrically in a Desaga Quick Scan densitometer, Heidelberg, FRG.

Enzymatic assays: The incubation medium for Ca^{++}-activated ATPase assay contained 40mM Tris-HCl (*p*H 7.5), 40mM KCl, 10mM $CaCl_2$, 0.2 ml of myofibrils and 3 mM of ATP. The detailed procedure was same as reported earlier (Talesara & Goldspink 1978). The assay procedure for SDH was essentially based on that of Nachlas et al. (1960) using INT [2(p-Iodophenyl) 3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride] as the artifical electron acceptor. Protein concentration was determined following Gornall et al. (1949).

Results and Discussion

Histochemical results of the two enzymes, SDH and m-ATPase on the myotomal muscles of carp, *Labeo rohita*, are shown in figures 1 and 2. The staining intensity of these enzymes give preliminary impression of the ratio of energy producing and energy utilizing reactions, respectively. SDH activity gives a measure for the oxidative energy production and m-ATPase is related to the speed of shortening.

The results can be correlated with the biochemical estimation of the above two enzymes (table 1). The relative predominance of the oxidative metabolism for energy production in the muscle fibretypes was Red > Pink > White, while the level of activity of m-ATPase was White > Pink > Red.

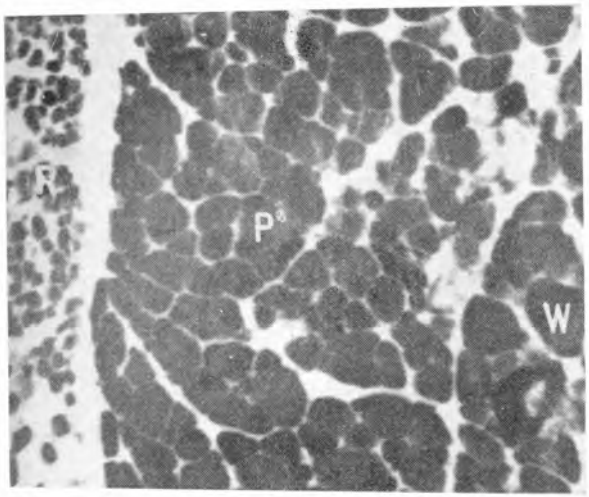
Table 1 *Succinic dehydrogenase and Myofibrillar-ATPase activity* in the various muscles of five teleost fishes*

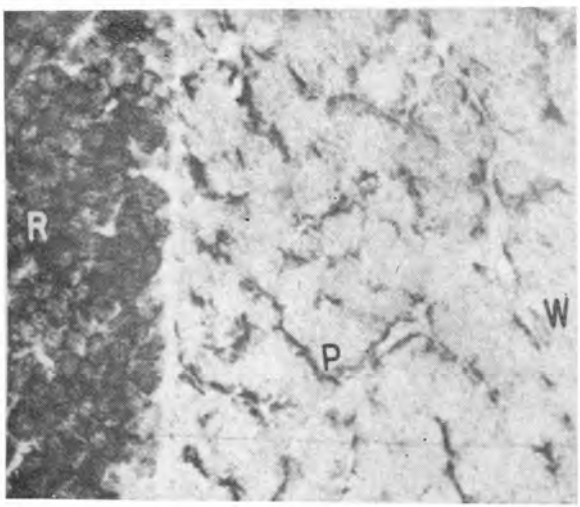
Fishes	*Muscles*		
	Red	*White*	*Pink*
Heteropneustes fossilis			
SDH	2.255 ± 0.107	0.815 ± 0.024	—
m-ATPase	0.192 ± 0.016	0.454 ± 0.028	
Clarias batrachus			
SDH	3.045 ± 0.435	0.792 ± 0.188	—
m-ATPase	0.142 ± 0.015	0.685 ± 0.049	
Channa punctata			
SDH	1.575 ± 0.185	0.821 ± 0.132	—
m-ATPase	0.217 ± 0.022	0.815 ± 0.118	
Mastacembelus pancalus			
SDH	1.771 ± 0.096	0.333 ± 0.052	—
m-ATPase	0.205 ± 0.015	1.051 ± 0.018	
Labeo rohita			
SDH	2.221 ± 0.217	0.560 ± 0.049	1.092 ± 0.232
m-ATPase	0.218 ± 0.011	0.807 ± 0.020	0.578 ± 0.046

*Values are mean ± S E
SDH: μg formazan/mg protein/min
m-ATPae: μ mole Pi/mg protein/min

In the light of above as well as our previous studies (Talesara & Shashi Kirah 1982, 1983, 1986, Shashi Kiran & Talesara 1984a & b, 1985), the red muscle of teleosts may be classified as slow twitch (Type I) and white as the fast twitch (Type IIB), the pink muscle in *L. rohita* distinguished as a separate class with distinct histochemical and biochemical characteristics as fast twitch type IIA or C.

