MYOSIN LIGHT CHAINS OF DEVELOPING FAST AND SLOW RABBIT SKELETAL MUSCLE

Gudrun PELLONI-MUELLER, Marco ERMINI and Eduard JENNY
Institute of Pharmacology and Biochemistry, Vet.-Med. Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland

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1. Introduction

Vertebrate fast and slow skeletal muscles are composed of fibres whose myosin is enzymatically and structurally distinct from each other. Enzymatically fast myosin is distinguishable from slow myosin by several-fold higher specific ATPase activity [1]. Structural differences are found in the subunit composition, in that after electrophoresis on SDS-polyacrylamide gels fast myosin is split into four bands, one corresponding to the heavy chains (mol wt. approx. 200 000), and the other three to the light chains (approx. 25 000; approx. 18 000; approx. 16 000; according to mobility), while slow myosin shows three bands in the mol. wt. regions of 200 000, 27 000 and 20 000 [2].

During development ATPase activities of both myofibrils and myosin were shown to be enhanced several times in fast muscle, while in slow muscle the enzymatic activities increase much less conspicuously [3,13].

Structural analyses of embryonic myosin apparently revealed two light chains only [4]. From that, and in view of the low ATPase activities, it was concluded that the embryonic myosin either is the same as or is similar to adult slow myosin. In both cases it was believed to occur in both fast and slow muscle uniquely [4,5]. In more recent papers, however, biochemical [6], physiological [7] and histochemical [8] data on vertebrate skeletal muscle development raise doubts about the existence of a unique fiber type or myosin in embryonic muscles. We present here a reinvestigation on the subunit composition of mammalian myosin during development by using purified myofibrils instead of isolated myosin for electrophoresis on SDS–polyacrylamide gels. In addition, the specific activities of the myofibrillar ATPase of the gastrocnemius and soleus have been determined at different postnatal developmental stages.

On the basis of the low mol. wt. subunits it is shown that at birth the gastrocnemius contains exclusively fast myosin. The light chain pattern of the neonatal soleus, however, is composed of the two light chains of the slow myosin plus the three light chains of the fast myosin, indicating that at birth the soleus contains both types of myosin.

At this stage the proportion of slow to fast myosin was calculated as 1:2. During development fast myosin is gradually eliminated from the soleus, and at the age of 30 days only slow myosin is seen to be present.

The stoichiometric evaluation of the light chains of the gastrocnemius revealed that from birth to adult the ratio of the three light chains changes considerably, while the two light chains of the slow myosin of the soleus remain constant in their proportion during postnatal development.

The specific activity of the myofibrillar ATPase of the gastrocnemius increases about four-fold from birth to adult, while the soleus exhibits only a temporary increase within the first 20 days after birth. This increase can be related to the initially strong presence of fast myosin in the soleus.

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2. Material and methods

2.1. *Animals and muscles*

The Mm. gastrocnemius (caput laterale) and soleus from New Zealand rabbits have been used as fast and slow muscles, respectively.

2.2. *Preparation of highly purified myofibrils*

After mincing and homogenization of the muscles in 'high ionic strength buffer' (100 mM KCl, 1 mM EDTA, 1 mM DTT, 7 mM KH_2PO_4, pH 7.0), the myofibrils were first washed twice in the same solution, followed by ten washings in 'low ionic strength buffer' (5 mM Tris-HCl, pH 7.0, 1 mM DTT). The solutions were prepared according to Morimoto and Harrington [9].

2.3. *SDS-polyacrylamide gel electrophoresis of the myofibrils*

For electrophoresis the myofibrils were heated for 5 min at 90°C in a solution containing (end concentrations) 25% glycerol, 0.5% (w/v) SDS, 100 mM phosphate buffer, pH 7.0, 0.168 mM mercaptoethanol, and traces of bromophenol blue. Gels, 5 mm in diameter, contained 7.5% acrylamide, 0.2% bisacrylamide, 0.1% SDS, 0.05% ammonium persulfate, 0.05% TEMED. Electrode buffer was 20 mM in phosphate buffer, pH 7.0, and 0.1% in SDS. Gels were run for 60 min at 6 mA per gel. Staining was performed in a solution of 0.25% Coomassie brilliant blue, 45% methanol, and 9% acetic acid, by heating for 60 min at 37°C. Destaining was performed by diffusion against 9% acetic acid.

