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Myosin isoforms in mackerel (*Scomber scombrus*) red and white muscles

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Myosin extracts of red and white muscles from mackerel (*Scomber scombrus*) were analyzed by native electrophoresis to investigate the existence of myosin isoforms in both kind of muscles, and by two-dimensional and SDS gel electrophoresis to study their subunit composition. Two isoforms were found in red muscle comprising one type of heavy chain and two light chains. Four isoforms were found in white muscle made up of one type of heavy chain and three types of light chains. The heavy chains from white muscle showed a higher electrophoretic mobility than that of red muscle in SDS-PAGE. Both heavy chains had an intermediate mobility between those of slow and fast myosins from rat diaphragm.

Myosin; Gel electrophoresis; Subunit composition; (*Scomber scombrus*)

1. INTRODUCTION

The existence of several myosin isoforms, with different electrophoretic mobilities under non-denaturing conditions, has been well established in a variety of muscle tissues of mammalian, avian, and amphibian origin [1] and recently in fish hearts [2]. These myosin isoforms have been shown to differ in light chain or heavy chain composition or both. In fish muscles the myotommal musculature is made up in most species by two different groups of fibers, white and red muscle fibers. It is generally accepted that the red muscle fibers are specialized in the low-speed cruising, while the white muscle fibers are mainly responsible for the short bursts of maximum speed [3]. Previous studies on myosin isolated from white and red muscles of the mackerel *Pneumatophorus japonicus japonicus* [4,5] have shown the light chain pattern of these myosins to resemble respectively those of the fast and slow muscle myosin of rabbit. Differences in Ca^{2+}- and EDTA-ATPase activities and in thermal stability were also detected between myosin from red and white muscles [4]. In carp (*Cyprinus carpio*), differences among the pink, red and white muscles have been demonstrated histochemically in their ATPase activity, in their myosin heavy chains by peptide mapping and immunohistochemical reactivity, and in their light chains [6]. These facts strongly suggested that the red and white muscles of fish have different myosin isoforms. The present work was undertaken to establish the possible existence of myosin isoforms within the red and white muscles from mackerel (*Scomber scombrus*) and to study their subunit composition.

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2. MATERIALS AND METHODS

2.1. *Animal samples; myosin extraction*

Mackerels (*S. scombrus*) of about 150 g were purchased in June from a local market. A sagittal cut was made at the level of the anal region and samples of white and red muscle tissues (approx. 200 mg) were taken. Other samples used as standards for comparison were soleus, diaphragm, and skeletal muscles from adult rat. Myosin extraction was carried out on ice according to [7]. Protein content in the extracts was determined by the Bradford method [8], using the Bio-Rad protein assay.

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2.2. *Gel electrophoresis under non-dissociating conditions*

Polyacrylamide gel electrophoresis under non-dissociating conditions was carried out as described [9,10].

2.3. *Analysis of myosin light chains*

The myosin light chains were analyzed by two different methods: two-dimensional electrophoresis, and SDS-PAGE of gel slices containing the myosin isoforms first isolated by native electrophoresis. Crude myosin extracts or gel slices containing myosin isoforms first separated by electrophoresis under non-dissociating conditions and detected by light staining and destaining were loaded on the first dimension tubes (10 × 0.27 cm). The gels contained 2% ampholytes, 1% Servalyt 4.5–5.0 and 1% Servalyt 5.0–5.5. Isoelectric focusing was run for 7500 V·h according to [11]. The second dimension was run in 0.75 mm thick slab gels 15% in acrylamide and 0.0864% *N,N'*-methylenebisacrylamide according to [12], at 20 mA per slab over about 5 h. Gels were silver stained as described in [13].

Gel slices containing the myosin isoforms first separated by electrophoresis under non-dissociating conditions were loaded in sample wells of 0.75 mm thick slab gels (15% acrylamide, 0.0864%, *N,N'*-methylenebisacrylamide) according to [12], run at 20 mA per slab for 5 h, and silver stained according to [13].

2.4. *Myosin heavy chain electrophoresis*

SDS-PAGE was carried out according to [14] with the following modifications: the composition of the separating gel was 6% acrylamide, 0.087% methylenebisacrylamide, 375 mM Tris-HCl (pH 8.8), 36.75% glycerol, 0.1% SDS, 0.07% ammonium persulfate and 0.1% Temed. The stacking gel was 3% acrylamide and 0.13% *N,N'*-methylenebisacrylamide. The extracts were diluted in 20% glycerol, 5% β-mercaptoethanol, 5% SDS, 1 mM EDTA, boiled for 2 min, and 0.15–0.2 μg protein was loaded. Electrophoresis was started at 70–80 V and constant intensity (about 10 mA per slab) until it reached 100 V, thereafter being continued at a constant 100 V for about 16 h. Gels were silver stained according to [13].