The results of the SDS-PAGE of the muscle types in *Labeo rohita* along with their respective densitometric scans is shown in figure 3. A 'finger-print' of the light chains of the muscle types in different teleost species examined is shown in figure 4. The classification of these light chains has been based on their electrophoretic mobilities on SDS- polyacrylamide gels with approx. molecular weights ranging from 15000–27000 daltons. It is also noticed that the DTNB-LC is found with almost same molecular weight (about 18,000) in myosin from all striated muscles. The





Figures 1–2. Transverse section of the myotomal muscles of fish *Labo rohita* (× 120) **1**, stained for SDH; **2**, stained for mATPase. Incubation *p*H 9.4, preincubation *p*H 10.3 (5–10 sec) (R, red; P, pink, W, white muscle)

two other light chains (alkali) are subject to larger species variations.

Also, in fishes there seems to be greater variations with regard to molecular weight among light chains relative to birds and mammals (Perrie & Perry 1970, Lowey & Risby 1971). It has been reported that fast twitch muscles from rabbit (Lowey & Risby 1971), sheep (Weeds & Pope 1971), cat (weeds et al. 1974) and chicken (Lowey & Risby 1971) all show three light chain components of identical mol. wts, 25000 (LC_1); 18000 (DTNB-LC) and 16000 (C_3). In fish fast muscle myosin, only one light chain, of a mot. wt. 18000 daltons, is found homologous in the different fishes. In carp and pike (Focant & Huriaux 1976), this light chain is removed by treatment with DTNB and can be phosphorylated as the mammalian DTNB light chain. In contrast, the 'alkali' light chain components of fish fast muscle myosin show a range of mol.wt. for each subunit between species. These mass differences may be attributable at least in part to the greater phylogenetic diversity of fish as a group. Accordingly, the present results showing great variability in the pattern of 'alkali' light chains (essential) in different fish species may be related to the adaptations in the enzymatic activities of myosins. This is so because the fish red and white muscles of different species of teleost show considerable adaptive differences, reflected in swimming behaviour and contraction speed (Johnston & Tota 1974) between homologous muscles. Fishes being poikilotheamic, the compensatory adjustments in ATPase activity in different species (Johnston et al. 1975) may result in evolutionary modification in the light chain components. Our findings are in agreement with the earlier ones (Weeds & Pope 1971, Focant et al. 1976, Johnston 1982) in that the light chains determine the response of a muscle through the regulation of the activc site of myosin.

Moreover, it is very clear that only in the carp (*Labeo rohita*) the histochemical, and electrohoretic studies can be correlated because of the segregation of all the three muscle layers, assessable for the individual type analysis. This kind of ready assessibility is nowhere found in the muscles of higher vertebrates. That is to say, the teleost fishes, especially the carps can be exploited to a much greater extent for studies comparative of different characteristics of the individual muscle types.

As far as one can generalize from a limited number of species tested, identical muscles from fishes of different origins display similar myosin light chains electrophoretic pattern in SDS–PAGE. Furthermore, these light chain components of fishes can be well correlated with those of higher vertebrates, thereby revealing that the differentiation of light chains is a common feature in all vertebrates and therefore

