Myosin Isoforms in White, Red and Pink Muscles of the Teleost Sea Bass (*Dicentrarchus Labrax, L.*)

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Abstract

Myosin isoforms of white, red and pink muscles from sea bass (*Dicentrarchus labrax*) were analyzed by means of two-dimensional gel electrophoresis of myosin light chains, SDS-PAGE and peptide mapping of myosin heavy chains. The isoforms detected in white and red muscles seemed to follow the pattern characteristic for mammalian myosins. By contrast in myosin of pink muscle white and red myosin light chains were found to be associated with one type only of myosin heavy chain, thus suggesting for the first time the existence at molecular level of a "hybrid" myosin in this muscle.

Key words: Isomyosin; Myosin heavy chains; Myosin light chains; Fish; Pink muscle; *Dicentrarchus labrax*

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The existence of several myosin isoforms has been well established in a variety of muscle tissues of mammalian, avian and amphibian origin [10]. In contrast fish skeletal muscle has received much less attention. It is well known that the lateral muscle of fish differs from the skeletal muscle of other Vertebrates for its myotomal organisation and because the two main types of fibres in the myotome (red and white) are not intermigled, but are separated into a superficial (red muscle) and a deep (white muscle) layer. Another type of muscle, usually located in the so called pink (intermediate) muscle, has been described. In most Teleosts this muscle which lies between the superficial red muscle and the deep white muscle layer of the myotome [13], can be composed either by a mixture of white and red fibers or by a fiber with typical histochemical reactivity [8].

On the basis of their specific metabolic and structural properties and recruitment pattern, it is universally accepted that in fish the red and white fibres are responsible for slow swimming (cruising) and fast swimming (darting, escape) respectively, whereas the functional significance of the intermediate (pink) muscle is not yet completely clear [12].

As in other Vertebrates, fish myosin molecule consists of two heavy chains (MHC, Mw about 200 kDa) and of two pairs of light chains (MLC, Mw 15-30 kDa).

The different composition of myosin subunits is at the basis of its polymorphism in different muscle types. Myosin molecule contains at least one type of heavy chain, different in red and white muscle, and two or three types of light chain in red and white muscle respectively [10].

The present work was undertaken in order to analyze myosin isoforms within the red, white and pink muscle of the Teleost *Dicentrarchus labrax* (the sea bass) and to study in details their subunity composition.

Materials and Methods

Animal sample and myosin extraction

Muscle samples were taken from grown-up individuals of *Dicentrarchus labrax* obtained from fish farm. The fish were anesthetized, killed by decapitation and the skin carefully removed. Red, white and pink samples of lateral muscle were isolated.

Myosin purification was carried out according to the method described in Dalla Libera et al. [5]. Protein concentration was determined according to the Lowry method, using bovine serum albumine as standard.

Two-dimensional electrophoresis of myosin light chains

The myosin light chains were analysed as described by O'Farrell [9], using in the first dimension (IEF) a pH gradient obtained from a mixture composed by one part of ampholines pH 3-10 and four parts of ampholines pH 3-5. The second dimension (SDS) was performed in a 15% polyacrylamide gel. The gels were stained with Coomassie Blue.

One-dimensional electrophoresis of myosin heavy chains

The SDS-PAGE was performed in 6% polyacrylamide gels. The gels were prepared according to Laemmli [6]. Glycerol was added to a final concentration of 37.5%, as suggested by Carraro and Catani [1], in order to improve the separation of myosin heavy chains.

The gels were stained with Coomassie Blue.

