COEXISTENCE OF FAST AND SLOW TYPE MYOSIN LIGHT CHAINS IN SINGLE MUSCLE FIBRES DURING TRANSFORMATION AS INDUCED BY LONG TERM STIMULATION

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1. Introduction

Weeds et al. have shown that the myosin light chain pattern of single fibres from rabbit psoas muscle, which is composed predominately of fast-twitch fibres, is uniform and corresponds to that of fast type myosin [1]. Microelectrophoretic analyses of total myofibrillar proteins from microdissected and histochemically typed rabbit muscle fibres have recently been performed in our laboratory and revealed that only one type of myosin is present in individual fibres [2]. These findings raise the question whether changes in the myosin species of a muscle as observed in cross-innervation [3–5] or in chronic electric stimulation experiments reflect a rearrangement of the fibre population by substitution or by transformation of the existing fibres. Only in the latter case might myosins of different types be expected to coexist transiently within individual fibres.

Sréter et al. [6,7] observed that myosin extracted from fast rabbit muscles which had been stimulated 3–10 weeks with a frequency pattern similar to that of a slow motoneuron revealed light chains of slow type myosin and additionally, at decreased concentrations and varied ratios also light chains of fast type myosin. These results were confirmed in our laboratory [8]. Moreover, it was shown that changes in the myosin light chain pattern correlate with quantitatively evaluated changes in fibre population [8,9]. In one animal an almost complete transformation of the fibre population was observed but electrophoresis of the extracted myosin revealed, aside from the predominance of the slow type light chains, a persistence of the slowest migrating fast type light chain. This finding was interpreted as indicating the coexistence of different myosins in individual fibres during the the process of their induced transformation [8,10]. This interpretation is supported by the single fibre analysis of the present investigation.

2. Materials and methods

Single muscle fibres were microdissected from freeze-dried samples of stimulated (left) and contralateral unstimulated tibialis anterior muscle. The muscles were from a rabbit (No. 3007 [8]) which had been exposed to continuous stimulation [8] of its left lateral popliteal nerve with a frequency of 10 Hz during 56 days. Analyses performed on the same muscles with regard to contractile parameters, morphometric and histochemical evaluation of fibre population as well as electrophoresis of myosin light chains have been published recently [8]. After dissection and typing the single fibres, soluble proteins were pre-extracted and microelectrophoresis of total myofibrillar proteins was performed in polyacrylamide (PAA) gel in the presence of 0.1% sodium dodecylsulphate (SDS) according to the methods described [2].

3. Results and discussion

Figure 1 shows the stained band patterns of SDS–polyacrylamide gel microelectrophoreses of total myofibrillar proteins from two single fibres of control

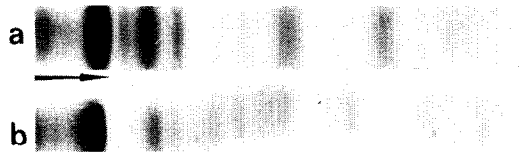

Fig.1. SDS–Polyacrylamide gel microelectrophoreses of total myofibrillar proteins from single rabbit muscle fibres: (a) normal fast-twitch fibre from control; (b) transformed fibre from 56 d stimulated tibialis anterior muscle. The stained gels were magnified through a cylindrical lens. Only that part is shown which contains actin, tropomyosin, troponin, and the myosin light chains.

(fig. 1a) and of stimulated (fig. 1b) tibialis anterior muscle. The protein pattern of the fast-twitch fibre from the unstimulated control muscle reveals the typical bands of the three fast type myosin light chains. According to calibration by reference proteins [2], the apparent molecular weights of the light chains LC_1, LC_2 and LC_3 were determined in this experiment as 22 000, 18 300 and 16 100, respectively. The protein pattern of the stimulated fibre is characterized by a greater number of bands in the same region (fig. 1b), but obviously the position of some of these bands does not correspond to that of the control. This can be seen more clearly from the densitometric evaluation of the two gels shown in fig.2. No peptides corresponding to light chains LC_2 and LC_3 are present in the electrophoretic pattern of the stimulated fibre. The two peak No 9 and 10 have not yet been identified. On the other hand, there is a peak (No. 7, fig. 2) at exactly the same position as light chain LC_1 of fast type myosin. The identity of this peptide with the latter is confirmed by the value of its app. mol. wt, 22 000. Further similarities exist with regard to peak No 1 and 2 representing actin and tropomyosin.

The densitometric scan of the electrophoresis derived from the stimulated fibre, reveals aside from the afore mentioned fast type myosin light chain LC_1 (peak No. 7) three peptides (peak Nos 5,6,8) which may be envisaged according to their apparent molecular weights (25 200, 24 000, 18 900) as light chains LC_{1a} LC_{1b} and LC_2 of the slow type myosin. These values fit well with those which were determined recently for the respective light chains by means of single slow-twitch fibre analysis [2].

These results provide evidence that light chains of

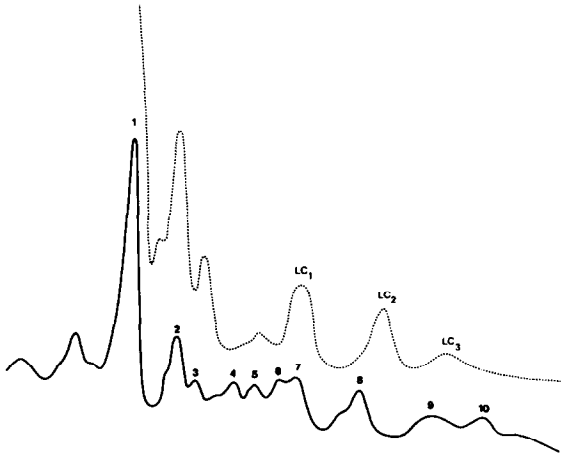

Fig.2. Densitometric scans of SDS-Polyacrylamide gel microelectrophoreses of myofibrillar proteins from single muscle fibres of control (dotted line) and of 56 d stimulated (solid line) rabbit tibialis anterior muscle. LC_1, LC_2, LC_3; light chains of fast type myosin with app. mol. wts, 22 000, 18 300, and 16 100, respectively. Apparent molecular weights of peaks 1–10: (1) 44 000; (2) 34 000; (3) 31 800; (4) 27 800; (5) 25 200; (6) 24 000; (7) 22 000; (8) 18 900; (9) 16 800; (10) 15 200.

different myosin species may be present under certain conditions within a single fibre. The transformation of the myosin from fast to slow type which has been observed in the stimulated muscle, is obviously not due to a change in fibre population by a substition of fast for slow-twitch fibres but rather results from a transformation of the existing fast into slow-twitch fibres. Simultaneous presence of different myosin types within a single fibre is assumed to be transitory, since the myosin extracted from muscles stimulated for prolonged periods, consisted only of the slow type according to analyses of the light chain pattern [11]. The fact that in the present case only one fast-type light chain is seen in the stimulated fibre suggests that the synthesis of at least this light chain is under control of a separate gene.

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