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Myosin structure in the eel (*Anguilla anguilla* L.)

Demonstration of three heavy chains in adult lateral muscle

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Myosin extracts from central white fibers and peripheral red fibers of the lateral muscle of eel (*Anguilla anguilla*) were analysed by electrophoresis under non-dissociating conditions, which demonstrated a polymorphism of myosin isoforms. The light and heavy subunit content of the isomyosins was established using SDS-PAGE and two-dimensional electrophoresis. In the central white muscle, 3 myosin isoforms FM3, FM2, FM1, were characterized by 3 types of fast light chain and one fast heavy chain HCf; the existence of a fourth isomyosin is discussed. In the peripheral red muscle, two myosin isoforms were found, SM1 and SM2, each characterized by a specific heavy chain, HCs1 or HCs2, and containing the same slow light chain content. This work demonstrates for the first time the existence of 3 heavy chains in the skeletal muscle of a fish.

Myosin; Gel electrophoresis; Myosin subunit composition; *Anguilla anguilla*

1. INTRODUCTION

Myosin polymorphism has been well established in mammals, birds and amphibians, in which various myosin isoforms, differing by their heavy chain and/or their light chain composition, were characterized in pyrophosphate gels [1,2]. In fishes the myosin structure is less clear. Light chain pattern, ATPase profile and immunological properties were analysed in the white (fast) and red (slow) muscles from a few species [3-6]. Red, pink, and white muscular fibers in the fish, are likely to be related respectively to the mammalian fiber types I, IIA and IIB [4], and seem to be characterized by different heavy chains as suggested by ATPase profile and peptide mapping [7]. In mammals, the 3 fiber types were shown to be each characterized by a specific heavy chain [8]. In the fish, recent studies using SDS-PAGE in the presence of glycerol have demonstrated the existence of two heavy chains, HCs and HCf, respectively related to red and white muscles [9,10]. Fish myosin presents a high lability when compared to myosin from mammals [11], and only few publications [9,10] analysed fish isomyosins in native conditions. In the present work, myosin isoforms were studied in the lateral muscle of the eel (*Anguilla anguilla* L.), within the peripheral part of the muscle which contains the red fibers, and the central region of the muscle containing the white fibers. The light chain content of the isomyosins was clearly established. The existence of 3 distinct heavy chains, two of which being specific of the red fibers and one characterizing the white fibers, was demonstrated for the first time in the fish skeletal muscle.

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2. MATERIALS AND METHODS

2.1. *Animals, ATPase profile, sampling and myosin extraction*

Yellow eels (*A. anguilla*) of about 0.8 kg were captured in their natural biotope in Brittany and kept under laboratory conditions. Study of the myofibrillar ATPase profile [12] was used for control of the muscular sampling. The peripheral region of the lateral muscle was composed of red fibers, and the central region of white fibers (Fig. 1). Pink fibers, in very small amounts, were suspected to be disseminated in the border part between the peripheral and central regions, which had never been quoted by other authors [4]. Samples of peripheral red muscle and of the deep white muscle were carefully dissected and immersed in liquid nitrogen. Preparation of crude myosin was performed in ice, according to [13].

2.2. *Electrophoretic analysis of native myosin*

Non-dissociating conditions were used according to [14] but with a few modifications. Running buffer was 20 mM $Na_4P_2O_7$ (pH 8.5), 10% glycerol, 0.01% 2-mercaptoethanol, 2 mM $MgCl_2$ and 2 mM ATP. Cylindrical gels (6 × 0.5 cm) were 4% in polyacrylamide. Electrophoresis was carried out at 80 V for 18 h at −2°C/−3°C.

