MYOSIN LIGHT CHAINS IN NORMAL AND ELECTROSTIMULATED CULTURES OF EMBRYONIC CHICKEN BREAST MUSCLE

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1. Introduction

Fast and slow twitch fibres of adult skeletal muscles are characterized by electrophoretically distinct patterns of fast- and slow-type myosin light chains [1]. The expression of fibre-type specific myosins in embryonic skeletal muscles is still a subject of controversy. By applying electrophoretic methods, several authors have shown that fast-type myosin is predominant in embryonic muscles even in precursors of future slow twitch muscles [2–7]. These findings were interpreted to mean that embryonic skeletal muscles initially express only fast-type myosin and the synthesis of slow-type myosin is initiated only after innervation. Only fast-type myosin light chains were detected in non-innervated myotubes cultivated from embryonic precursors of both fast and slow chicken muscles [8].

Transformations of fast and slow twitch muscles with transitions in the myosin light chain patterns can be induced in adult mammalian muscles by cross-reinnervation [9,10] or by long-term indirect electrostimulation [11,12]. Electrostimulation of noninnervated myotubes in vitro has been shown to induce changes in the myosin light chain pattern [13]. After 24 days stimulation at 3 Hz, slow-type myosin light chains were detected in addition to the predominant fast-type light chains. These changes might be explained by a specific activation of genes coding for slow-type myosin. On the other hand, it was reported that electrostimulation of myotubes in vitro leads to an overall activation of myofibrillar protein synthesis [14]. The alterations seen in the myosin light chain pattern [13] might therefore be brought about by a quantitative change. This study examines this latter possibility. It is shown that slow-type myosin light chains can be detected at extremely low amounts in nonstimulated myotubes and that their cellular level increases by electrostimulation.

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2. Materials and methods

Myoblasts from breast muscle of 11-day-old chicken embryos were seeded, induced for fusion and cultured as in [15]. Cytosine arabinoside (10^{-6} M) was added 1 day after fusion in order to inhibit fibroblast proliferation. The cells were cultured in a 8% CO_2 atmosphere in minimum essential medium (Dulbecco's modification) supplemented with 10% horse serum and 5% embryo extract. Myotubes (11-day-old) were continuously stimulated by platinum electrodes at a frequency of 3 Hz (pulse duration 40 ms). This frequency was chosen because myotubes did not respond with single contractions at frequencies >6 Hz.

Myosin was purified from the myotubes by $(NH_4)_2SO_4$ precipitation (34–45%) according to [16].

Defined amounts of myosins were subjected to isoelectric focusing in the first dimension [17] followed by SDS–polyacrylamide gel electrophoresis in the second dimension [18].

3. Results

Fig.1a,b shows two-dimensional electrophoreses of myosin purified from 35-day-old unstimulated (fig.1a) and stimulated (fig.1b) myotubes. The myosin light chain patterns in fig.1a,b were obtained from equal amounts (120 μg) of protein applied to the gels. Under these conditions, only myosin light chains LC_{1f} and

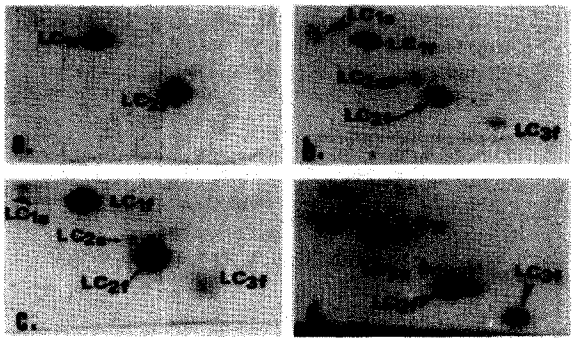

Fig.1. Two-dimensional electrophoreses of myosin purified from control and long-term electrostimulated myotubes of cultured embryonic chicken breast muscle (only the light chain regions of the Coomassie brilliant blue-stained gels are shown): (a) myosin light chains of 35-day-old non-stimulated myotubes (120 μg protein applied); (b) myosin light chains of 35-day-old stimulated (24 days) myotubes (120 μg protein applied); (c) myosin light chains of 10-day-old myotubes (520 μg protein applied); (d) coelectrophoresis of (a) with 120 μg adult chicken breast muscle myosin.
Abbreviations: $LC1_s$, $LC2_s$; slow myosin light chains 1, 2; $LC1_f$, $LC2_f$, $LC3_f$, fast myosin light chains 1, 2, 3

