DIFFERENTIAL DISTRIBUTION OF TROPOMYOSIN SUBUNITS IN FAST AND SLOW RAT MUSCLES AND ITS CHANGES IN LONG-TERM DENERVATED HEMIDIAPHRAGM

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1. Introduction

Skeletal tropomyosin, a rod-shaped protein which lies in the grooves of the double-stranded F-actin filament, is involved in the calcium regulatory system of muscle contraction [1]. The native molecule is a dimer in which the two subunits α (34 000 M_r) and β (36 000 M_r) are assembled as $\alpha\alpha$, $\beta\beta$ or $\alpha\beta$; in the slow and fast mammalian muscles the ratio of molar amount of α and β subunits of tropomyosin differs, the β-subunit being more represented in the slow type and during the development [2,3]. The distribution of tropomyosin subunits has been studied in several mammalian species by immunohistochemical studies and electrophoretic analysis of extensively purified tropomyosin. Results have been collected by two-dimensional electrophoresis [6]. The position of the tropomyosin subunits have been identified in a two-dimensional electrophoretogram of crude myofibrils from rat muscle, using tropomyosin purified by standard techniques [7]. However, their relative proportion in the fast and slow muscles has not been determined. We have performed the two-dimensional electrophoresis of actomyosin from normal and long-term denervated rat muscles. The tropomyosin subunit pattern was thus compared with the myosin light chain pattern in the same preparation. The two-dimensional electrophoresis shows that adult rat soleus, a predominant slow type muscle, contains almost exclusively the β-subunit of tropomyosin, while adult fast and immature muscles contain both α- and β-subunits in about the same proportion. This is a further evidence that tropomyosin subunit ratio differs in fast and slow skeletal muscles, even though with a distribution peculiar to each mammalian species. The analysis of tropomyosin subunits and myosin light chains shows that there is a loss of slow type components in long-term denervated hemidiaphragm.

2. Experimental

We have analyzed actomyosin prepared according to [8] by the two-dimensional technique in [9] as modified [10]. Relative amounts of tropomyosin subunits in the slabs were determined by the television system in [11].

3. Results and discussion

The two-dimensional electrophoretogram of actomyosin from an adult rat fast-twitch muscle, the extensor digitorum longus, shows the peculiar myosin light chains 1f, 2f and 3f; α and β tropomyosin subunits are present in about the same proportion (fig.1a). The actomyosin from the soleus, a predominant slow-twitch muscle, shows besides the peculiar myosin light chains 1s and 2s, trace amount of the fast type components. The tropomyosin shows a so large preponderance of the β-subunit that, taking into account the percentage of fast fibers in the muscle [12], we can suggest that tropomyosin must be present as the $\beta\beta$ dimer in the most of the slow fibers of the rat soleus (fig.1d). Since this situation seems to be peculiar to rat slow muscle fibers we have verified the identity of the indicated β-subunit with a purified rabbit marker. Fig.1h shows that rat and rabbit tropomyosin subunits comigrate in our conditions on two-dimensional electrophoresis The increase of app. M_r of tropomyosin subunits when the slab gel contains 3 M urea (fig.1g) confirms the correct identification of the tropomyosin spots [6,13].