2.4. *Assay of the myofibrillar ATPase*

The myofibrils (0.3–0.7 mg protein [10]) were incubated for five minutes with 5 mM $MgCl_2$, 0.2 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.6, 2.5 mM ATP. The reaction was started upon the addition of the ATP, and terminated after the addition of 25% TCA. The liberated phosphate was determined according to Lowry and Lopez [11], modified by Mattenheimer [12].

3. Results and discussion

3.1. *General considerations on the use of myofibrils for SDS-polyacrylamide gel electrophoresis*

As shown in fig.1a, b, and c, electrophoresis of extensively washed myofibrils results in a very good resolution of the light chains both of fast and slow skeletal muscle myosin.

From the tropomyosin–troponin complex only tropomyosin and troponin-T remain detectable on the gels, but not troponin-I nor troponin-C. This is also indicated by the complete absence of EGTA-sensitivity in ATPase assays [13].

On the other hand, electrophoresing directly myofibrils for the analysis of the myosin light chains has several advantages over the use of isolated myosin:

(1) Quantities as small as 0.1 g of muscle provide sufficient material to perform gel electrophoresis, hence fast and slow muscles can be prepared separately even from embryonic animals. This is in contrast to myosin isolation, where yield is low, and mixed muscle has to be taken from young animals for comparison with adult fast and slow muscle. In addition, selective extraction, which never can be ruled out in myosin isolation, is unlikely to occur in myofibrillar preparation.

(2) Myosin complexed with actin, as in myofibrils, is better protected from denaturation, thus eventual loss of myosin components during isolation due to e.g. higher susceptibility to dissociation, as shown for embryonic myosin [14], is avoided.

3.2. *The light chains of developing soleus and gastrocnemius muscle*

The light chain pattern of gastrocnemius myosin does not change between birth and maturity (figs.1 and 2 a–e). The three typical bands of light chains LCf1, LCf2 and LCf3 are always present.

Five light chain bands are seen, however, in the soleus muscle of newborn rabbits (fig.1 and 2 f–j). Mixing of myofibrils of young soleus with either adult fast or slow myofibrils demonstrates that the five bands are identical with LCs1, LCf1, LCs2, LCf2, and LCf3, in the order of mobility from top to bottom of the gels (fig.1). Therefore no evidence exists, on the basis of light chain composition, that a third type of myosin is present in embryonic skeletal muscle. Thus it is obvious that the soleus muscle,