2.3. *Electrophoretic analysis of myosin heavy chains*

This was performed according to [15] but with a few modifications, using a 6% polyacrylamide separation slab gel in the presence of 0.1% SDS and 37.5% glycerol. The extracts were diluted in 20% glycerol, 5% SDS, 5% 2-mercaptoethanol, 1 mM EDTA and boiled for 2 min. Electrophoresis was carried out at 55 V for 18 h. For studying the heavy chains content of myosin isoforms, these were first separated under non-dissociating conditions and stained with Coomassie brilliant blue G-250. Bands of myosin iso-enzymes were cut out with

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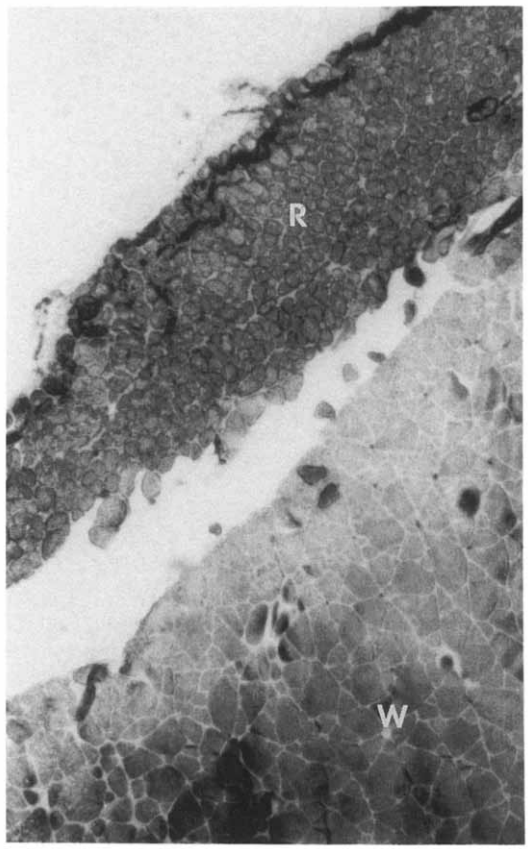

Fig. 1. Cryostat section stained for ATPase. The preincubation at 4.6 was suitable to discriminate simultaneously all different fiber types and muscular regions. R = Red muscle, W = White muscle. (×120). Some fibers with a lower myofibrillar ATPase profile, are located between the red and the white muscle and suspected to represent pink fibers.

a razor blade, and each gel slice was placed on SDS-polyacrylamide after a 5 min treatment with the SDS solution.

2.4. *Electrophoretic analysis of myosin light chains in one-dimensional gel*

Electrophoresis was performed as described in [16], but with a few modifications. Myosin iso-enzymes were first separated in non-dissociating conditions and stained with Coomassie brilliant blue G-250. Bands of myosin iso-enzymes were cut out with a razor blade. Each gel slice was placed on SDS-polyacrylamide gel for further electrophoresis, after a 5 min treatment with SDS solution containing 2% 2-mercaptoethanol and 20 mM Tris-HCl, pH 6.8. Electrophoresis was performed using 12.5% polyacrylamide gels, in a discontinuous Tris-glycine system [17]. Gels were stained with Coomassie brilliant blue R-250 or with silver nitrate (Amersham).

2.5. *Two-dimensional electrophoretic analysis of myosin light chains*

Crude myosin extracts were dissolved in 9 M urea, 2% nonidet P-40, 2.2% ampholines (pH 4–6.5) and 5% 2-mercapetoethanol. Analysis of myosin light chains was performed in horizontal electrophoretic system [18] using 2.2% ampholines in the first dimension and 14% or 15% polyacrylamide, 0.1% SDS in the second dimension.

3. RESULTS

3.1. *Myosin isoforms*

In the white fibers from the central part of the lateral muscle, electrophoresis under non-dissociating conditions revealed 3 myosin isoforms identified as FM3, FM2 and FM1, considering their increasing electrophoretic mobility, and a faster migrating band to be discussed further (Fig. 2). The peripheral part of the muscle, that contains the red fibers, demonstrated two myosin isoforms, the predominant form, SM1, migrating faster than the minor form SM2. Co-electrophoresis of myosins respectively extracted from the peripheral and the central part of the muscle showed that SM1 has electrophoretical mobility similar to FM3.