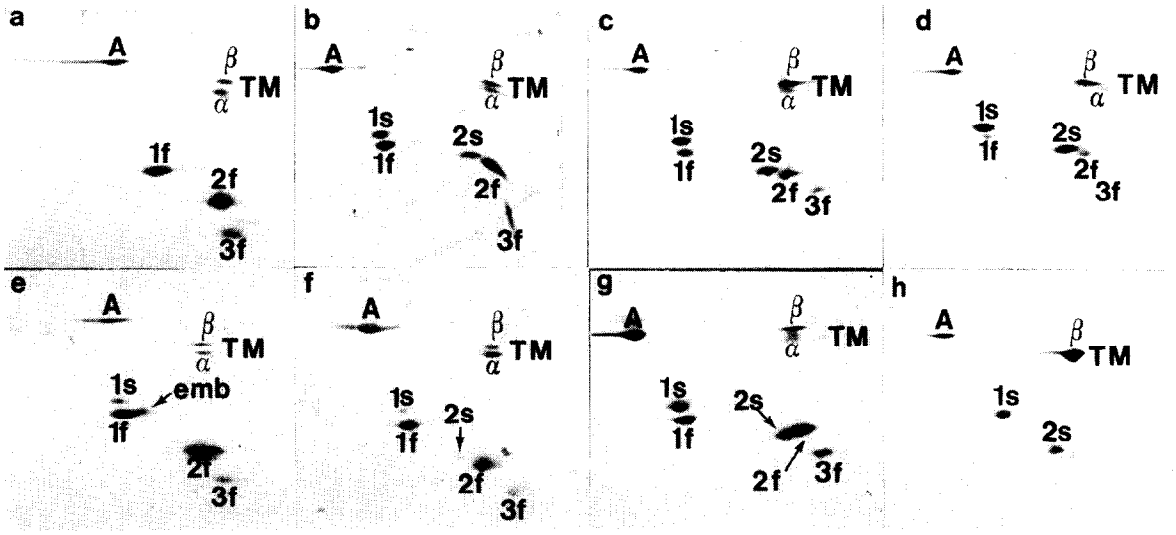

Fig.1. Two-dimensional gel electrophoresis of actomyosin from rat muscles. Actomyosins extracted as in [8] from pooled muscles, were subjected to two-dimensional gel analysis according to [9] with the modifications in [10]: (a) adult rat extensor digitorum longus; (b) adult rat diaphragm; (c) extensor digitorum longus and soleus coelectrophoresis; (d) adult rat soleus; (e) leg muscles from one day old rat, (f) 12-month denervated adult rat diaphragm; (g) soleus and extensor digitorum longus coelectrophorezed in the presence of 3 M urea in the SDS dimension; (h) β-subunit of rabbit tropomyosin and adult rat soleus coelectrophoresis; (A) actin, (1f–2f–3f) myosin light chains that distinguish the fast muscles; (1s–2s) myosin light chains that distinguish the slow muscles; (emb) myosin light chain characteristic of embryonic muscles; (α–β TM) tropomyosin subunits. Only the acidic low molecular mass region of the slabs is presented, thus the minor basic component 1's light chain [7] is not shown. About 80 μg actomyosin/slab was used, in the coelectrophoresis experiments ~40 μg of each sample were used. Where used the purified β subunit of rabbit tropomyosin (generous gift of Dr R. K. Roy) was 1.5 μg.

Note that the β-subunit is present as a double spot in this gel-coanalysis of fast and slow actomyosin (fig.1g). The same splitting of the tropomyosin spots is present occasionally in some slabs, but without any consistent difference among muscles, so that it can result from artifacts during isoelectrofocussing. On the other hand microheterogeneity [4] or phosphorylation [14] of tropomyosin subunits have been reported; so it remains to be elucidated whether the single spot pattern or the splitting is representative of the in vivo condition. As a consequence of this uncertainty, we consider the ratio between α and β subunit in table 1.

Fig.1b shows the two-dimensional electrophoresis of actomyosin from rat diaphragm, a muscle which contains fast and slow fibers, the fast type being the slightly major proportion [15]. Note the 5 spots pattern of the fast and slow myosin light chains and the slightly greater proportion of the β tropomyosin subunit. After chronic denervation, besides the loss of slow-type myosin light chains and the decreased content of the 3f light chain, note the predominance of the α tropomyosin subunit (fig.1f). These effects may result from the selective loss of slow type fibers in long-term denervated hemidiaphragm. However, while the myosin light chain pattern of denervated diaphragm is similar to that of extensor digitorum longus, the tropomyosin subunit ratio is different (cf fig.1a and 1f). Thus, since

Table 1
Ratios of amounts of α and β subunits of tropomyosin from rat muscles

Muscle	Ratio of α/β	p
Extensor digitorum longus	1.17 ± 0.04 (8)	<0.001
Soleus	0.26 ± 0.06 (6)	
Diaphragm	0.99 ± 0.07 (7)	<0.001
12 months denervated diaphragm	4.15 ± 0.84 (5)	
Leg muscles from 1 day old rat	1.06 ± 0.10 (8)	<0.001

