

SUBUNIT COMPOSITION OF FISH MYOFIBRILS: THE LIGHT CHAINS OF MYOSIN

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Abstract—1. The myosin light chains from red and white skeletal muscles myofibrils, actomyosin and myosin of five fish species have been investigated by polyacrylamide gel electrophoresis.

2. Three light chains were found in myosins of white muscle. Red muscle myosins possess two light chains which do not correspond to any of the light chain components of white muscle myosins.

3. Fish myosins have the same light chains pattern as mammal myosins but show a greater variability of mol. wt.

INTRODUCTION

Mammalian and chicken skeletal muscle myosins contain two large polypeptide chains (heavy chains) of mol. wt around 200,000 daltons (Gazith *et al.*, 1970) together with a variable number of small polypeptide chains (light chains) of about 20,000 daltons each. Myosin extracted from white (or fast twitch) skeletal muscles possesses three different light chain subunits (Lowey & Risby, 1971; Weeds & Lowey, 1971) which appear to be implicated in the regulation of the enzymic activity of myosin (Stracher, 1969; Dreizen & Gershman, 1970). Red (or slow twitch) muscle myosins possess only two distinct light chains different from that of white muscles but similar to that from cardiac myosin (Lowey & Risby, 1971; Sarkar *et al.*, 1971).

In most fish, distinct red and white muscles, composed of homogeneous populations of fibre types, are easily recognizable in the trunk musculature. In general, red muscle is used for slow speed swimming, while locomotion at higher speeds and during bursts of activity is achieved by the contraction of increasing numbers of white fibres (Greer-Walker & Pull, 1973; Johnston & Goldspink, 1973a,b).

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‡ Abbreviations used: SDS, sodium dodecyl sulphate; EDTA, ethylenediaminetetracetic acid; Tris, Tris(hydroxymethyl)aminomethane; ATP, adenosine-5'-triphosphate; DTNB, 5,5'-dithio-(bis 2-nitrobenzoic acid).

There have been few comparative studies on fish red and white muscle myosins probably because of the instability of these proteins in many species (Hamoir *et al.*, 1960; Connell, 1961; Syrový *et al.*, 1970). To the authors knowledge no previous systematic studies on the light chain components have been undertaken. Preliminary experiments have established that the myosin light chains are different in the white, red and cardiac muscles of carp (Huriaux & Focant, 1974). In view of the considerable variety in locomotory behaviour shown by different fish it was thought to be of interest to investigate the myosin light chains from homologous muscles of a number of species. Myosin light chain components of white and red swimming muscles of carp, pike, dogfish, mackerel and angler-fish have been compared by polyacrylamide gel electrophoresis in urea and SDS.‡

MATERIALS AND METHODS

Five species of fish were used in these investigations, two freshwater species, carp (*Cyprinus carpio* L.) and pike (*Esox lucius* L.) (obtained locally in Belgium) and three marine species, dogfish (*Scyllium canicula* L.), mackerel (*Scomber scombrus* L.), and angler-fish (*Lophius piscatorius* L.) (obtained from the English Channel). The fish were stunned by a blow to the head and killed by decapitation. White muscle was dissected from the whole lateral dorsal muscle. Red muscle was excised from the lateral line, care was taken to exclude any of the so called pink fibre types which are situated between the red and white muscle regions. Myofibrils, actomyosins and myosins were prepared from the muscles immediately after excision. All preparations were carried out at 4°C unless otherwise stated in the presence of 2 mM 2-mercaptoethanol.

Preparation of myofibrils

Muscle was minced with scissors and homogenised at 0°C with a Polytron blender (Kinética GmbH, Luzern, Switzerland) in 10 vol of 0.1 M KCl; 5 mM EDTA; 10 mM Tris-HCl pH 7.0. Homogenisation was for 3 periods of

25 sec interspersed with periods of cooling in ice. The homogenate was spun at 2000 *g* for 10 min and the myofibrils prepared from the residue as described previously (Johnston & Tota, 1974). All stages of the preparation were monitored by careful microscopical examination. Myofibrils were stored in a medium of 0.1 M KCl; 5 mM Tris-HCl pH 7.0; 1 mM 2-mercaptoethanol at 0°C at a concentration of approx 5 mg/ml.