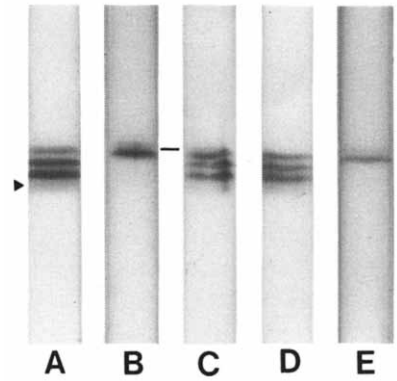

Fig. 2. Electrophoresis under non-dissociating conditions of myosins extracted from lateral muscle of the eel. A, D – White muscle; B, E – Red muscle; C – Co-electrophoresis of myosins extracted from red muscle and white muscle. > Faster migrating band. – SM2.

3.2. *Heavy chains*

In the white fibers from the central part of the muscle, one heavy chain only, called HCf, was detected. In the peripheral red fibers, 3 heavy chains were demonstrated: a predominant faster moving form referred to as HCs_1, again HCf and a heavy chain with lower electrophoretic mobility referred to as HCs_2 (Fig. 3). Reelectrophoresis of SM1 and SM2 in SDS-PAGE confirmed this interpretation (Fig. 4).

3.3. *Light chains*

In the peripheral fibers, two-dimensional electrophoresis revealed two light chains. LC1s and LC2s

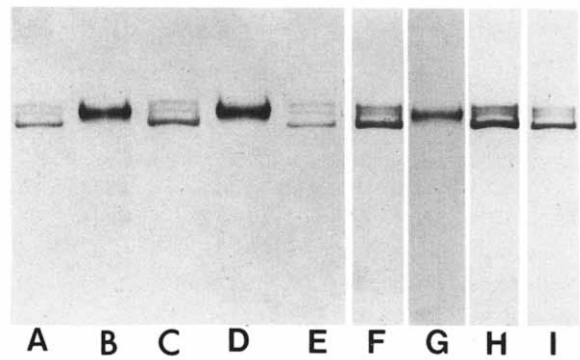

Fig. 3. SDS-PAGE in presence of 37.5% glycerol of myosin heavy chains extracted from the lateral muscle of the eel. A, C, E, F, H, I – Red muscle; B, D, G – White muscle.

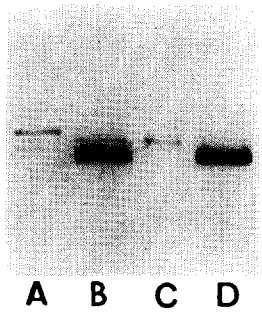

Fig. 4. Analysis of myosin heavy chains by re-electrophoresis in the presence of SDS of myosin isoforms first separated under non-dissociating conditions (heavy chains), and control. A, Control heavy chains extracted from chicken ALD; B, SM1 + SM2; C, SM2; D, SM1.

