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Myosin heavy chain isoforms in white, red and ventricle muscles of barbel (Barbus barbus L.)

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Abstract

1. Actomyosin extracts of trunk, heart, and head muscles from barbel *(Barbus barbus* L.) were analyzed by SDS-polyacrylamide gel electrophoresis to study their myosin heavy chain composition.

2. Four heavy chain isoforms were found: trunk white, trunk red, and ventricle muscles yielded one heavy chain typical of the muscle type; head muscles devoid of red fibers displayed two heavy chain isoforms, the slow migrating one corresponding to the trunk white muscle type.

3. The electrophoretic mobility of red and ventricle myosin heavy chains related to that of white isoforms appeared highly modified by the glycerol content of the gels.

INTRODUCTION

Myosin is a polymorphic molecule which exists in a large number of isozymic forms in fast skeletal, slow skeletal, and cardiac muscles of mammals and birds. These isoforms differ in their heavy and/or light chain composition and are synthesized in response to various physiological requirements such as the muscle contractile activity (for a review, see Swynghedauw, 1986). In fish, white, red, and ventricular muscle fibers always exhibit distinct myosin light subunits (Huriaux and Focant, 1974; Focant *et al.,* 1976, 1981, 1983; Huriaux *et al.,* 1983, 1988; Watabe *et al.,* 1984; Dinh *et al.,* 1985; Rowlerson *et al.,* 1985; Karasinski, 1988; Martinez *et al.,* 1989, 1990). A single isoform of myosin heavy chain was recently detected in white and red muscles from some teleost fish (Karasinski, 1989; Karasinski and Kilarski, 1989; Martinez *et al.,* 1989, 1990). But the migration of the red heavy chain on SDS-polyacrylamide gel electrophoresis was found to be slower, identical or faster than that of the white one, probably due to differences in fish species or experimental conditions.

We recently studied the myosin light chains of several trunk and head muscles from two barbel populations (hatchery and river) (Huriaux *et al.,* 1988, 1990). We showed that the electrophoretic characteristics of myosin light chains and the red fiber content of heterogeneous muscles were similar in specimens of the same size and did not appear to be influenced by their speed of growth or mode of life. The present work was

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undertaken to determine the distribution of heavy chain isoforms within white, red, and ventricle muscles of barbel *(Barbus barbus* L.). Additionally, the effect of pH and glycerol concentration on heavy chain electrophoretic mobilities was investigated.

MATERIALS AND METHODS

Muscle samples and actomyosin isolation

Barbels *(Barbus barbus* L.) of 19-23 cm standard length were obtained from a hatchery (CERER, University of Liège) or caught in the river Ourthe (Belgium). The river barbels were 4'years+ old. The hatchery barbels raised under accelerated growth conditions were 1 year + old. The following muscles were dissected: the dorso-lateral white muscle located in front of the dorsal fin, the superficial red muscle located at the side of the body at the level of the lateral line, the cardiac ventricle muscle, the jaw adductor mandibulae A 1, A 2 and A3, and the levator hyomandibulae. The adductor A 1 which looked heterogeneous was further divided according to the colour: A 11 (white-upper), A 12 (white-lower) and A 13 (red-central). The dissection and preservation of muscle samples and the isolation of actomyosin were performed according to Huriaux *et al.* (1990). .

Myosin heavy chain electrophoresis

Actomyosin samples were dissolved in 62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol pH 6.8, and boiled for 3 min. Polyacrylamide gel electrophoresis was carried out according to Danieli Betto *et al.* (1986) with the following modifications: three different glycerol concentrations (30%, 40% or 50% w/v) were used in the separating and stacking gels as well as two pH values (8.8 or 8.4) in the separating gel buffer. Electrophoresis was started at 5 mA for 2 hr and continued at 10 mA for 16 hr (slab gel of 18x8x0.2cm). The proteinic bands were stained with Coomassie Brilliant Blue R-250.