Peptide mapping of myosin heavy chains

Myosin heavy chains were electrophoretically purified in 5% polyacrylamide gel. Peptide mapping was performed

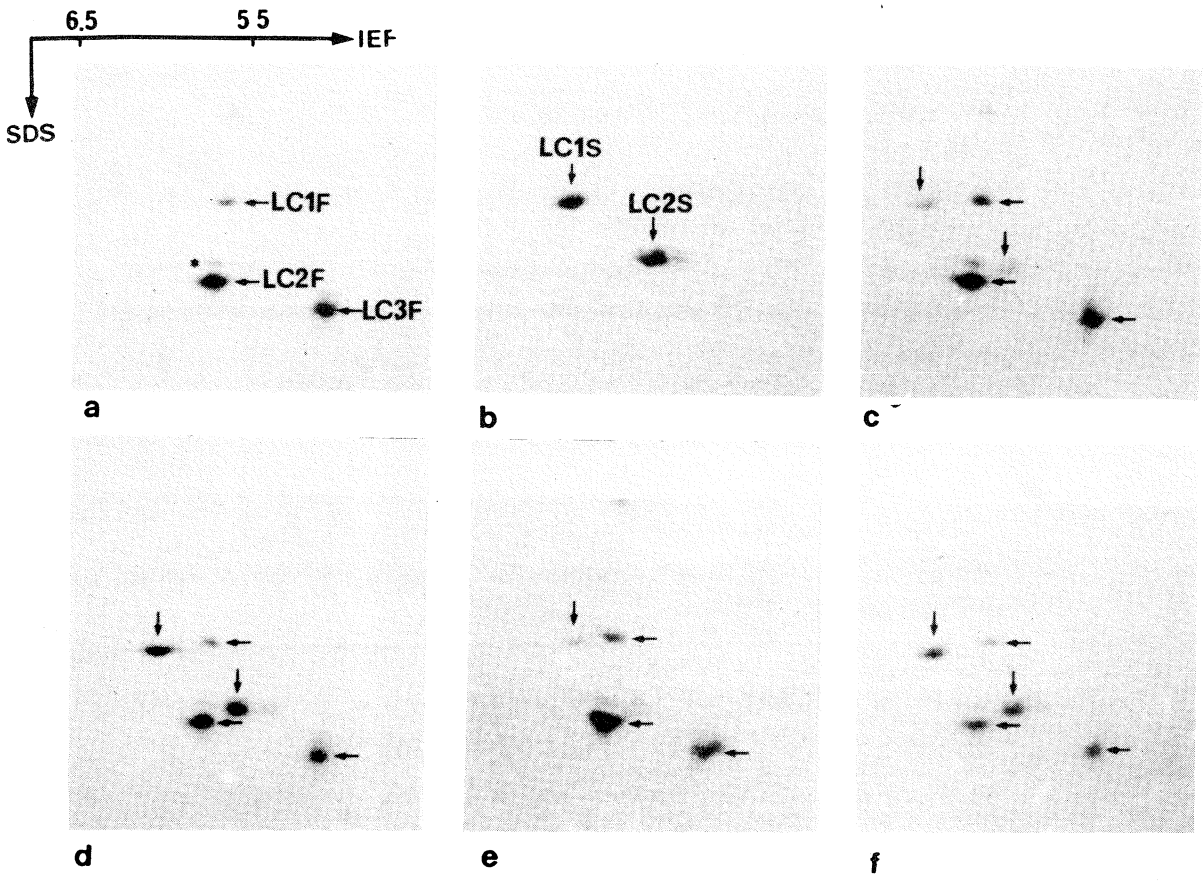

Figure 1. Two-dimensional electrophoresis of myosin light chains.
Key: (a) white muscle; (b) red muscle; (c) pink muscle; (d) a + b; (e) a + c; (f) b + c. About 50 µg of protein were analyzed in each slab. LC1F, LC2F, LC3F: light chains peculiar to myosin from white muscle (leftward arrows); LC1S, LC2S: light chains peculiar to red muscle (downward arrows). IEF: isoelectric focusing; SDS sodium dodecyl sulphate. The asterisk locates a minor spot, the nature of which is unknown, just above LC2F in (a).

according to Cleveland et al. method [3] using *S. aureus* V8 protease, as detailed in [2] and [4].

In the case of co-analysis experiments, we made a comigration of myosin heavy chains and loaded one slice, containing both MHC, on the second gel.

