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Subunit composition of native myosin isoenzymes of some striated mammmalian muscles

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Summary

Six "native" isomyosins of characteristic electrophoretic mobility are present in various proportions according to the muscle type (fast, slow or mixed). SM2 and SM1 contain MHC1; IM contains MHC2A; FM1, FM2 and FM3 contain MHC2A and MHC2B. SM2, SM1 and IM contain MLC1s; SM1, IM, FM2 and FM3 contain MLC1f; FM2 and FM1 contain MLC3f. The six isomyosins appear to separate on the base of their myosin light chain content.

Introduction

Myosin exists in several isoenzymatic forms which differ in heavy and light chain content (1,2,3). Fast and slow subunits can be co-expressed in a single fiber and existence of myosins hybridized in light chains has been postulated (4,5). We report in this paper that two of the so called "native" myosins are such hybrid forms which occur in sizeable proportions in several mammalian muscles, together with four other common forms. All six forms have characteristic electrophoretic mobilities which can be measured with reproducible accuracy and are specific of the form but not of the animal species.

Methods.

The muscles were extracted in 4 vol of a Guba-Straub solution (0.3M NaCl, 0.1M NaH_2PO_4, 1mM $MgCl_2$, 10 mM $Na_4P_2O_7$, 0.1% NaN_3 pH 6.5, 0.1% 2-mercaptoethanol). After centrifugation (20.000 rpm, 20 min) the supernatant was mixed with 1 vol glycerol and stored at -20°C. This crude extract of myosin was used for pyrophosphate polyacrylamide gel electrophoresis as previously reported (3). All myosin samples contained in addition 20% of a crude extract of taenia coli used as internal standard. The electrophoretic profiles were scanned at 536 nm and the mobility as well as the relative proportions of the isomyosins were obtained using a computerized densitometer. The method has been described in details in earlier works (3,6).

For the analysis of subunit composition of single isoenzyme native myosin zones were cut out from gels run in non-dissociating conditions and remigrated in 15 or 5% SDS-polyacrylamide gel-electrophoresis (7).

Results.

1. Mobilities and proportions.

The main isomyosin of rat soleus, SM2, is well known (2,3,11). We decided to use its rate of migration as a reference point (arbitrarily set equal to 100) to evaluate the electrophoretic mobilities of the native isomyosins. Since the mobility of taenia coli myosin is quite high, 147 ± 1 relatively to that of SM2, it migrates well ahead of the striated isomyosins with which it never interferes: it was then convenient to use it as an internal standard to which we referred the mobilities of the isoforms extracted from all the muscles which we investigated (Table 1). SM2 is the only zone present in soleus of guinea-pig, rabbit and cat, and it is the prominent band in all slow muscles; it is also present in most mixed muscles. The small variations in mobility (96 to 102) are probably due to some subtle variations in the method. SM1 with a mobility of 102-106 is observed in rat soleus and in several mixed muscles. IM (for intermediary myosin) with a mobility of 112-114

TABLE 1. Mobility of adult myosins.

Means for the number of experiments indicated between brackets. The standard errors of the means are between 0.5 and 2.
a) Form observed in 2 experiments; b) in 3 experiments.

Muscle	Mobility of myosin form in % of rat SM2					
	SM2	SM1	IM	FM3	FM2	FM1
SLOW MUSCLES						
SOLEUS						
mouse (4)	99		114			
rat (7)	100	105	112			
g.-pig (4)	99					
rabbit (8)	96					
cat (4)	101					
FAST MUSCLES						
PSOAS						
rabbit (5)				115	121	127
cat (4)				114	120	126
EDL						
g.-pig (4)				120	124	129
MIXED MUSCLES						
EDL						
mouse (4)		104a		121	125	130
rat (7)	99			118	122	128
rabbit (4)	100			112	118	123
cat (4)	101			114	118	125
dog (5)	99	105b		116	122	129
FLEXOR DIGITORUM COMMUNIS						
dog (5)	97	102		112	119	126
QUADRICEPS						
man (6)	100	105		112	118	124
TRICEPS SURAE						
man (10)	101	106		114	119	125

is a rarer form, found in rat and mouse soleus. The triplet FM3 (mobility 114-120), FM2 (mobility 120-124) and FM1 (mobility 126-129) is observed in all fast and mixed muscles. The relative proportions of the native isomyosins widely vary according to the type of muscle, but homologous muscles of different animals appear to be similar (Table 2).

TABLE 2. Proportions of isomyosins in % of total myosin.

Means ± standard error of the mean, for the number of experiments indicated between brackets except for 2(a) and 3(b) experiments.