RESULTS

Trunk, and heart muscles

In all the electrophoretic conditions investigated, only one heavy chain was found in myosin from barbel white, red, and ventricle muscle (Fig. 1). At pH 8.8 (Danieli Betto *et al.,* 1986) in the presence of 30% glycerol, the isoforms from red and ventricle muscles exhibited a higher mobility than that from white muscle (Fig. 2). Densitométrie scans of comigration of white myosin with red or ventricle myosin revealed a small difference in mobility between these last two heavy chains, the ventricle isoform being the fastest one (Fig. 3). The relative mobilities of the three isoforms at pH 8.8 progressively varied according to the glycerol content (30%, 40% or 50%) of the gels (Fig. 1A). Red and ventricle heavy chains migrated more slowly than the white one in the 50% glycerol gel system where all electrophoretic bands appeared very diffuse. Similar results were found at pH 8.4 [pH used for barbel light chain separation (Huriaux *et al.,* 1990)] except for the ventricle isoform in 40% glycerol (Fig. IB). The best separation of white and red isoforms was obtained using pH 8.8 and 30% glycerol. No difference was detected

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between corresponding myosin heavy chains from hatchery or river barbels.

***Figure 1**. Schematic picture extrapolated from SDS-PAGE patterns of barbel myosin heavy chain migration depending on pH and glycerol concentration. (A) pH 8.8; (B) pH 8.4. Clear bands represent the white muscle, black bands the red muscle, and striped bands the ventricle muscle. Red and ventricle heavy chains were located in comparison with white ones.*

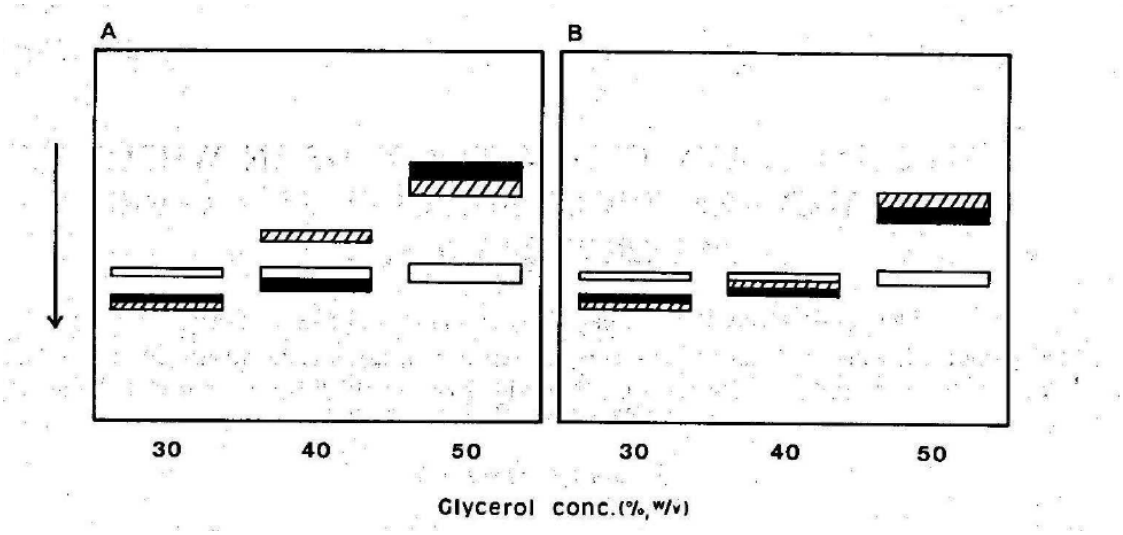
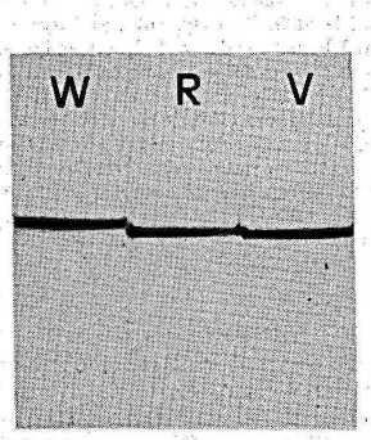