Preparation of actomyosin

The minced muscle was extracted for 30 min with 6 vol of 0.5 M KCl; 0.03 M NaHCO₃ (I = 0.53, pH 8.1) and left overnight at the laboratory temperature. The muscle debris was removed by centrifugation for 10 min at 27,000 *g*. The supernatant was diluted by 6 vol of water and the pH lowered to 6.3 with N acetic acid. The precipitated actomyosin was isolated by centrifugation for 10 min at 14,700 *g*, washed twice by resuspension in water and in 0.03 M KCl, dissolved and dialysed against a phosphate-NaCl buffer of I 0.35 and pH 7.1 (0.0308 M Na₂HPO₄; 0.0077 M NaH₂PO₄; 0.25 M NaCl).

Preparation of myosin

White muscle myosin. This was prepared by neutral ammonium sulphate fractionation of the actomyosin in the presence of 0.4% ATP and 0.02 M MgSO₄. The myosin isolated between 35 and 60% saturation was dialysed against the I 0.35 phosphate-NaCl buffer described above.

Red muscle myosin. Since red muscle myosin cannot be isolated, with a good yield and purity, by ammonium sulphate fractionation, the following faster method was used.

The mince was extracted for 10 min with 3 vol I 0.7 phosphate buffer of pH 6.7 (175 mM KH₂PO₄; 175 mM K₂HPO₄) containing 2 mM disodium ATP. After elimination of the muscle pulp by centrifugation at 27,000 *g* for 10 min, the extraction fluid was diluted by the same volume of water and dialysed 24 hr against a phosphate-NaCl buffer of I 0.25 and pH 7.2 (0.022 M Na₂HPO₄; 0.0055 M NaH₂PO₄; 0.179 M NaCl). The precipitated actomyosin was removed by centrifugation at 27,000 *g* for 10 min. The supernatant was diluted to I 0.05 with water and acidified to pH 6.3. The myosin precipitate was isolated and washed as for the actomyosin, dissolved and dialysed against the usual phosphate-NaCl buffer of I 0.35 and pH 7.1. A final clarification was carried out by centrifugation at 105,000 *g* for 1 hr.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed in a vertical gel slab of 18 × 8 × 0.2 cm under two different sets of conditions.

(a) **Urea system.** Electrophoresis was carried out in 8 M urea at pH 8.6 by the method of Akroyd (1967), as modified by Perrie & Perry (1970). Both running and gel buffers contained 0.02 M Tris and 0.12 M glycine. Gel buffer and protein samples (10 mg/ml) were made 8 M in urea. The gel was defined giving respectively the acrylamide (Acr) and bisacrylamide (Bis) percentages in the gel solutions. In these experiments Acr × Bis = 10 × 0.26.

(b) **SDS system.** Molecular weights were determined by electrophoresis in the presence of sodium dodecyl sulphate (SDS) using a discontinuous buffer system and two superimposed gels of different polyacrylamide concentrations according to Neville (1971). Buffers were made as follows: upper cathodic buffer: 0.04 M boric acid, 0.041 M Tris, 0.1% SDS (w/v), pH 8.64; upper gel buffer: 0.027 M

H₂SO₄, 0.054 M Tris, pH 6.1; lower gel and anodic buffer: 0.4244 M Tris, 0.0308 M HCl, pH 9.18. Compositions of the gels (Acr × Bis) were 3.2 × 0.2 for the upper gel and 15 × 0.1 for the lower gel.

Protein samples at 10 mg/ml were dissolved in 0.05 M Tris-HCl pH 7.5 and made 2% in SDS and 4% in 2-mercaptoethanol. They were heated at 100°C for 3 min. Before application on the gel, a few crystals of saccharose were added to increase the density.