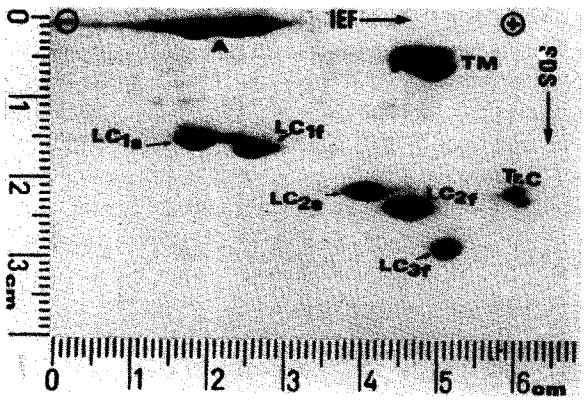

Fig.2. Two-dimensional coelectrophoresis of myofibrillar proteins from chicken slow anterior latissimus dorsi (ALD) and fast posterior latissimus dorsi (PLD) muscles.
Abbreviations: $LC1_s$, $LC2_s$, (slow myosin light chains 1, 2 from ALD); $LC1_f$, $LC2_f$, $LC3_f$, fast myosin light chains 1, 2, 3 from PLD; A, actin; TM, tropomyosin; TRC, troponin 'c'; IEF, isolectric focusing; SDS, sodium dodecyl sulphate

LC_{2f} can be detected with certainty in the electrophoresis of myosin from control myotubes (fig.1a). On the contrary, myosin of the stimulated myotubes (fig.1b) contains some additional peptides at low concentration. These were identified tentatively from coelectrophoresis with myosin purified from fast adult chicken breast muscle (fig.1d) and slow anterior latissimus dorsi (fig.2) as light chains $LC3_f$, $LC1_s$ and $LC2_s$. The faint spot adjacent to light chain $LC2_s$ at the acidic side could represent light chain 1 of fibroblast myosin although the fibroblast content of the cultures extremely low. It cannot be excluded that this faint spot represents the phosphorylated form of $LC2_s$. No changes in the myosin light chain pattern could be detected under these experimental conditions in myotubes which had been stimulated for shorter periods (10 and 15 days). In spite of the predominance of $LC1_f$ and $LC2_f$, long-term stimulated myotubes appear thus to be characterized by the expression of the complete sets of light chains both of fast and slow myosin. Results shown in fig.1c suggest that the detection of additional light chains in long term stimulated myotubes (fig.1b) is not due to a qualitative change. In contrast to the electrophoresis depicted in fig.1a,b, a much larger amount of myosin (520 μg) was applied to the gel in fig.1c. A light chain pattern was obtained under these conditions similar to that shown in fig.1b.

Non-stimulated myotubes (10-day-old) evidently contain light chains $LC3_f$, $LC1_s$ and $LC2_s$. However, the concentration of these light chains is much lower than in long-term stimulated myotubes since they can be detected only by overloading the gels.

4. Discussion

The view that embryonic muscles initially synthesize only fast-type myosin light chains has been questioned by several authors. Immunochemical studies with specific antibodies suggested that slow and fast myosins coexist in embryonic muscles [19–21]. Our results confirm the predominance of fast-type myosin light chains $LC1_f$ and $LC2_f$ in noninnervated myotubes [8]. Furthermore, we show that non-innervated myotubes contain also slow-type myosin light chains, although at very low concentration. This could explain why slow-type myosin light chains were not detected in other electrophoretic studies on myosin from myotube cultures [7,8]. Our results agree with those in [22]. These authors were able to show by incorporation studies that myotubes cultivated from embryonic precursors of fast and slow muscles both synthesize the complete set of myosin light chains, although with a predominance of $LC1_f$ and $LC2_f$.

These results suggest that the cellular levels of the slow myosin light chains as well as that of the third

fast myosin light chain can be increased by long-term electrostimulation (fig.1b). In view of the stimulation-induced increase in overall myofibrillar protein synthesis [14], increases in these light chains might reflect altered rates of their synthesis and/or degradation. These findings are not comparable with the pronounced alterations induced by long-term indirect stimulations of adult muscles in vivo [11,12]. Unlike the in vivo stimulation, the myotubes were stimulated directly. Possible nerve-mediated influences were thus excluded. Furthermore, a frequency of only 3 Hz was applied as compared to the 10 Hz stimulations in vivo [11,12]. It remains open whether the induced changes are specifically related to the applied frequency or merely represent a response to increased contractile activity.

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