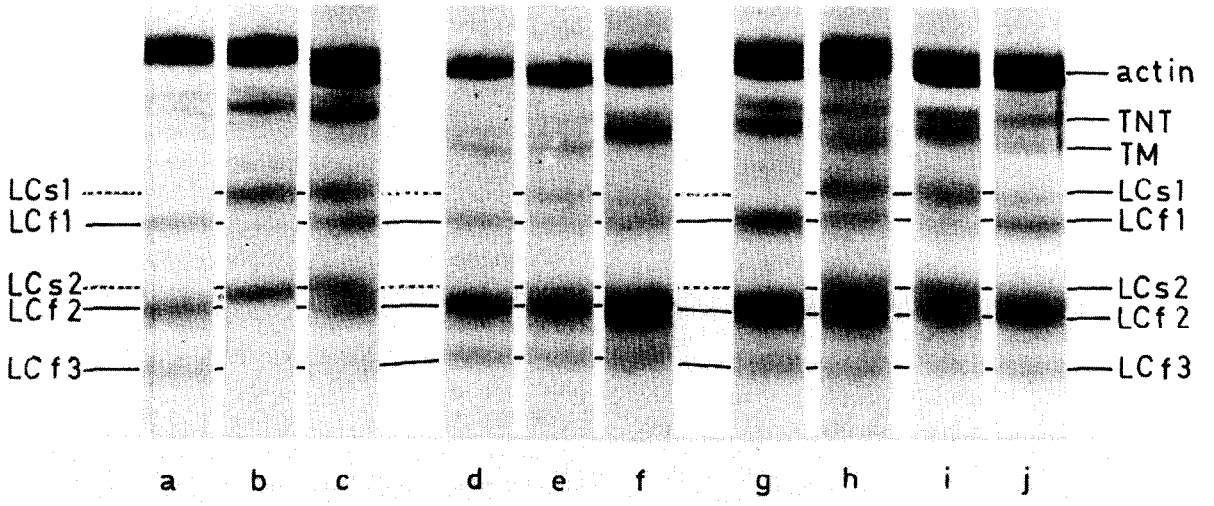

Fig.1. The light chains of rabbit gastrocnemius and soleus myosin after electrophoresis of purified myofibrils on 7.5% SDS-polyacrylamide gels. Migration from top to bottom. The pictures of gels were cut above the actin band. 20 micrograms of myofibrillar protein of each muscle type were loaded onto the gels (i.e. mixtures of the two were 40 μg). LCf1, LCf2, LCf3, light chains of fast myosin; LCs1, LCs2, light chains of slow myosin. TNT, troponin T; TM, tropomyosin. (a) Adult gastrocnemius; (b) adult soleus; (c) mixture of (a + b); (d) 3 days old gastrocnemius; (e) 3 days old soleus. (f) mixture of (d + e); (g), (d + a); (h), (d + b); (i), (e + b); (j), (e + a).

