Electrophoretic separation by an improved method of fast myosin HCIIb-, HCIId-, and HCIIa-based isomyosins with specific alkali light chain combinations

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An improved method of electrophoresis under nondenaturing conditions separated three electrophoretically distinct isomyosin triplets when applied to rat fast-twitch muscles displaying a predominance of one of the fast myosin heavy chain isoforms HCIIb, HCIId or HCIIa. The three isomyosin triplets, named FM1b-FM3b, FM1d-FM3d, FM1a-FM3a, corresponded to the three possible alkali light chain (LC) combinations (LC1f homodimer, LC1f LC3f heterodimer, and LC3f homodimer) with each fast HC isoform. Different proportions of these various isomyosins suggested specific affinities of light chains LC1f and LC3f for the fast heavy chain isoforms.

Myosin heavy chain; Isomyosin electrophoresis; Myosin heavy chain-based isomyosin; Myosin alkali light chain

1. INTRODUCTION

Three fast isomyosins (FM1, FM2, FM3) have been distinguished to date by electrophoresis under nondenaturing conditions in adult mammalian skeletal muscles [1–3]. These isomysins differ with regard to their light chain (LC) complements: FM1 is the LC3f homodimer, FM2 the LC1f/LC3f heterodimer, and FM3 the LC1f homodimer [2,4,5]. In view of the existence of several fast myosin heavy chain (HC) isoforms (HCIIb, HCIId, HCIIa) [6,7], we were interested in investigating the role of the heavy chain complement on the electrophoretic mobility of fast isomyosins. For this purpose, myosin extracts from skeletal muscles of adult rats, displaying a predominance of one of three fast HC isoforms, were analysed with an improved method of electrophoresis under nondenaturing conditions.

2. MATERIALS AND METHODS

2.1. *Muscles and myosin extraction*

Fast-twitch muscles were dissected from adult male Wistar rats: superficial portion of vastus lateralis, deep portion of masseter, extensor digitorum longus subjected to 56 days of chronic low-frequency stimulation [8], tibialis anterior, and hypothyroid 35-day low-frequency stimulated tibialis anterior [9]. Crude myosin extracts were prepared as previously described [7].

2.2. *Myosin heavy chain and light chain analysis*

Myosin heavy chain isoforms were electrophoretically separated in the presence of SDS using a 5–8% gradient polyacrylamide slab gel system [6–8]. Gels were silver-stained [10] and evaluated densitometrically.

Myosin light chains were assessed by two-dimensional electrophoresis as previously described [11]. Gels were stained with Serva blue R-250 (Serva Heidelberg, FRG) and evaluated by integrating densitometry using a computer-assisted video camera system (Syncotec, Aßlar, FRG). The total extinction of each LC was corrected by division by its molecular mass and expressed as percentage of total light chains [11].

2.3. *Isomyosin electrophoresis*

Isomyosins were separated on 1.5 mm thick vertical slab gels containing 20 mM $Na_4P_2O_7$, 1 mM EDTA and 10% glycerol, pH 8.8 (Desaphor VA150 apparatus, Desaga, Heidelberg, FRG). Polyacrylamide and bisacrylamide concentrations were 4.4% and 0.14%, respectively. The running buffer (pH 8.8) contained 20 mM $Na_4P_2O_7$, 1 mM EDTA, 10% glycerol, and 0.02% 2-mercaptoethanol. Myosin extracts were diluted in sample buffer (pH 8.8) containing 20 mM $Na_4P_2O_7$, 0.5 mM EDTA and 25% glycerol. After pre-electrophoresis for 30 min, 5–10 μg protein was loaded on the gel. Electrophoresis was carried out at −0.5°C with a constant voltage (11 V/cm) for 66 h. Gels were silver-stained [10] and evaluated densitometrically (LKB 2202 Ultroscan densitometer).