***Figure 2.** SDS-PAGE at pH 8.8 and 30% glycerol of myosin heavy chains from barbel white (W), red (R), and ventricle (V) muscle.*



Head muscles

In all head muscle parts examined by electrophoresis in 30% glycerol pH 8.8, two heavy chains were detected except for in the small A 13 fragment; here only one band was observed (Fig. 4). The slow migrating isoform had the same mobility as the myosin heavy chain from trunk white muscle; it was absent in A 13. The fast migrating isoform exhibited a slightly higher mobility than the trunk red muscle isoform. This fast migrating isoform comigrated with the red isoform in the heterogeneous muscle parts A 13, A3 and levator hyomandibulae which contained, according to light chain analysis,

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12%, 10% and 8% red fibers, respectively (Huriaux *et al.,* 1990). This heavy chain characteristic of head muscles displayed the fastest electrophoretic mobility in our several experimental conditions. It was especially well resolved in the 100% white muscle parts A 11, A 12 and A 2 where it represented 47 ± 8%, 61 ± 4% and 76 ± 9% of both heavy chains, respectively. Similar results were obtained with hatchery or river barbel muscles.

***Figure 3**. Densitométrie scans of comigrating myosin heavy chains from barbel white (W), red (R), and ventricle (V) muscles in SDS-PAGE at pH 8.8 and 30% glycerol.*

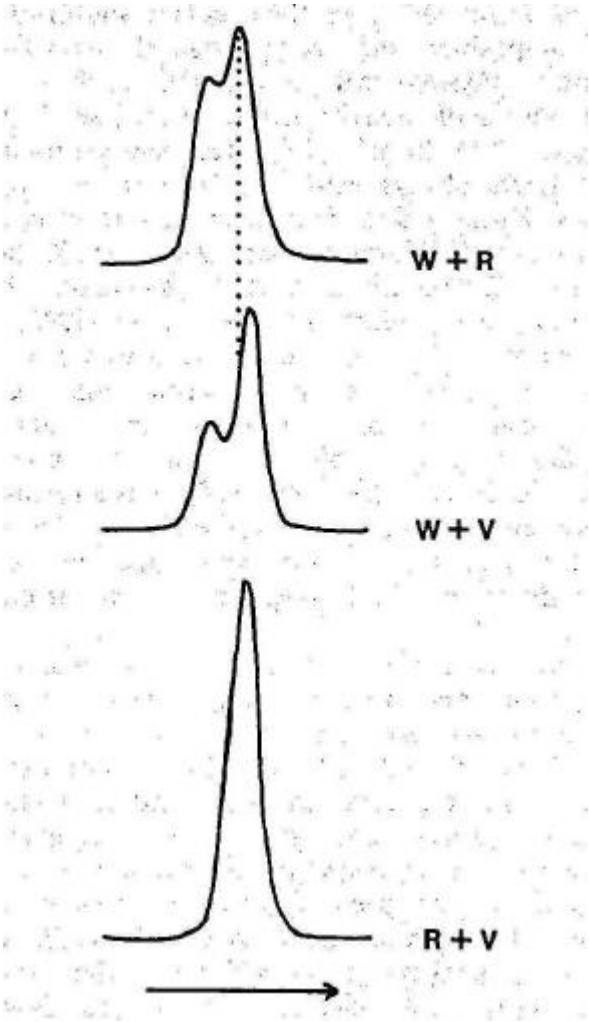

***Figure 4.** SDS-PAGE at pH 8.8 and 30% glycerol of barbel myosin heavy chains from trunk white muscle (W), add. mand. A 11 part (a), add. mand. A 12 part (b), add. mand. A 13 part (c), add. mand. A 2 (d), add. mand. A 3 (e), levator hyomandibulae (f), and trunk red muscle (R).*

