CHANGES IN MYOSIN LIGHT AND HEAVY CHAIN STOICHIOMETRY DURING DEVELOPMENT OF RABBIT FAST, SLOW, AND CARDIAC MUSCLE

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1. Introduction

The structural and enzymatic properties of myosin change during development and differentiation of striated rabbit skeletal muscles [1]. Prior to postnatal differentiation in both, prospective fast and slow muscles, one single myosin species is synthesized. It possesses three light chains with the same electrophoretic mobility as those of adult fast myosin, but with a different stoichiometry [1]. Around 30 days after birth in fast muscles the adult values of light chain proportions are reached, while in slow muscles the original myosin has been gradually eliminated and replaced by the typical slow myosin [1].

We present here an extension of the myosin light chain analysis to the rabbit fetal heart muscle. We have found that cardiac myosin of rabbits at the age of 9–3 days before birth contains a mixture of light chains as found in adult fast and slow skeletal muscle myosin. In this respect it resembles the neonatal soleus myosin [1]. In addition we report on fundamental changes in the quantitative relationship of myosin heavy to light chains of developing fast, slow, and cardiac muscle. These results were obtained after electrophoresis of highly purified myofibrils on SDS-polyacrylamide gels.

2. Materials and methods

2.1. *Animals and muscles*

The hearts from New Zealand rabbits were used, as well as the Mm. gastrocnemius (caput laterale) and soleus as fast and slow muscles, respectively.

2.2. *Preparation of highly purified myofibrils*

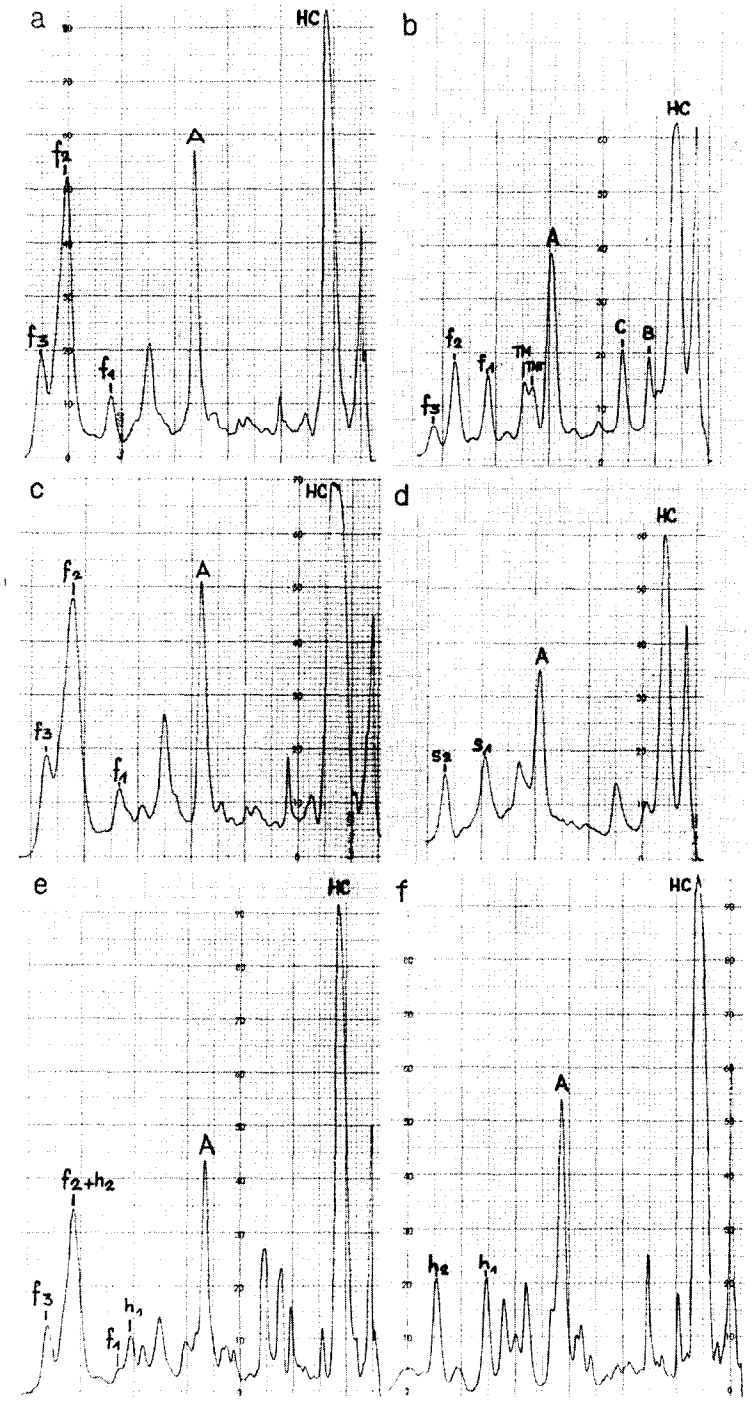
After mincing and homogenization of the muscles in 'high ionic strength buffer' (100 mM KCl, 1 mM EDTA, 1 mM DTT, 7 mM KH_2PO_4, pH 7.0), the myofibrils were first washed twice in the same solution, followed by ten washings in 'low ionic strength buffer' (5 mM Tris–HCl, pH 7.0, 1 mM DTT) [2].