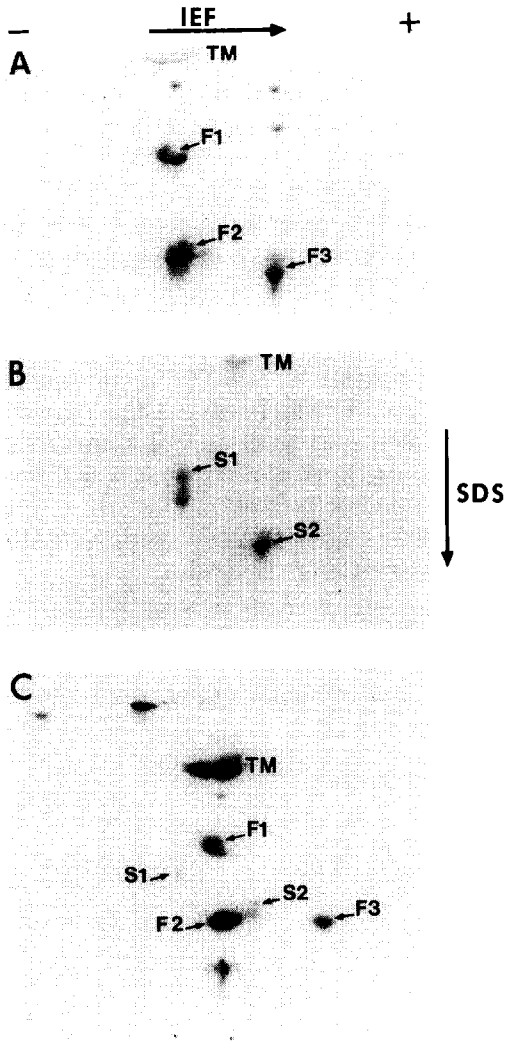

Fig. 5. Two-dimensional electrophoresis of myosin light chains extracted from the lateral muscle of the eel. A, White muscle; B, Red muscle; C, Co-electrophoresis of myosin light chains extracted from red muscle and white muscle. F1, F2, F3: Fast light chains type 1, 2, 3; S1, S2: Slow light chains type 1, 2; Tm: Tropomyosin.

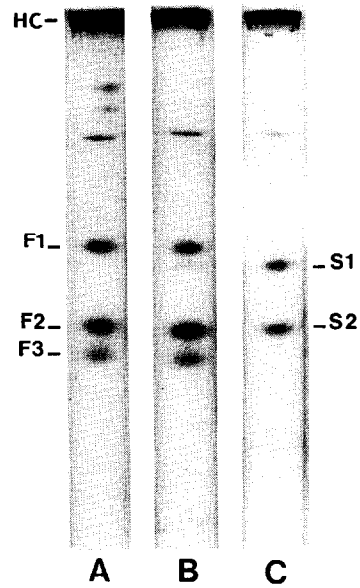

Fig. 6. Analysis of light chains by re-electrophoresis in presence of SDS of myosin isoforms first separated under non-dissociating conditions (light chains). A, White muscle; B, Coelectrophoresis of myosins extracted from red muscle and white muscle; C, Red muscle. HC: Heavy chains; F1, F2, F3: Fast light chains type 1, 2, 3; S1, S2: Slow light chains type 1, 2.

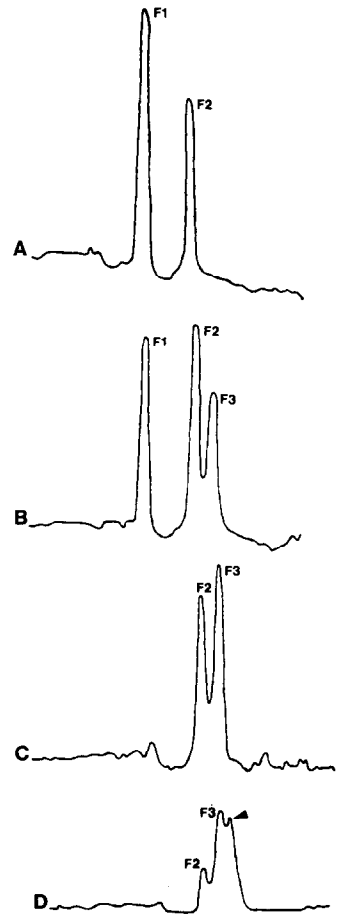

Fig. 7. Densitometric profile of the light chains contained in the different fast myosin isoforms first separated under non-dissociating conditions. A, FM3; B, FM2; C, FM1; D, Faster migrating band. F1, F2, F3: Fast light chains type 1, 2, 3; ◄ Peptide of 15 500 Da.

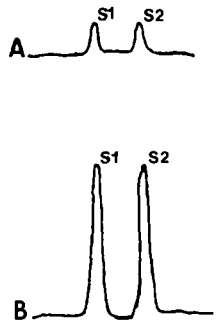

Fig. 8. Densitometric profile of the light chains contained in the two slow myosin isoforms first separated under non-dissociating conditions. A, SM2; B, SM1; S1, S2, slow light chain types 1, 2.