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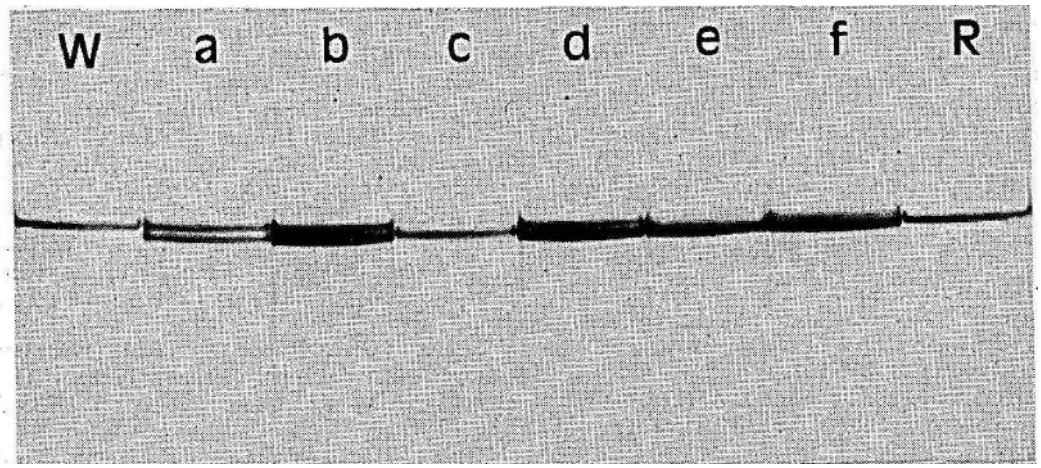

DISCUSSION

The present investigation establishes the existence of four myosin heavy chain isoforms within adult barbel striated muscles. Distinct types were already described in white and red muscles of other teleost fish (Scapolo and Rowlerson, 1987; Karasinski, 1989; Karasinski and Kilarski, 1989; Martinez *et al.,* 1989). However, to our knowledge, this is the first paper demonstrating the existence of heavy chains specific to ventricle and head muscles. Contrary to higher vertebrates, fish ventricle muscle myosin differentiates from red or slow-twitch muscle in both its light (Huriaux and Focant, 1974, 1985; Huriaux *et al.,* 1983) and heavy subunits. A peculiar myosin heavy chain isoform was detected in jaw adductors and levator hyomandibulae which are the most important buccal cavity muscles acting in fish feeding (Ballintijn *et al.,* 1972). This additional heavy chain could be related to the great variety of fiber types which have been histochemically and immunohistochemically identified in head muscles of various fish, and especially with pink fibers which are present in substantial amounts (Akster and Osse, 1978; Akster, 1983; Scapolo *et al.,* 1989). This last hypothesis is supported by Scapolo and Rowlerson (1987), who showed that myosin from carp pink lateral muscle exhibits three light chains indistinguishable from those of white muscle, but differ from white and red muscle myosin by peptide maps of its heavy chains. It is well known that the ATPase activity of myosin and therefore its heavy chain composition can be correlated with the speed of fiber shortening (Barany, 1967; Reiser *et al.,* 1985). Thus, this heavy chain isoform, which represents 50-75% of both heavy chains in the head muscles devoid of red fibers, might be a significant factor in the characteristic functions of these muscles.

In the course of this work we determined that the glycerol concentration added in both stacking and separating, polyacrylamide gels modified the electrophoretic migration distance of red and ventricle muscle heavy chains relative to the white muscle isoforms and their resolution. Such a phenomenon may explain the variability of the relative mobility of fish white and red heavy chains in previous studies (Karasinski, 1989; Karasinski and Kilarski, 1989; Martinez *et al.,* 1989). The heavy chain electrophoretic pattern might vary due to other technical treatments of gels and also depends upon the

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fish species studied. We showed that the best differentiation of the barbel heavy chain isoforms was obtained using 30% (w/v) glycerol, pH 8.8 or 8.4. Under these experimental conditions, white, red, and ventricle heavy chains from *Cyprinus carpio* (L.) were found migrating together (unpublished personal results). Consequently, the evaluation of the molecular mass of heavy chains using this electrophoretic method must be taken with caution.

Finally, as already observed for the myosin light chains and the parvalbumins (Huriaux *et al.,* 1990), raising the barbel experimentally in fast growth conditions does not modify the physicochemical characteristics of myosin heavy chains.

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