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

For electrophoresis (essentially according to Maizel, 1974 [3]) the myofibrils were heated for 5 min at 90°C in a solution containing 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.

3. Results and discussion

The purified myofibrils merely contain myosin, actin, tropomyosin, troponin T, and some B- and C-protein (fig.1b). These proteins were identified as described earlier [1]. In cardiac myosin 5 light chains, of which two form the large asymetric band (f2 + h2), are present at 3 days before birth (fig.1e). They



Gastroc-
nemius

a = -3 d
b = adult

Soleus

c = -3 d
d = adult

Heart

e = -3 d
f = adult

Fig.1

Table 1
Quantitative evaluation of myosin light chains from electrophoresed myofibrils of fetal and adult rabbit heart muscle

Age (days from birth)	(n)	LCh1	LCf1	LCh2	LCf2	LCf3
−9	(3)[a]	4.4	5.0	70.2		20.5
−3	(3)[a]	12.5	4.8	66.2		16.4
Adult (+ 120)	(2)	45.9	0	54.1	0	0

[a]Pooled together

The single light chains were calculated as percentage of total light chain mass. LCh1, LCh2: light chains of the heart myosin, with apparent molecular weights of 27 000 and 20 000, respectively. LCf1, LCf2, LCf3: light chains of the fast type myosin, with apparent molecular weights of 26 000, 18 000, and 16 000, respectively. At 9 and 3 days before birth, LCf2 is so abundant that the peaks of the two bands of LCh2 and LCf2 were too close to be integrated separately.

correspond in their electrophoretic mobility to the myosin light chain of adult fast and cardiac muscle. In myosin of hearts from adult rabbits the 3 fast light chains have disappeared, and the 2 typical cardiac myosin light chains are present alone (fig.1f).

In table 1 the cardiac myosin light chains from rabbits at 9 and 3 days before birth, as well as from adult animals are compared quantitatively to each other. Since the bands of the intermediate fast (LCf2) and the smaller cardiac light chain (LCh2) of the fetal cardiac myosins overlap due to the abundance of LCf2 (fig.1e), only the sum of the two could be obtained from the densitograms. It can be seen that at 9 days before birth the fast components are present to a larger extent than at the age of 3 days before birth. We conclude that the heart synthesizes, similar to the prospective slow soleus muscle, first a fast type myosin with 3 light chains. However, its replacement by the slow type myosin with 2 light chains typical for the adult heart muscle, occurs at an earlier developmental stage than in the soleus muscle [1].

The similar developmental pattern in myosin light chain composition of cardiac and slow skeletal muscle is very striking. It leads to the question as to what the regulatory factor determining fiber differentiation during development might be. It has been shown that in mammals like rabbits whose neuromotor apparatus apparently is immature at birth, all skeletal muscles exhibit a uniform fiber pattern both in terms of structural and metabolic properties [4,5]. Concomitant with the maturation of the neuromotor system and the increasing motor activity of the young animals, the muscle fibers become differentiated into fast, slow, and intermediate, according to the function of the respective muscle. During this period the light chain stoichiometry of the fast myosin changes towards the proportions found in adult fast muscle. In slow muscles the slow myosin type becomes synthesized in increasing amounts and gradually displaces the fast type myosin, which originally was present alone [1] (fig.1c). This functional adaptation, and the fact that in adult animals fast muscles can be changed experimentally into slow ones by continuous electrical stimulation [6], let us deduce that the steady excitation of a muscle