3. RESULTS

The slab gel system chosen for isomyosin separation made it possible to detect slight differences in electrophoretic mobilities by subjecting different samples to electrophoresis in adjacent lanes on the same gel. Using the established methods [1–5] of isomyosin separation (21 h run, Coomassie blue-staining), no conspicuous differences were detected between the electrophoretically separated isomyosins of three muscles (Fig. 1, panel a). According to myosin heavy chain analyses, these muscles were characterized by the predominance of one of the three fast HC isoforms. Muscles with a

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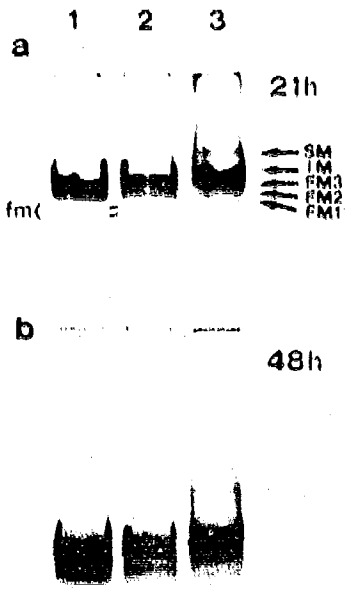

Fig. 1. Coomassie blue-stained isomyosin electrophoreses of extracts from three different rat fast-twitch muscles. (Lane 1) tibialis anterior, rich in HCIIb; (lane 2) deep masseter, rich in HCIId; (lane 3) 35-days stimulated hypothyroid tibialis anterior, rich in HCIIa. (a) Separation after 21 h electrophoresis. (b) Separation after 48 h. *Abbreviations:* fM, fetal isomyosins; FM1, FM2, FM3, fast isomyosins; IM, intermediate isomyosin; SM, slow isomyosin.

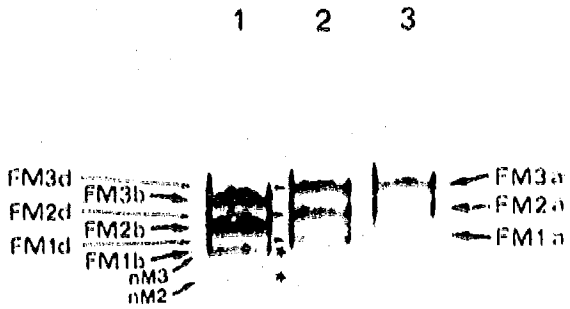

Fig. 2. Myosin heavy chain-based fast isomyosins. Myosin extracts from three rat muscles with predominance of one of the three myosin HC isoforms were subjected to 66 h electrophoresis under nondenaturing conditions and silver-stained. (1) Superficial vastus lateralis, rich in HCIIb; (2) deep masseter, rich in HCIId; (3) 56-day electrostimulated extensor digitorum longus, rich in HCIIa. *Abbreviations:* FM1b-FM3b, HCIIb-based isomyosins; FM1d-FM3d, HCIId-based isomyosins; FM1a-FM3a, HCIIa-based isomyosins; nM, neonatal isomyosins (indicated by asterisks).

predominance of HCIIb and HCIId (Fig. 1, lanes 1 and 2) contained three fast isomyosins which were named FM1, FM2, and FM3. The muscle with a predominance of HCIIa (Fig. 1, lane 3) contained, in addition to the three fast isomyosins, a fourth slower migrating band, termed IM (intermediate isomyosin). The separation was slightly improved when the time of electrophoresis was increased from the usual 21 h duration to 48 h (Fig. 1, panel b).

The resolution of the isomyosin pattern was further improved by prolongation of the electrophoretic run to 66 h (Fig. 2). An additional factor contributing to the improved separation was a reduction in the amount of protein applied from normally 20–25 μg to 5–10 μg. Detection of such low protein amounts was only possible when the gels were silver-stained [10]. Electrophoresis of extracts from three different muscles on adjacent lanes of the same slab gel produced three well-separated isomyosin bands. However, the mobilities of the isomyosin triplets differed between the three muscles under study. The isomyosin triplet of the superficial vastus lateralis, a muscle with a high content of HCIIb (Table I), displayed the highest mobility (Fig. 2, lane 1), whereas the isomyosin triplet of the long-term electrostimulated extensor digitorum longus with the highest amount of HCIIa (Table I) had the lowest mobility (Fig. 2, lane 3). The isomyosin triplet of the deep masseter, a muscle with a predominance of HCIId (Table I), dispalyed an intermediate electrophoretic mobility (Fig. 2, lane 2).