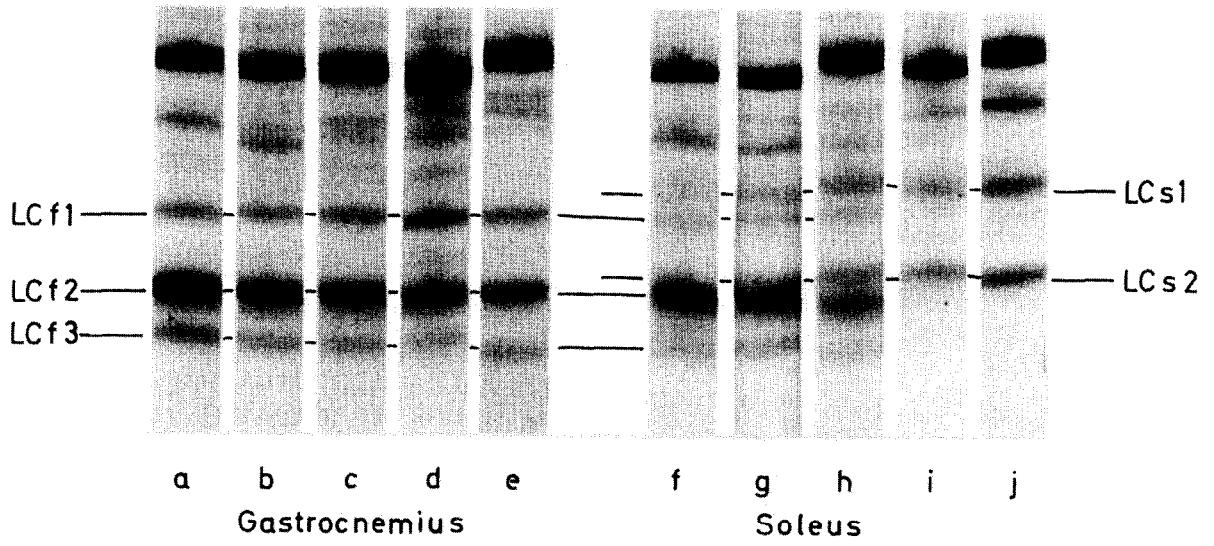

Fig.2. Light chain patterns of rabbit gastrocnemius and soleus myosin in the course of postnatal development after electrophoresis of purified myofibrils on 7.5% SDS-polyacrylamide gels. Migration from top to bottom. The pictures of the gels were cut above the actin band. LCf1, LCf2, LCf3, light chains of fast myosin. LCs1, LCs2, light chains of slow myosin. (a, b, c, d, and e) Gastrocnemius of 1, 3, 11, 25 days old, and adult rabbits. (f, g, h, i and j) Soleus of 1, 3, 11, 25 days old, and adult rabbits.

Table 1
Quantitative evaluation of light chains from electrophoresed myofibrils of gastrocnemius (a) and soleus muscle (b) from rabbits in the course of postnatal development

(a) *M. gastrocnemius*

Age	(n)	LCf1 (mol. wt. = 25 000)	LCf2 (mol. wt. = 18 000)	LCf3 (mol. wt. = 16 000)
0– 4 d	(8)	17.8 (± 2.2)	64.2 (± 2.4)	18.5 (± 2.6)
8–11 d	(5)	24.0 (± 3.1)	58.9 (± 2.4)	17.1 (± 2.5)
16–25 d	(3)	31.4 (± 1.6)	53.1 (± 1.2)	15.3 (± 2.2)
Adult	(2)	33.7	50.8	15.6

(b) *M. soleus*

Age	(n)	LCs1 (mol. wt. = 27 000)	LCf1 (mol. wt. = 25 000)	LCs2 (mol. wt. = 20 000)	LCs2+LCf2	LCf2 (mol. wt. = 18 000)	LCf3 (mol. wt. = 16 000)
0– 4 d	(8)	12.1 (± 3.1)	10.8 (± 1.9)	[16.3]	63.5 (± 3.9)	[47.2]	13.6 (± 2.2)
8–11 d	(5)	24.5 (± 5.0)	11.1 (± 1.3)	[20.0]	54.4 (± 2.1)	[34.4]	10.0 (± 2.1)
16–25 d	(3)	37.2 (± 4.8)	7.9 (± 1.7)	31.5 (± 4.9)	48.5 (± 3.6)	17.0 (± 2.8)	6.4 (± 1.3)
Adult	(2)	56.1	0.0	43.9	43.9	0.0	0.0

Gels were scanned, and peak areas integrated. Standard deviation is indicated in parentheses. In table 1b, LCs2 and LCf2 of the first two age groups were integrated together. The sum has been resolved into its two peaks of LCs2 and LCf2 by calculations based on the stoichiometric relationship of the light chain pattern from gastrocnemius myosin of the corresponding age groups of table 1a. These values are indicated in square brackets.

prior to postnatal differentiation, is composed of a mixture of slow and fast fibres.

From quantitative evaluation, performed by gel scanning and peak integration, we calculated that the soleus contains at birth about two thirds of fast myosin, and one third of slow myosin (table 1). At the age of ten days their distribution is about equal, and at 20 days two-thirds are already slow. For these calculations a uniform staining is assumed for the three light chain proteins.

Quantitation of gastrocnemius light chains reveals that in the course of development the three light chains change in their stoichiometric relationship. In adult fast myosin the proportion of LCf1 : LCf2 : LCf3 is 33.7:50.8:15.6 (table 1a), which is in agreement with the ratios reported by others [2,15]. At birth, however, the proportion is 17.8:64.2:18.5. With development the relationship continuously shifts towards that found in adult fast myosin, which is reached around 30 days after birth.