All electrophoresis was carried out at a constant current of 20 mA (about 200 V) for about 4 hr. Eight samples (0.1–1 mg) and a tracking dye were applied. Gels were stained with Coomassie brilliant blue and destained as described by Weber & Osborn (1969). Mobilities and relative proportions of the bands were determined by scanning the gels with a Quick-Scan densitometer (Helena laboratories, Beaumont, TX). Molecular weights were calculated using a calibration curve (distance of migration vs log. mol. wt) made by running various proteins of known mol. wt (11,700–68,000) under the same electrophoretic conditions. The estimated mol. wts were determined from the means of a large number of gels (usually more than 10) from at least 6 different (only 3 for angler-fish) preparations of myosins, actomyosins, and myofibrils from different fish.

Reagents

All reagents were of analytical grade. Sodium dodecyl sulphate, acrylamide, N, N'-methylenebisacrylamide and Coomassie Brilliant Blue were purchased from Serva, Heidelberg, Germany. N, N', N'-tetramethylenediamine from Fluka, A. G., Buchs SG, Switzerland. Urea and glycine were from Merck, Darmstadt, Germany. Tris and ATP from Sigma Chemical Co., St Louis, MO, U.S.A., 2-mercaptoethanol from Calbiochem, Los Angeles, CA, U.S.A.

Various highly purified proteins were used for the calibration curve of the mol. wt determinations: serumalbumin and ovalbumin from Sigma, carboxypeptidase A, myoglobin and cytochrome C from Schwarz Mann, Division of Becton-Dickinson and Co, Orangeburg, NY, U.S.A., trypsin from Worthington Biochemical Corp., Freehold, NJ, U.S.A. and β-lactoglobulin from Calbiochem.

Determination of protein concentration

The protein content was determined by the biuret method (Gornall *et al.*, 1949) for the myofibrils and by refractometry with sodium light at 25°C, assuming a refractive index increment of 0.182 ml/g (Hamoit, 1955) for the actomyosins and myosins.

RESULTS AND DISCUSSION

The species of fish used in this present study are all native to North-West Europe and its continental shelf and consequently have the unstable type of myosins. Conventional chromatographic techniques used to isolate mammalian myosins yield fish myosin preparations of low enzymic activity due to the formation of aggregated and denatured products.

Myofibrils which contain all the proteins of the contractile machinery, mainly actin, myosin and proteins of the calcium regulatory tropomyosin-troponin complex, are far more stable than the corresponding myosins (Johnston *et al.*, 1972). A parallel study of the myosin light chains from myofibrils has the

advantage of allowing the detection of any possible changes in electrophoretic mobility in these subunits which may have resulted from the denaturation of myosins. Subtraction of myosin components from gel patterns from myofibrils and actomyosins enables the identification of actin, tropomyosin and a few proteins probably belonging to the troponin complex on the basis of mol. wt. together with the myosin light chains. The migration of light chain components were found to be similar from preparations of myofibrils, actomyosins and myosins. Species variation in the mobility of the light chain components were therefore not attributable to any differences in myosin stability between the different fish.

The gel patterns obtained upon electrophoresis of myofibrils, actomyosins and myosins in 8 M urea at pH 8.6 are relatively simple since only tropomyosin and light chains enter the gel; other proteins have either too large a mol. wt or else form complexes. Proteins migrate in this type of gel according to their size, shape and charge. Interpretation is made difficult however, since each band does not necessarily correspond to a different light chain. Indeed, recent studies on carp (Focant *et al.*, 1974) and pike white muscle myosins have indicated that the same light chain can afford two or three bands according to its level of phosphorylation or its state of preservation. The number of sub-bands also increases with time of storage in 8 M urea.

However, it is possible to obtain a "finger print" of the light chains from urea gels which is characteristic of the species examined. In the white muscle of the fish in the present study the number of bands seems to vary between species (Fig. 1). The red muscles investigated showed a more uniform pattern of bands (Fig. 1): generally there were two bands,

one migrating slightly in front of tropomyosin and another faster migrating band.