3. RESULTS

3.1. *Myosin isoforms*

Two bands were found after electrophoresis under non-dissociating conditions in myosin extracts from red muscles, the slowest migrating form being predominant. In white muscles four isoforms were detected, the fastest and slowest migrating forms being present in lower amounts than the two intermediate migrating forms, which appeared to be in similar amounts. The slowest migrating form found in red muscle had the same mobility as the second fastest migrating form from white muscle. All myosin isoforms showed relatively higher electrophoretic mobilities than those from rat skeletal muscle (fig.1).

3.2. *Light chains*

Two main spots were detected in red muscle and three in white muscle after two-dimensional electrophoresis (fig.2), and after isolation of the myosin isoforms and subsequent analysis of the slices by SDS-PAGE (fig.3). In red muscle, both isoforms showed the same light chains, but with different relative intensities, and the same happened for the four isoforms from white muscle. The main spots are supposed to be the light chain types 1, 2 and 1–3 from red and white muscle, respectively. Three additional spots, named A, B, and B', and one termed C, were detected in red and white muscles, respectively. The calculated molecular masses of the light chains and spots are listed in table 1.

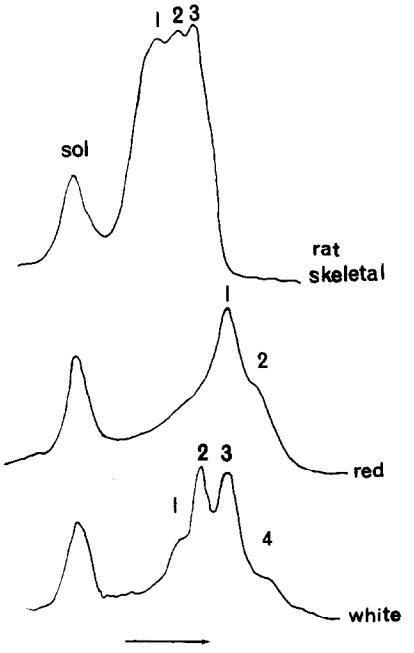

Fig.1. Comparison of gel electrophoresis under non-dissociating conditions of rat skeletal and mackerel red and white muscles. Rat soleus (sol) was included as an internal standard. Numbers represent the myosin isoforms in order of increasing mobility. The arrow indicates the direction of migration.

3.3. *Heavy chains*

In both red and white muscles, only one heavy chain was detected. The isoform from red muscle had a slightly higher molecular mass than that from white muscle (fig.4A). However, upon co-migration, both forms seemed to migrate as one

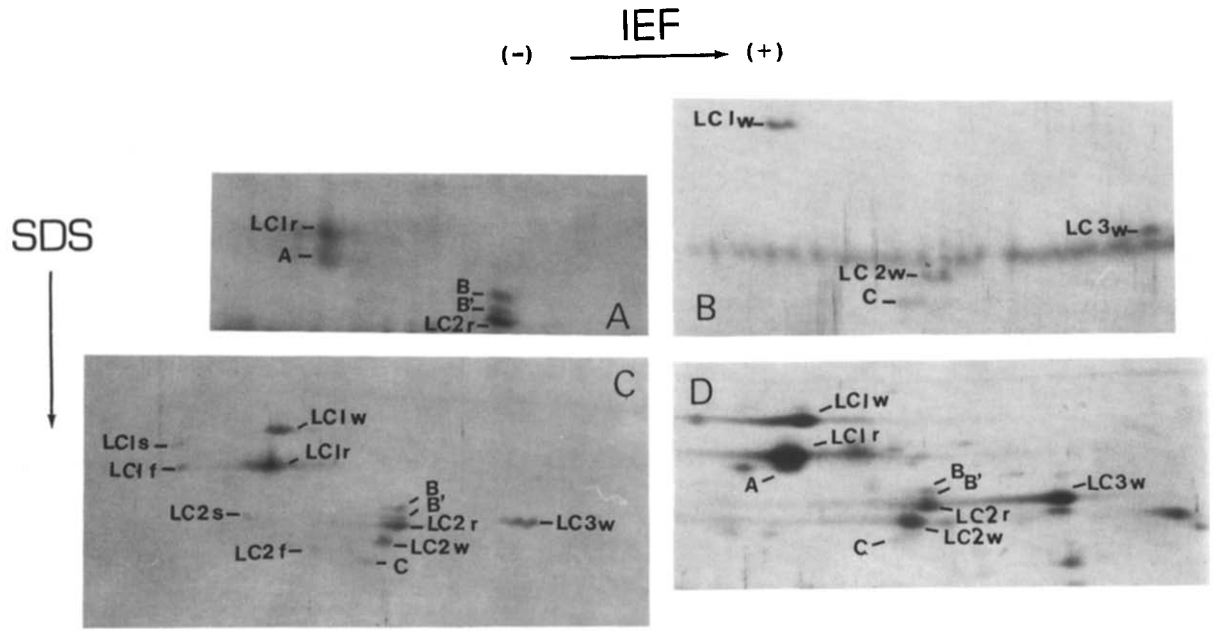

Fig.2. Two-dimensional electrophoresis gels showing the area of the light chains. Samples: myosin from mackerel red muscle (A), myosins from mackerel white muscle (B), and comigration of myosins from red and white muscle of mackerel and rat diaphragm (C), first isolated by native electrophoresis. (D) Comigration of red and white myosin extracts of mackerel. LC, light chain; r, from red muscle; w, from white muscle; s, slow and f, fast from rat diaphragm. A, B, B′, and C are the unidentified spots.