(Fig. 5). Re-electrophoresis of SM1 + SM2 in SDS-PAGE confirmed this result, since only LC1s and LC2s were observed (Fig. 6). In the white central fibers, two-dimensional electrophoresis and re-electrophoresis in SDS-PAGE of the total myosin isoforms demonstrated 3 light chains: LC1f, LC2f and LC3f (Fig. 5, 6). The light chain composition of each fast myosin isoform was studied by SDS-PAGE of 5 gel slices containing the individual bands first isolated in pyrophosphate gels (Fig. 7). Three bands corresponding to FM3, FM2 and FM1 contained respectively LC1f-LC2f. LC1f-LC2f-LC3f and LC2f-LC3f. The fourth faster migrating band observed in pyrophosphate gel electrophoresis of myosin extracted from white muscle contained LC2f, LC3f and a peptide of molecular mass 15 500 Da. This band might correspond to a LC2f degradation product, as suggested by the densitometric profile which account for the phosphorylatable light chain instability [19]. SM1 and SM2 were both shown to contain equal proportions of LC1s and LC2s, as demonstrated by densitometric profiles (Fig. 8). The apparent LC molecular masses as deduced from standard marker proteins run in parallel, were respectively 26 000 Da for LC1f, 23 000 Da for LC1s, 17 700 Da for LC2s, 17 500 Ca for LC2f and 16 500 Da for LC3f.

4. DISCUSSION

In this work we demonstrate a polymorphism of myosin isoforms in the lateral muscle from the eel, and we establish their light and heavy subunit content.

In the white part of the eel muscle, electrophoresis under non-dissociating conditions has revealed 4 bands, as already observed for other fish species [9,10]. In the mackerel white muscle, 4 isomyosins were described; it has been suggested [10] that they might correspond to the intermediate and the 3 fast isomyosins usually observed in mammals [20]. In *Rutilus rutilus*, it was proposed [9] that the faster migrating band observed in pyrophosphate gel was to be reported to embryonic/neonatal myosin. In the eel, analysis of the stoichiometry of the light chains present in the different bands excludes these two interpretations. We consider that 3 myosin isoforms only are present in the white fibers: FM3, FM2 and FM1, composed respectively by $(LC1f)_2$ $(LC2f)_2$, (LC1f)$(LC2f)_2$ (LC3f) and $(LC2f)_2$ $(LC3f)_2$, associated apparently with one single heavy chain HCf as observed in the fast muscle of mammals [14] and urodelan amphibians [21]. The faster migrating band can be interpreted in different ways: it could be a myosin isoform characterized by a specific LC of 15 500 Da, but this interpretation is contradictory with work of [4,6] that demonstrated 3 fast light chains only in the white muscle of adult eel; it could be considered as embryonic/neonatal myosin, but it is not present in the lateral part of the glass eel muscle (results not shown). The hypothesis that finally has been retained after analysing the stoichiometry from the densitometric evaluation of the LC pattern, is that the fast migrating band corresponds to the FM1 degradation product, resulting from LC2f degradation. Indeed the important lability of LC2f from the eel has already been mentioned [6].

The peripheral red fibers are characterized by two slow myosin isoforms, SM1 and SM2, composed of the same light chains content $(LC1s)_2$ $(LC2s)_2$ according to [10]. Previous reports of SDS-PAGE analysis distinguished two distinct heavy chains only, HCf and HCs, related to white and red muscles respectively [9,10]. We indicate for the first time the existence of 3 distinct heavy chains in the peripheral red muscle, and demonstrate that SM1 and SM2 are characterized by distinct heavy chains. It is interesting to note that two myosin isoforms, SM1 and SM2 were demonstrated in the anterior *latissimus dorsi* (ALD) slow tonic muscle of the chick [22], composed by the same light chains content (LC1s and LC2s) and two different heavy chains encoded by separate mRNAs [23]. We cannot exclude that the presence of HCf in the peripheral part of the muscle might account for contamination during dissection and sampling, but another possible interpretation would be the presence of hybrid isomyosins (HCf, LC2).

A clear understanding of the myofibrillar heavy chains localization implies immunological studies using antibodies directed against the 3 different heavy chains. This first work on the eel myosin structure is part of a general analysis of muscular differentiation in this species.

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