Densitometric analyses

Densitometric analysis scanning is performed on four different gels using a dual-wavelength chromato scanner model CS-930 from Shimadzu linked to a data recorder DR-2 always from Shimadzu.

Results

Myosin light chains

Two-dimensional analysis of myosin light chains (MLC) from the lateral muscle of *Dicentrarchus labrax* is shown in Fig.1. The white muscle (fast muscle) contains three MLCs: LC1F (27 kDa), LC2F (19 kDa) and LC3F (17 kDa) (Fig.1,a). The red muscle (slow muscle) contains two MLCs: LC1S (26 kDa), LC2S (20 kDa) (Fig.1,b). The percentages of each light chain, as determined by densitometric analyses, were: LC1F 13%, LC2F 55%, LC3F 32%; LC1S 53%, LC2S 47%. A minor spot (asterisk), the nature of which is unknown, is present just above LC2F (Fig.1,a). Anyway the co-analysis of red and white muscle myosin (Fig.1,d) excludes the possibility that this component may be a slow-type light chain. It is worthy to be noted that LC1F displays a higher electrophoretic mobility than LC1S and that LC3F is present in a greater amount than LC1F: this latter finding is of some interest since in the mammalian white myosin it happens always the contrary. Moreover LC3F, at variance with other fish correspondent subunits [11], is characterized by a molecular weight value lower than LC2F and an isoelectric point more acidic than LC2F. In these respects LC3F behaves as mammalian counterpart.

The pink muscle is characterized by a completely different situation. Indeed the myosin present in this muscle displays mainly four MLCs: LC1F (11%), LC1S (7%), LC2F (53%)

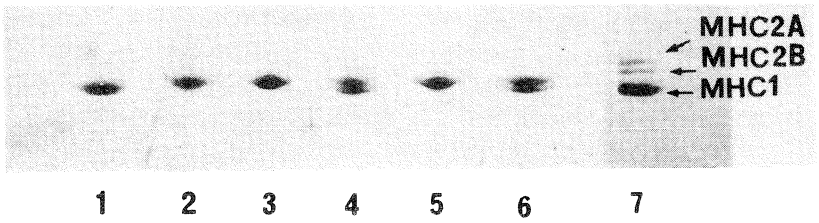

Figure 2. One-dimensional electrophoresis of myosin heavy chains.
Key: [1] red muscle; [2] pink muscle; [3] white muscle; [4] 1 + 2; [5] 2 + 3; [6] 1 + 3; [7] rat soleus + rat EDL. About 0.5 µg of protein were analyzed in each lane. MHC 1: myosin heavy chain peculiar to slow myofibers; MHC 2A: myosin heavy chain peculiar to fast oxidative myofibers; MHC 2B: myosin heavy chain peculiar to fast glycolytic myofibers.

and LC3F (29%) (Fig.1,c). The nature of these light chains was confirmed by co-analysis experiments with white and red myosin (Fig.1,e and Fig.1,f, respectively). The possibility that also LC2S exists as a minor component is suggested by the fact that in the two-dimensional electrophoresis a tiny spot is observed above on the right of LC2F (Fig.1,c). However also at naked eye it is evident that this spot is present in an amount definitely much lower than LC1S. By means of densitometric analyses it is therefore possible to quantify as 7% and 93% the presence of MLCs of slow (LC1S and possibly LC2S) and fast-type (LC1F, LC2F, LC3F), respectively, in the myosin of pink muscle.

Myosin heavy chains

The myosin heavy chains (MHCs) have been analysed by SDS-PAGE (Fig.2). The heavy chains of myosin from rat EDL and Soleus were used as standard.In both red and white muscle, one type only of heavy chains was detected (Fig.2, lanes 1 and 3). The myosin of the red muscle contains a specific heavy chain with an electrophoretic mobility higher than that characteristic of myosin of white muscle, as demonstrated by coelectrophoresis experiments (Fig.2, lane 6). Similar results for white and red myosin heavy chains were recently reported by Martinez et al. [7] in some fishes. According to this electrophoretic method the myosin isoform found in pink muscle was made up of one type only of heavy chain (Fig.2, lane 2). Moreover pink MHC electrophoretic mobility is the same as that of white MHC (Fig.2, lane 5) and different from that of red MHC (Fig.2, lane 4). In the pink muscle no MHC of slow type is observed. However we can not exclude the presence of 2% of red MHC, as this percentage is the lower detection limit of the electrophoretic method.