Fig.1. Densitometric tracings of the gastrocnemius, soleus and heart myofibrils after electrophoresis on SDS–polyacrylamide gels. Exactly 20 μg of myofibrillar protein was loaded always onto the gels. −3 d = three days before birth. Peaks: HC = heavy chains, B = B-protein, C = C-protein, A = actin, TNT = troponin T, TM = tropomyosin, fl, 2, 3 = light chains of fast myosin with apparent molecular weights of 25 000, 18 000, and 16 000, respectively; sl, 2 = light chains of slow myosin with apparent molecular weights of 27 000, and 20 000, respectively; hl, 2 = light chains of heart slow myosin (molecular weights corresponding to sl, 2). The proteins were identified by comigration with marker proteins of known molecular weights as described in [1].

initiates the synthesis of the characteristic slow type myosin with two light chains and low ATPase activity. This has already been suggested some years ago on the basis of physiological experiments [7]. The properties of heart myosin are very similar to those of slow skeletal muscle myosin [8]. The heart representing the most extreme case of a continuously contracting muscle contains, as expected, the slow type of myosin exclusively. On the other hand, at an early time of embryonic development, when the heart muscle fibers have not yet been contracting for a longer period, only the fast type myosin is synthesized, as in the undifferentiated slow skeletal muscles.

However, since the heart starts functioning within the first 2 days of embryonic life, it is somewhat surprising that still 3 days before birth embryonic (fast type) myosin is detectable. This could be explained by the fact that in heart at late fetal stages, and even during early postnatal development, the fiber number is still increased by myoblast fusion [9]. These new fibers have to go through the stage of embryonic myosin synthesis too, and, consequently, still contribute embryonic myosin at relatively late stages to the otherwise slow myosin.

In extending the quantitative analysis to the whole set of myosin subunits, we have found that in all 3 muscles the proportion of heavy to light chains is considerably different between the stage of 3 days before birth and maturity (fig.1). This is further substantiated by the figures in table 2. In both the gastrocnemius and soleus myosin the heavy chains increase from approx. 50% of total myosin to approx. 70–76%. In heart myosin a similar development is observed if late fetal stages are compared to adult ones. From the total of myofibrillar proteins loaded onto the gels the percentage of actin plus myosin was always 60–62%, while the ratio of myosin to actin was constantly 1:3.2. These values did not differ either during development or between the three muscle types. We can therefore conclude that there is an actual increase of heavy chains and a simulataneous decrease of light chains going on during postnatal development of skeletal muscle. In heart muscle the same course of development takes place at an earlier age.

An approximation of the molar relationship between the heavy and light chains reveals that a considerable change occurs from the embryonic to the adult stage. In myosin of late fetal or neonatal rabbits the mean molar ratio of heavy to light chains (average mol. wt. values: 200 000 and 20 000, respectively) was found to be around 1:10, in myosin of fully developed animals 1:2. It is known that the heavy and light subunits of myosin are characterized by different turnover rates [10–12,4], and it is suggested that different genes are coding for the various subfractions [13].

From our results it must be concluded that the synthesis of the single myosin fractions is either not yet coordinated in undifferentiated fibers, or it follows a pattern specific for embryonic myosin.

Table 2
Myosin heavy chains as a percentage of total myosin from rabbit gastrocnemius, soleus, and heart muscle

Age (days from birth)	(n)	Gastrocnemius	Soleus	Heart
– 9	(3)[a]	– –	– –	54.0
– 3	(3)[a]	48.4	51.6	54.0
0– 4	(8)	56.1 ± 1.5	54.0 ± 1.4	–
8–11	(5)	70.6 ± 2.7	66.7 ± 1.9	–
16–25	(3)	75.7 ± 2.6	69.1 ± 3.9	–
Adult (+ 120)	(2)	76.6	69.5	81.2

[a]Pooled tothe
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(Calculated at various developmental stages, from the densitograms of electrophoresed myofibrils on SDS–polyacrylamide gels. Mean values ± SD.)

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