In SDS gels myofibrils dissociate into their constituent components, principally myosin heavy and light chain subunits, actin, tropomyosin and the troponin subunits. A comparison of myofibrils, actomyosins and myosins allows a classification of these proteins. Figure 2 shows the part of the myofibril gel pattern containing proteins with mol. wts lower than actin and tropomyosin. The components of mol. wt 21,000 and 34,000 daltons present in myofibril gels were absent in gels of the corresponding myosins. From a consideration of the mol. wts of these bands and information in the literature (Greaser & Gergely, 1971), it seems likely that they correspond to components of the troponin complex. It is interesting to note that the 21,000 daltons component was absent from red muscle myofibrils. Summarizing the band patterns for all five fish we find 3 light chains in the white muscle myosins with the exception of the angler-fish. However, the fast migrating band of this species was very large and may well correspond to 2 bands of similar mol. wt. Comparison of electrophoretic mobilities with proteins of known mol. wt gives values from 16,000 to 27,000 daltons for their mol. wts (Fig. 2). Red muscle myosin possesses two light chains of mol. wt ranging from 17,000 to 24,000 daltons which have no counterpart in the white muscles. The slow component sometimes appears as a double band as in rabbit red muscle myosin (Sarkar *et al.*, 1971).

Thus fish white and red muscle myosins have a characteristic light chain pattern which is similar to that found in mammalian white (fast twitch) and red (slow twitch) muscles respectively (Lowey & Risby, 1971; Sarkar *et al.*, 1971). Also as in mammals the

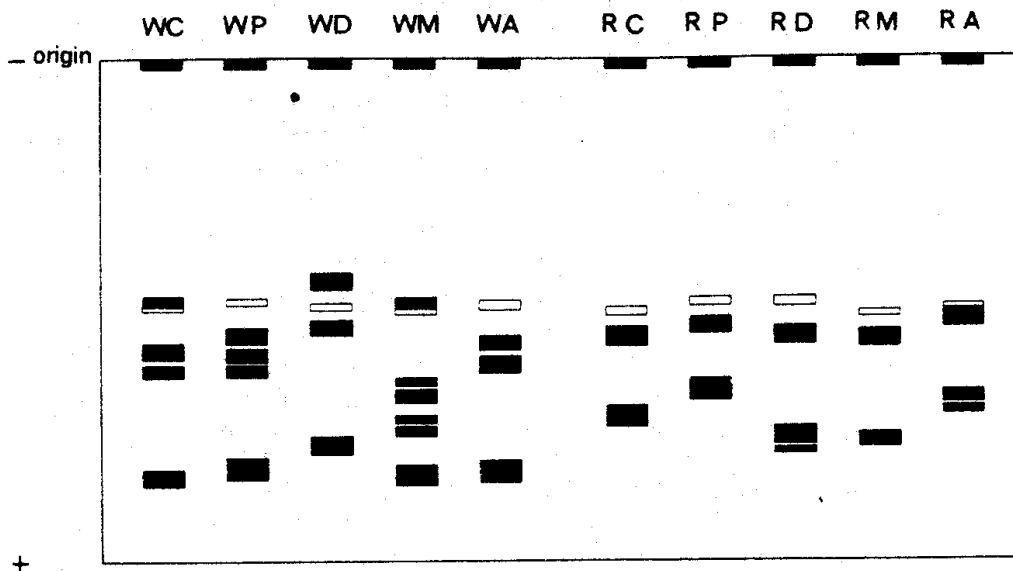


Fig. 1. Polyacrylamide gel electrophoresis in 8 M urea. Schematic picture extrapolated from myofibrils, actomyosins and myosins electrophoretic patterns of white (W) and red (R) muscles of carp (C), pike (P), dogfish (D), mackerel (M) and angler-fish (A). Clear bands represent the tropomyosin, black bands the light chains (except bands at origin corresponding to heavy chains).