Two-dimensional electrophoresis of actomyosin was carried out in the condition described in text and fig.1. Relative amounts of the α- and β-subunits of tropomyosin were determined by comparing the intensities of staining with Coomassie brilliant blue of electrophoretic spots, using the television system in [11,25]. In fact it is possible to compute the effective colour quantity ratio of the electrophoretic spots by means of threshold areas and of the corresponding step voltage. Each value is the mean ± SEM (no. obs.) p was calculated *vs* 12-month denervated hemidiaphragm by Student's t-test

the effect of chronic denervation does not seem to be the result of mere fiber selection, we have tested the possibility of retrodifferentiation or regeneration events after chronic denervation. Fig.1e shows the two-dimensional pattern of actomyosin from developing rat muscle. The myosin light chains display a mixed pattern. Besides the subunits typical of the adult fast muscle (1f, 2f and, even if in smaller amount, 3f) trace amounts of slow and of the 'embryonic' [7] light chains are present (see [16]). The α- and β-subunits of tropomyosin are present in almost similar amounts. Thus long-term denervation seems to lead in rat diaphragm to a new steady-state synthesis of tropomyosin subunits in a ratio dissimilar to both adult fast and developing muscles (table 1). The tropomyosin analysis in long-term denervated diaphragm reveals the partial loss of the β-subunit, a component prevailing in slow muscles. Thus the selective loss of slow type characteristics in myosin light [15] and heavy [10] chains in chronically denervated muscle, is confirmed by tropomyosin analysis. These results corroborate the impulse-mediated mechanism of the neural influence on muscle differentiation [17]. However, the peculiar nature of the tropomyosin (i.e., the α subunit predominance) of chronically denervated muscle, makes interpretation difficult. The question is whether or not the change in contractile proteins (myosin and tropomyosin) results solely from one of these mechanisms: fiber selection, transformation of pre-existing slow fibers to fast ones, or regeneration. In fact while the tropomyosin subunit ratio of developing muscle (fig.1e) seems to exclude the relevance of regenerative events in the response of muscle to chronic denervation, the study of acutely regenerating muscle in innervated or denervated rat limbs ([18] our preliminary results) strongly suggest that these events take place in long-term denervated diaphragm. Two-dimensional electrophoretograms of actomyosin from rat skeletal muscles have been presented in a study of myosin light chains from developing [19] and regenerating muscle of denervated or still innervated limbs [18]. Although not discussed in [18,19], the results concerning the tropomyosin subunits agree with these data. In fact, two-dimensional electrophoretogram of actomyosin from 1 month regenerating rat soleus in a denervated limb displays not only a tropomyosin subunit ratio (preponderance of α on β subunit) but also a myosin light chain pattern (lower amount of 3f) very similar to that we found in chronically denervated hemidiaphragm (see fig.1f and [18]). Regenerative events have been suggested to explain the changes in myosin isoforms that occur after electrical phasic stimulation of denervated muscles [20]. The absence in our long-term denervated muscles of the embryonic myosin light chain, a protein characteristic of the early stages of muscle development [7], could be attributed to the fact that the development of muscle fibers seems to be a multistage process even in the absence of the nerve, e.g., in in vitro cell cultures [7,21]. However, morphological aspects of regenerative events in long-term denervated diaphragm are difficult to demonstrate ([15,22], S. Lücke et al., unpublished) while split fibers and satellite fibers are observed more frequently during the earlier stages of the process [23,24]. By integrating the above results with our data in [10,15], we can suggest that in chronically denervated muscle the still surviving fibers, either pre-existing or regenerated after denervation, respond (in the absence of the main differentiative influence of the nerve) to the residual mechanical and humoral stimuli that not only allow their survival, but, probably, induce a new steady-state in the synthesis of the contractile proteins. Thus, while the change in the gene expression of the myosin and tropomyosin isoforms in chronically denervated hemidiaphragm is clearly established, further experiments are required for understanding the physiological inferences of long-term denervation experiments.

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