Muscle		Proportion of myosin form SM2	SM1	IM	FM3	FM2	FM1
SLOW MUSCLES							
SOLEUS							
mouse	(4)	70±6		30±6			
rat	(7)	73±4	16±3	11±3			
g.-pig	(4)	100					
rabbit	(8)	100					
cat	(4)	100					
FAST MUSCLES							
PSOAS							
rabbit	(5)				44±2	37±1	19±8
cat	(4)				52±4	34±2	14±3
EDL							
g.-pig	(4)				52±1	33±2	15±1
MIXED MUSCLES							
EDL							
mouse	(4)		8±5a		48±4	25±2	20±3
rat	(7)	4±1			35±2	38±2	20±2
rabbit	(4)	18±7			37±7	27±6	18±3
cat	(4)	3±1			40±2	39±3	18±5
dog	(5)	21±4	10b		40±14	22±10	6±2
FLEXOR DIGITORUM COMMUNIS							
dog	(5)	16±3	14±9		25±5	33±6	12±2
QUADRICEPS							
man	(16)	40±7	8±5		25±4	17±3	10±4
TRICEPS SURAE							
man	(10)	47±11	7±4		22±6	14±3	10±4

2. Light and heavy chain distribution.

The subunit composition of the six native isomyosins is shown in Table 3. SM2 was analyzed in soleus of mouse, rat, guinea-pig and cat: it is uniformly slow, containing the slow MHC1 and the two slow MLC1s and MLC2s. SM1 has been analyzed only in dog EDL, since the muscles of other species contain very little of it: its composition is that of SM2 with some MLC1f: it is therefore a hybrid slow myosin with regard to the alkaline light chains. IM was analyzed in mouse and rat soleus: it contains a fast myosin 2A molecule hybrid with regard to slow light chain, MLC1s, and in some analyses, with MLC2s. The triplet FM3, FM2 and

FM1 was analyzed in mouse, rat and dog EDL, and also in dog flexor digitorum communis: the results (Table 3) confirm numerous earlier reports [chicken (8), rabbit (10), rat (11) mouse (9)].

TABLE 3. Subunit composition of adult isoenzymes.

Myosin heavy (MHC) and light (MLC) chains in native myosins. The distribution of myosin subunits was determined electrophoretically in 5 or 15% polyacrylamide gels from native isoforms separated in non-dissociating conditions.

	SM2	SM1	IM	FM3	FM2	FM1
MHC1	+	+				
MHC2A			+	+	+	+
MHC2B				+	+	+
MLC1s	+	+	+			
MLC2s	+	+	(+)			
MLC1f		+	+	+	+	
MLC2f			+	+	+	+
MLC3f					+	+

Discussion.

Adult mammalian muscles contain up to six isoforms of myosin that can be separated by electrophoresis under non-dissociating conditions. The three slowest migrating forms (SM2, SM1 and IM) are typical of slow-twitch muscle, whilst the three fastest forms (FM3, FM2, FM1) are typical of fast-twitch muscle; mixed muscle contains the three fast forms and one or two slow forms. The electrophoretic mobility of the six forms are reproducible and accurate, even when they are compared between muscles of different animals which contain them in widely varying proportions. Thus, the electrophoretic mobility can be used to help identifying a particular native isomyosin.

The analysis of the subunit composition shows that four forms (SM2, FM3, FM2, FM1) are homogeneous, containing subunits either of the slow type or of the fast type, whilst two forms (SM1, IM) are hybrids in their light chains (Table 3). SM2 is the "true" slow-twitch isoenzyme, both because it contains the slow subunits of myosin and because it is the major component of slow-twitch muscles. SM1 appears to be a myosin in which one slow light chain of SM2 is replaced by one fast light chain: its existence has been postulated in single human fibers (4) and it seems similar to a slow myosin component present in It fibers described in 60 days chronically stimulated fast-twitch muscles (12). IM has been previously observed in mouse soleus (9) and is perhaps restricted to small mammals, since we observed it only in mouse and rat; it is presumably located in the Type IIA fibers. Since it is absent in mouse EDL, one has to conclude that Type IIA fibers of mouse EDL are different from Type IIA fibers of the mouse soleus with regard to their light

chains content, although they are histochemically indistinguishable. We confirm that the fast triplet are homodimers in MLC1f (FM3) and MLC3f (FM1) and heterodimer MLC1f-MLC3f (FM2), and we add that each fast form is a mixture of the heavy chains MHC2A and MHC2B.

Table 3 shows the six isomyosins arranged in order of increasing electrophoretic mobility: it is clear that their content in myosin light chains could be the base for their different rates of migration. We did not measure accurately the relative proportions of the myosin light chains in the hybrid myosins SM1 and IM, but it is probable that they are heterodimers of the type MLC1s-MLC1f combined either with MHC1 or MHC2A. If this hypothesis is accepted, it is easy to infer the quantitative composition in light chains from the proportions of the native isomyosins: MLC1s = SM2 + (SM1 + IM)/2; MLC1f = FM3 + (SM1 + IM + FM2)/2 and MLC3f = FM1 + FM2/2.

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