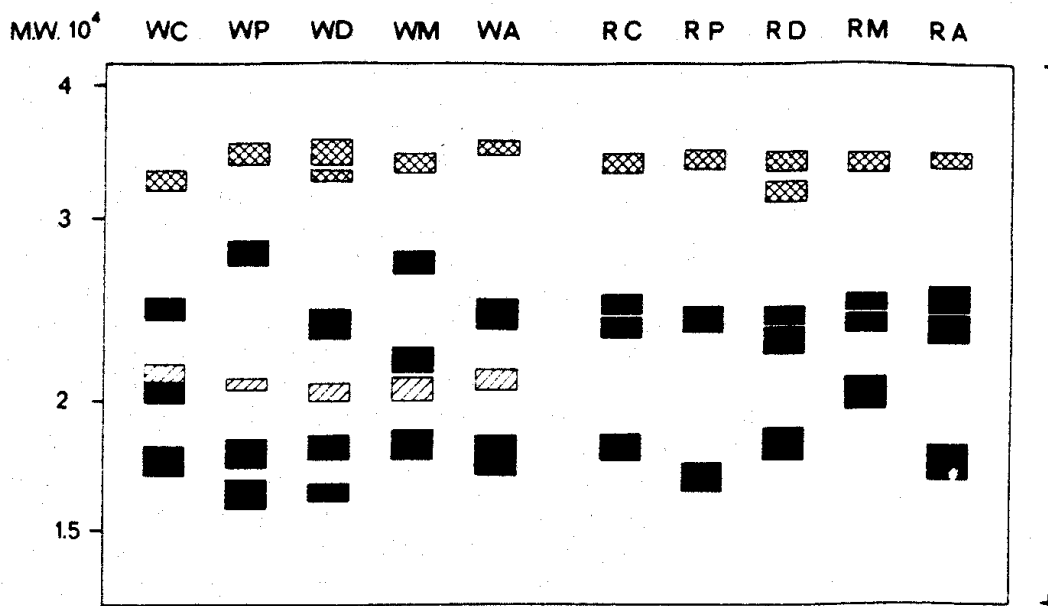


Fig. 2. Polyacrylamide gel electrophoresis in the presence of SDS (see analytical methods). Schematic picture of the lower part of the 15% acrylamide gel, extrapolated as in Fig. 1. Black bands represent light chains. The cross-hatched and striped bands correspond to components of the troponin complex. Estimated mol. wts are plotted in ordinate.

light chain components of the fish fast muscles did not correspond to any of the components in the slow muscles (Lowey & Risby, 1971). This differentiation appears to be a common feature in all vertebrates and therefore it constitutes a very good criterion for the characterization of muscle types.

However, there seems to be more variation both in charge and mol. wt between light chains from different fish relative to birds and mammals (Perrie & Perry, 1970; Lowey & Risby, 1971). For example, fast twitch muscles from the following animals: rabbit (Lowey & Risby, 1971), sheep (Weeds & Pope, 1971), cat (Weeds *et al.*, 1974) and chicken (Lowey & Risby, 1971) all have three light chain components of identical mol. wts, 25,000 (Alk 1), 18,000 (DTNB) and 16,000 (Alk 2). In fish fast muscle myosins, only one light chain, of a mol. wt of 18,000 daltons, is found homologous in the different fish. In carp (Focant *et al.*, 1974) and very likely in pike this light chain is removed by treatment with DTNB and can be phosphorylated as the mammals 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 (Fig. 2). These mass differences may be attributable at least in part to the greater phylogenetic diversity of fish as a group. The results obtained may also be related to various adaptations in the enzymic activity of the myosins of the different species. For example, there are considerable adaptive differences in locomotory function between red and white muscles among species. This is reflected in differences in swimming behaviour and possibly contraction speed (Johnston & Tota, 1974) between homologous muscles. Also, unlike mammals and birds, fish are poikilothermic and the compensatory

adjustments in ATPase activity found in species adapted to different environmental temperatures (Johnston *et al.*, 1975; Johnston & Goldspink, 1975) may result in evolutionary modifications in the light chain components. In either case these results are consistent with the view that the light chains determine the response of a muscle through the regulation of the active site of myosin (Stracher, 1969; Dreizen & Gershman, 1970; Lowey & Risby, 1971; Weeds & Pope, 1971).

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