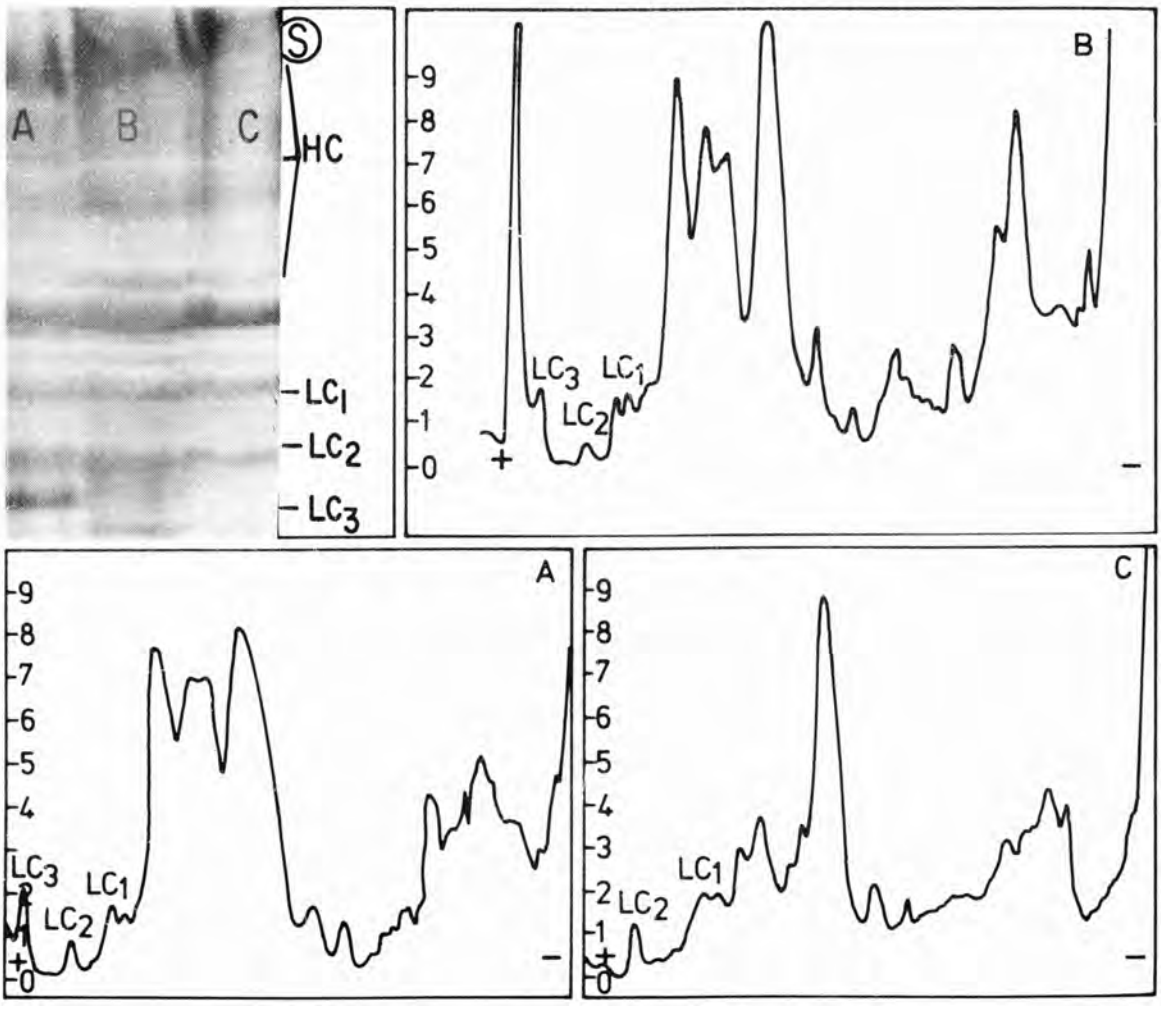


Figure 3 SDS-polyacrylamide gel electrophoregram of the myotomal muscles of the fish, *Labeo rohita* (slab gels A-C) showing myosin light chain (LC) and heavy chain (HC) patterns (S). The corresponding densitometric scans of the gels A-C (as shown in S) are shown separately representing different muscles. (A, White, B, Pink, C, Red muscle)

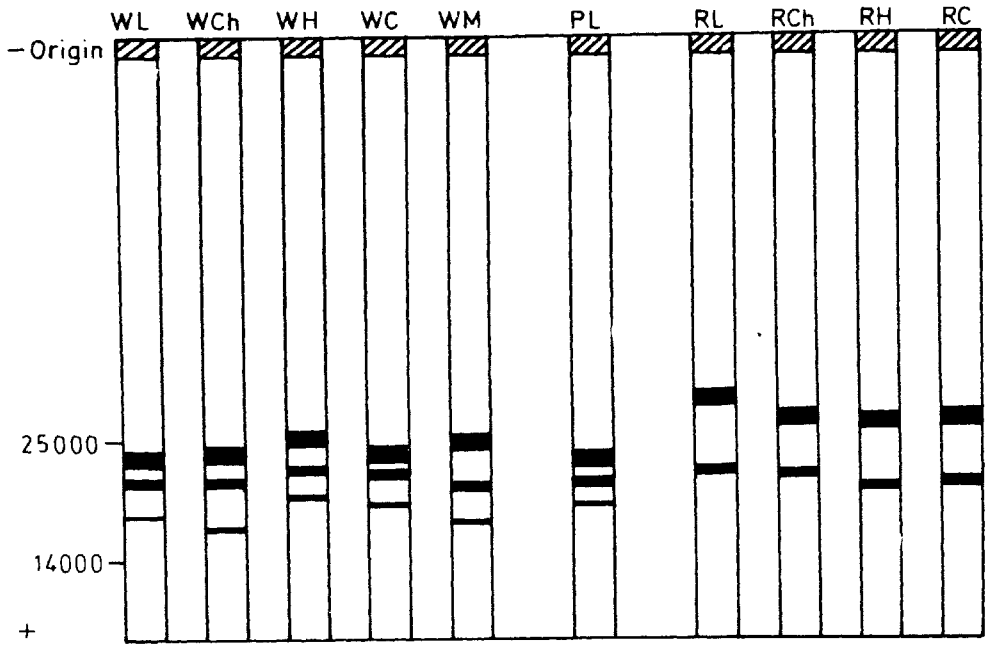


Figure 4 Schematic picture extrapolated from electrophoretic patterns of White (W), Pink (P), and Red (R) muscles of *Labo rohita* (L), *Channa punctata* (Ch), *Heteropneustes fossilis* (H), *Clarias batrachus* (C) and *Mastacembelus pancalus* (M). Black bands represent myosin light chains. Striped bands at origin correspond to heavy chains. Estimated molecular weights are represented in ordinate

constitutes a very good criterion for the characterization of different muscle types. Also, the easy electrophoretic characterization of myosin isozymes must allow the use of myosin as a decisive parameter in a variable number of biological studies.

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