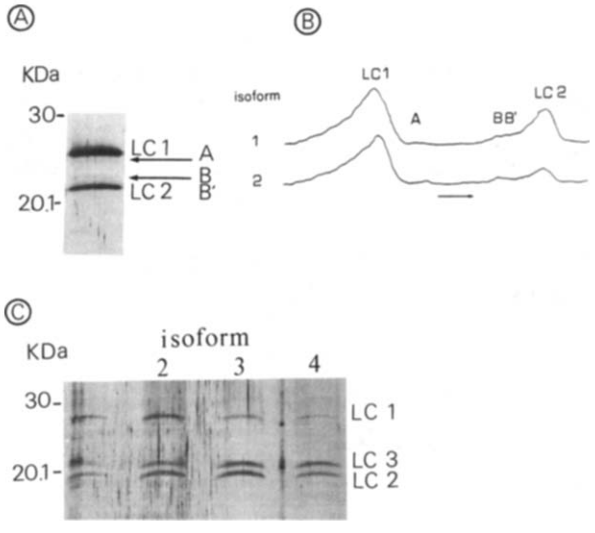

Fig.3. SDS-PAGE electrophoresis of gel slices containing the myosin isoforms first isolated under non-dissociating conditions. (A) The two myosins from red muscle, (B) densitometric scans of the light chains of isoforms 1 and 2 from red muscle, (C) light chains of each isoform from white muscle. Only the area of the light chains is shown. Symbols as in fig.2.

band, probably due to the small difference in molecular mass. Both forms showed a molecular mass intermediate between the fast and slow isoforms of rat diaphragm. Comigration with rat diaphragm confirmed the difference in molecular

Table 1

Calculated molecular masses of the myosin light chains (LC) and additional spots

		Molecular mass (kDa)
Red muscle	LC 1	23.6 (24.5)
	A	23.0
	B	21.2
	B′	20.7
	LC 2	20.2 (20.0)
White muscle	LC 1	26.0 (27.0)
	LC 2	19.2 (19.0)
	C	18.5
	LC 3	20.5 (21.0)

The molecular masses of the light chains of *Pneumatophorus japonicus japonicus*, reported by Watabe and co-workers [4,5], are shown in parentheses for comparison

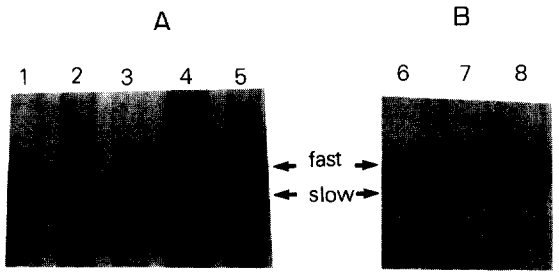

Fig.4. Heavy chain electrophoresis. (Lanes) Samples: (1,5,6) rat diaphragm, (2) mackerel red muscle, (3) mackerel white muscle, (4) comigration of red and white mackerel muscles, (7) comigration of mackerel red muscle and rat diaphragm, (8) comigration of mackerel white muscle and rat diaphragm. Fast and slow denote fast and slow heavy chains from rat diaphragm. (A) and (B) belong to different gels.

mass between the red and white isoforms from mackerel (fig.4B).

4. DISCUSSION

The present results establish the existence and subunit composition of different myosin isoforms in red and white muscle which differ in both their light and heavy chains. The isoforms detected in red muscle seemed to follow the pattern characteristic for slow mammalian myosin, i.e. only two light chains and one type of heavy chain. The fastest migrating isoform probably originated from the main isoform after partial dissociation of light chain 2. The isoforms detected in white muscle were made up by three types of light chains and apparently only one type of heavy chain. They probably correspond to the intermediate and to the three light chain isoforms usually observed in mammals [15]. The fact that a heavy chain specific for the intermediate form was not detected here is probably due to it being very close to the main heavy chain and representing only a small amount of total heavy chains present in the sample.

No attempt was made to identify the minor components (A, B, B′, C), which were found in the molecular mass and isoelectric point range of the light chains, since the samples were taken from post-rigor muscles and some proteolytic activity may have taken place producing polypeptides in that range.

To summarize, differences in activity and thermostability between red and white muscle myosins, such as those found by Watabe and Hashimoto [5], should be attributed to differences in both heavy and light chain composition of the myosin isoforms present in those muscles.

To our knowledge, this is the first work demonstrating the existence and subunit composition of myosin isoforms in both red and white fish muscles.

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