In soleus myosin of newborn rabbits the light chain pattern is complicated by the presence of both fast and slow myosin subunits (table 1b). Moreover, since in gels from young myofibrils the broader band of LCf2 overlaps slightly with that of LCs2, the sum of both peaks only has been integrated. That sum has been resolved into its two peaks of LCs2 and LCf2 by calculations based on the stoichiometric relationship of the light chain pattern from gastrocnemius myosin of the corresponding age groups. The light chains LCs1 and LCs2 of slow myosin remain virtually unchanged from birth to maturity in their quantitative relation. Fast myosin is eliminated from soleus muscle continuously during postnatal differentiation. These results indicate that slow myosin is structurally identical in embryonic and adult muscle with respect to its subunit composition. On the other hand, fast myosin is subject to changes in its light chain stoichiometry.

3.3. *The specific ATPase activity of myofibrils during development*

In the adult gastrocnemius and soleus the myofibrils exhibit very clear differences in ATPase activity [15,18], corresponding to the presence of fast and slow myosin, respectively. A mixture of the two types of myosin therefore should result in a specific activity, which is either lower or higher with respect to the one or the other type of myosin.

Consequently, in the early phase of postnatal development, the myofibrillar ATPase activity of the

Table 2
Specific activities of the gastrocnemius and soleus myofibrillar ATPase during postnatal development

Age (days)	Gastrocnemius	Soleus		
	Spec. act. ($\bar{x}$) ($\pm s\bar{x}$) (μmoles P_i/mg prot./5 min, 25°C)	Spec. act. ($\bar{x}$) ($\pm s\bar{x}$)	Theoretical spec. act.	Protein ratio $\frac{\text{fast myosin}}{\text{slow myosin}}$ (from the values in table 1b)
1 (n = 4)	0.66 (± 0.09)	0.56 (± 0.07)	0.64	2.52 : 1
8–11 (n = 4)	1.92 (± 0.04)	1.15 (± 0.06)	1.34	1.25 : 1
17–25 (n = 5)	2.07 (± 0.16)	0.94 (± 0.11)	1.07	0.45 : 1
Adult (n = 5)	2.40 (± 0.09)	0.62 (± 0.07)	0.62	0.00 : 1

The experimentally determined specific activities of the soleus myofibrillar ATPase are compared to the values estimated on the basis of the protein ratios of fast to slow myosin (as calculated from the values in table 1b) as well as of the corresponding specific activities of the gastrocnemius myofibrillar ATPase of the respective age groups. As basic value of the soleus (slow myosin) myofibrillar ATPase the one found in adult animals was taken. Specific activity is expressed as μmoles P_i liberated per mg protein per 5 min at 25°C.

soleus should be influenced by the initially strong presence of fast myosin.

We have compared the myofibrillar ATPase activities of both the soleus and gastrocnemius from birth to maturity (table 2).

The specific activity of the gastrocnemius myofibrillar ATPase is shown to increase from 0.66 at birth to 2.40 in the adult, with the steepest increase taking place within the first 14 days of age. Similar results have been obtained by others [16–18].

In the soleus, however, the behaviour of the myofibrillar ATPase is different. Around birth the specific activity is equal to that of the gastrocnemius. Within the first 10 days an approx. two-fold increase to 1.15 is observed, followed by a slow decrease to the value found in adult animals.

Since from the gels it can be deduced that in the adult soleus only slow myosin is present, we consider the specific activity of the adult soleus myofibrillar ATPase as representative for myofibrils containing exclusively slow myosin.

As indicated in table 2 we have taken into consideration on one hand the ratio of fast to slow myosin, as calculated for each age group on the basis of the light chain proportions on the gels, and on the other hand the respective gastrocnemius myofibrillar ATPase activities, for the calculation of the expected specific activities of the soleus myofibrillar ATPase of the four age groups. As basic value of the soleus (i.e. slow myosin) we have taken the one found in adult animals.

It can be seen that these theoretical values are compatible with the actually determined ones.

This can be taken as evidence that the five light chains found initially in the soleus indeed indicate the simultaneous presence of fast and slow myosin.

Additional support for this conclusion can be drawn from the publications of Buller et al. [19], and Gutmann et al. [7], where a temporary increase in contraction velocity was described for slow mammalian skeletal muscles in the early phase of postnatal development.