Densitometric evaluation revealed different proportions of the three isomyosin bands in the three muscles under study (Table I). FM2 was the most prominent band in the superficial vastus, whereas FM3 was predominant in the low-frequency stimulated extensor digitorum longus. The three isomyosin bands were present in nearly equal proportions in the deep portion of masseter muscle. These specific isomyosin distributions could be explained by differences in the alkali light chain content (Table I). The high FM3 content of the stimulated extensor corresponded to the highest relative concentration of LC1f and the low content of FM1 to

Table I

Densitometrically evaluated percentage distribution of myosin light and heavy chain isoforms of different heavy chain-based isomyosins in rat fast-twitch muscles

Muscle			LC1f/LC3f
Superficial vastus			
98% HCIIb	2% HCIId	0% HCIIa	
29% LC1f	53% LC2f	18% LC3f	1.6
36% FM3b	43% FM2b	21% FM1b	
Deep masseter			
7% HCIIb	74% HCIId	19% HCIIa	
23% LC1f	53% LC2f	23% LC3f	1.0
27% FM3d	28% FM2d	24% FM1d	
56-day stimulated m. extensor digitorim longus			
2% HCIIb	25% HCIId	70% HCIIa	
47% LC1f	44% LC2f	6% LC3f	7.8
54% FM3a	32% FM2a	10% FM1a	

Isomyosin electrophoresis of the same muscles is depicted in Fig. 2. Small amounts of neonatal and slow isoforms present in masseter and extensor digitorum longus muscle were not included.

the low amount of LC3f. Conversely, the relative high percentage of FM2 and FM1 in the superficial vastus and the deep masseter, respectively, corresponded to relative high amounts of LC3f in these muscles (Table I). As seen in Fig. 2 (lane 2), the deep masseter displayed two additional faint isomyosin bands with higher mobility than FM1. In view of the presence of low amounts of neonatal myosin in adult masseter [12], these bands were tentatively identified as neonatal isomyosins.

4. DISCUSSION

As previously shown, the electrophoretic mobilities of the fast isomyosins FM1, FM2 and FM3 relate to differences in their alkali light chain complement [2,4,5]. The present results on three different muscle types, each with a predominance of one of the fast myosin HC isoforms (HCIIb, HCIId or HCIIa), suggest that the observed differences in electrophoretic mobility of the fast isomyosins may also relate to differences in HC complement. Thus, we demonstrate the existence of three different HC-based isomyosin triplets yielding a total of nine fast isomyosins in rat fast-twitch muscle. It appears conceivable to explain the electrophoretic differences of the three isomyosins of each triplet by their specific LC complements and the differences which exist between the electrophoretic mobilities of the three triplets by their specific HC complements. We suggest a nomenclature indicating the HC-based differences of these fast isomyosins, i.e. FM1b, FM2b, FM3b, FM1d, FM2d, FM3d, FM1a, FM2a, and FM3a (Fig. 3).

An interesting observation relates to different proportions of FM1, FM2 and FM3 in the three HC-based isomyosin triplets. These can be explained by differences in the relative concentrations of alkali light chains LC1f and LC3f. In this regard, major differences exist between FMb and FMd isomyosins on the one hand, and FMa isomyosins on the other hand. Our data suggest different affinities of the fast HC isoforms for the two fast alkali LC species. Thus, it appears likely that HCIIb and HCIId display a higher affinity for LC3f than HCIIa. This conclusion is in agreement with

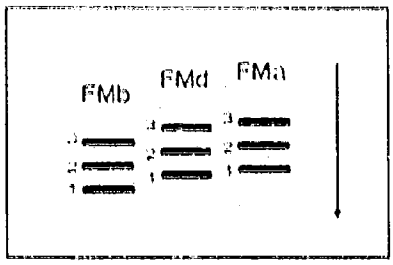

Fig. 3. Schematic illustration of the electrophoretic mobilities of HCIIb-, HCIId-, and HCIIa-based fast isomyosins. Three fast isomyosins result from the combination of the LC1f homodimer (FM3), the LC1f/LC3f heterodimer (FM2) and the LC3f homodimer (FM1) with each fast HC isoform homodimer. Arrow indicates direction of migration.

our previous observation that chronic low-frequency stimulation-induced transitions in myosin heavy chain expression in the order HCIIb → HCIId → HCIIa [8] are paralleled by increases in the LC1f/LC3f ratio [11].

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