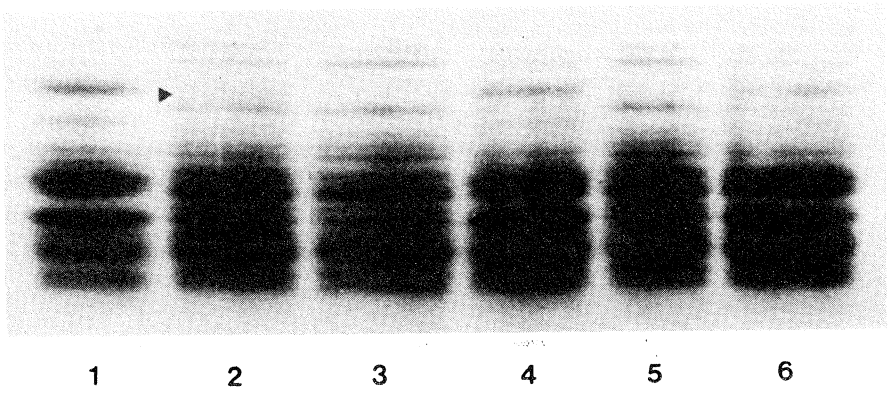

Figure 3. One-dimensional peptide mapping with Staphylococcus aureus V8 protease of electrophoretically purified myosin heavy chains.
Key: [1] red muscle; [2] pink muscle; [3] white muscle; [4] 1 + 2; [5] 2 + 3; [6] 1 + 3. About 50 µg of protein were analyzed in each lane. The triangle in lane [2] indicates a peptide unique in pink myosin heavy chain map.

Therefore the slow MLCs, that are present in 7% as stated above, are not correlated by a similar amount of slow MHC, thus negating the possibility that pink myosin is a mixture of white and red myosin isoforms.

In order to obtain more informations about the nature of the MHC of pink muscle, peptide mapping experiments have been performed on electrophoretically purified MHCs. The relative results are illustrated in Figure 3. White and red MHC gave origin to very different digestion patterns (Fig.3, lanes 1 and 3). By contrast pink MHC mapping (Fig.3, lane 2) is very similar if not identical to that of white MHC (Fig.3, lane 3). Only a small difference (triangle in Fig.3, lane 2), which could not be significant, is detected between white and pink MHC mappings. Similar results were obtained for carp pink MHC [12].

Discussion

The present results establish the existence and subunit composition of different myosin isoforms in red, white and pink muscle of sea bass. The isoforms detected in red and white muscles seem to follow the pattern characteristic for slow and fast mammalian myosin. On the contrary the isoform(s) in pink muscle, here analyzed for the first time, are different from the classical situation of slow or fast myosin. The myosin of pink muscle is not a mere mixture of red and white myosin, as demonstrated by densitometric analyses of myosin light and heavy chains. In fact, the heavy chain of pink muscle myosin contains one type of heavy chain which could be identical or different to the one of white muscle. This heavy chain is associated not only with MLCs of fast type, originating two homodimers $[(LC1F)_2$-$(LC2F)_2]$ or $[(LC3F)_2$-$(LC2F)_2]$ and one heterodimer [(LC1F)-(LC3F)-$(LC2F)_2$] but could be also associated with both fast and slow light chains, giving origin to a hybrid isoform. Therefore these results confirm and extend the myosin polymorphism in the skeletal muscle observed in mammalian, avian myosin and in some kinds of fish. This polymorphism is surely correlated to the different contractile properties of the different types of muscle. In this respect, the pink muscle appears of particular importance since its functional significance is not yet completely clear.

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