4. Concluding remarks

Two questions arise in connection with the results presented here:

(1) What is the reason for the low specific activity of myofibrils (and myosin) of the gastrocnemius at birth, since at least on the basis of light chain identification, no qualitative change is found in fast myosin?

(2) Do the results presented here imply that both, slow and fast myosin, are always present throughout skeletal muscle development?

For the first question we may consider that the change in stoichiometry of light chains in fast myosin during development affects the enzymatic activity.

Fast myosin, undergoing considerable intramolecular quantitative shifts in its subunit composition, exhibits an increase in activity of about ten times during development without any visible qualitative changes [13]. It has also been demonstrated in chicken skeletal muscle that the light chain pattern of fast myosin at even very young embryonic stages is qualitatively the same as in that from adult animals [6]. In contrast to rabbit fast myosin, however, also the ATPase activity is equal in embryonic and adult muscle. Although no quantitative data are given with respect to light chain stoichiometry it can clearly be estimated from the picture of the gels (fig.4 of ref. [6]) that no difference between embryonic and adult myosin light chain proportion exists.

Our findings on rabbit fast myosin structural and enzymatic developmental behaviour, together with those reported on chicken fast myosin development, may be indicative of a correlation between light chain stoichiometry and enzymatic activity of myosin.

To answer the second question an additional analysis of the light chain pattern of prospective slow muscles is required for embryonic stages. If, however, the observed constant increase of the ratio of fast to slow myosin towards the younger stages is extrapolated to the stages before birth, one might expect that at a certain embryonic stage no slow myosin is present anymore. This would imply that indeed one myosin type only is synthesized in undifferentiated skeletal muscle, but contrary to the current opinion, this would be a fast type myosin.

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References

[1] Barany, M., Barany, K., Reckard, T. and Volpe, A. (1965) Arch. Biochem. Biophys. 109, 185–191.
[2] Lowey, S. and Risby, D. (1971) Nature 234, 81–85.
[3] Müller, G., Ermini, M. and Jenny, E. (1975) Experientia 31, 723.
[4] Perry, S. V. (1970) in: The Physiology and Biochemistry of Muscle as a Food, 2nd. edn. (Briskey, E. J., Cassens, R. G. and Marsh, B. B., eds.), 537–553, University of Wisconsin Press, Madison.
[5] Trayer, I. P. and Perry, S. V. (1966) Biochem. Z. 345, 87–100.
[6] Sreter, F., Holtzer, S., Gergely, J. and Holtzer, H. (1972) J. Cell Biol. 55, 586–594.
[7] Gutmann, E., Melichna, J. and Syrovy, I. (1974) Physiol. bohemoslov. 23, 19–27.
[8] Karpati, G. and Engel, W. K. (1967) Arch. Neurol. 17, 542–545.
[9] Morimoto, K. and Harrington, W. F. (1973) J. Mol. Biol. 77, 165–175.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. biol. Chem. 193, 265–275.
[11] Lowry, O. H. and Lopez, J. A. (1945) J. biol. Chem. 162, 421.
[12] Mattenheimer, H. (1958) Acta biol. et med. Germanica 1, 405.
[13] Pelloni-Müller, G. (1976) Diss. Phil. Natw. Fak. Univ. Zürich.
[14] Dow, J. and Stracher, A. (1971) Proc. Nat. Acad. Sci. USA 68, 1107–1110.
[15] Pfister, M., Schaub, M. C., Watterson, J. G., Knecht, M. and Waser, P. G. (1975) Biochim. Biophys. Acta 410, 193–209.
[16] Trayer, I. P. and Perry, S. V. (1966) Biochem. Z. 345, 87–100.
[17] Ermini, M. and Schaub, M. C. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1266–1270.
[18] Jablecki, C. and Kaufman, S. (1973) J. biol. Chem. 248, 1056–1062.
[19] Buller, A. J., Eccles, J. C. and Eccles, R. M. (1960) J. Physiol. 150, 399–416.