Mechanisms underlying the asynchronous replacement of myosin light chain isoforms during stimulation-induced fibre-type transformation of skeletal muscle

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During the fibre-type transformation induced by chronic electrical stimulation of rabbit fast-twitch muscle, replacement of the fast forms of the two classes of myosin light chain by their slow isoforms occurs asynchronously. Studies of total cellular myosin light chains and of the slow-to-fast transition now justify the conclusion that the asynchrony is due to switching between the expression of fast and slow genes for the two light chain classes at sequential stages of the transformation process.

Myosin light chain *Polymorphism* *Muscle* *Stimulation*

1. INTRODUCTION

Continuous low-frequency electrical stimulation of a fast skeletal muscle via its motor nerve results in a transformation of its contractile properties [1–3]. During the course of this fast-to-slow muscle transformation, expression of the fast type of myosin gives place to that of the slow type within individual muscle fibres. Myosin is a hexameric protein, consisting of 2 heavy chains and a pair of each of 2 classes of light chain: the alkali light chains (ALC = LC1 + LC3) and the phosphorylatable light chains (PLC = LC2). All of these subunits can exist in fast and slow isoforms and the myosin transition observed during fibre-type transformation has been shown to involve changes in the isoforms of each subunit [4–9]. Although the stoichiometric composition of native myosin is maintained during fibre-type transformation, replacement of the individual myosin subunits has been shown to occur asynchronously [9]. There are 3 possible explanations for this. (i) Replacement of the fast by the slow isoform of each light chain class may occur simultaneously in the cellular pool, asynchrony in the replacement of these subunits in native myosin resulting from differences in the rates at which each subunit is incorporated. (ii) Switching from expression of the fast to the slow genes may occur after periods of stimulation that are different for each class of light chain. (iii) Switching of expression may occur simultaneously, with differences in the rates of synthesis and degradation of the 2 light chain classes being responsible for their asynchronous replacement. Here, we distinguish between these mechanisms by comparing the composition of the light chains in the total cellular pool with those bound into native myosin, and by examining the effect on myosin composition of cessation of stimulation after transformation has occurred.

2. MATERIALS AND METHODS

Tibialis anterior (TA) and extensor digitorum longus muscles of adult New Zealand White rabbits were stimulated continuously at 10 Hz by means of implanted stimulators as in [10]. In some experiments stimulation was discontinued after a period of 10 weeks and muscles allowed to recover for periods of up to 6 weeks. Muscles were homo-

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genised in 0.4 M NaCl, 0.05 M Tris-HCl (pH 6.8), 1% 2-mercaptoethanol, and samples of the homogenate subjected to 2-dimensional gel electrophoresis [11] as in [12,13]. Quantitation of the myosin light chains from these muscle samples was achieved by excision of the respective spots from stained gels, elution of the Coomassie blue dye with 25% (v/v) pyridine by overnight incubation, and spectrophotometric estimation at 605 nm. With this procedure there was a linear relationship between protein concentration and absorbance for A values between 0.01 and 0.39 for a test protein, bovine serum albumin. The light chain analyses were conducted such that measurements of the eluted dye fell within these limits. Since the amount of dye bound per mol depends upon the M_r of the peptide, and the absorbance depends upon the volume into which the dye is eluted, corrections were incorporated for these variables in the determination of light chain composition.

The muscle homogenate was also used for the preparation of a purified sample of myosin, the light chain composition of which was determined by densitometric scanning of the bands produced by 1-dimensional electrophoresis in SDS-polyacrylamide gels [9].

Both types of analysis were also performed on control muscles from 4 rabbits, and the results indicated that the relative amounts of the different light chains, expressed as percentages, could be determined in this way to better than ±1.5% (SE).

3. RESULTS

In muscles which had been stimulated for up to 10 weeks the relative proportions of the myosin light chains were determined both from the total cellular pool (fig.1a) and from the light chains bound in assembled myosin molecules (fig.1b). The figures for the myosin-bound light chains shown in fig.1b were derived from [9] and are reproduced here, modified by incorporation of additional values for control TA muscles and amalgamation of the values for the fast alkali (ALCf) and slow alkali (ALCs) light chains, for convenience of comparison.

During transformation there was a gradual decline in the proportion of the fast-type light chains with a concomitant rise in the proportions of the slow light chains. The asynchronous replacement of the myosin-bound light chain components was evident from a consideration of the proportions of ALCf and ALCs and of PLCf and PLCs (fig.1b). A comparison of the graphs for the total and for the myosin-bound light chains shows them to be superficially similar in terms of the time course of the transitions. However, some interesting differences also emerged:

(i) the proportions of the fast light chains in the cellular light chain pool of the control TA muscles did not correspond to those observed for the myosin-bound light chains. Comparison of the light chains ratios revealed a relative excess of PLCf in the cellular pool;

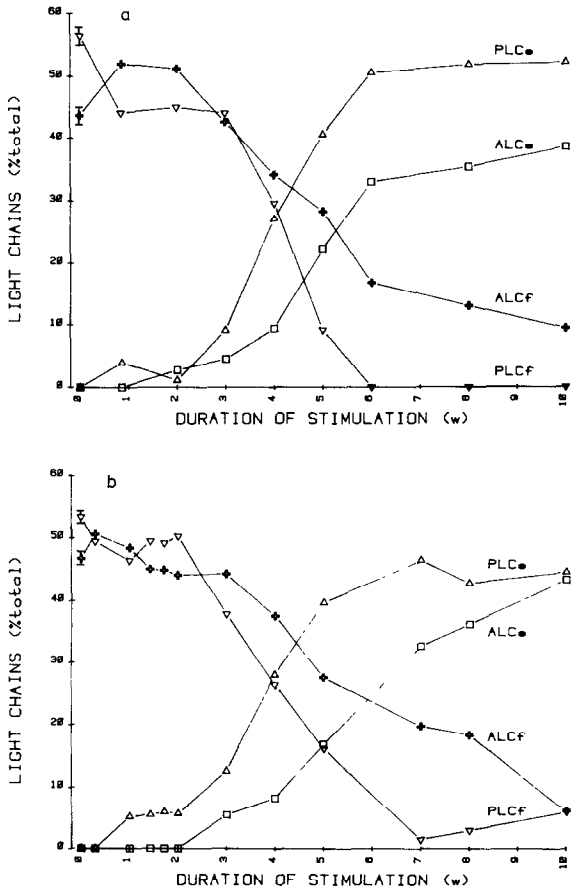


Fig.1. Proportions of individual myosin light chains as a percentage of the total light chains in TA muscles after various periods of stimulation: (a) total cellular pool, (b) myosin-bound pool. Values for control TA muscles (0 weeks stimulation) are the means ±SE of 4 animals.

(ii) the change-over point, at which fast and slow isoforms were transiently present in equal amounts, occurred earlier for the PLC isoforms than for the ALC isoforms in both fig.1a and b, but the delay between these 2 points was not as great in the total cellular extracts (1 week, vs nearly 2 weeks for the myosin-bound light chains);
(iii) the decrease in PLCf was observed after 3 weeks of stimulation in total cellular light chains, but after only 2 weeks in the myosin-bound light chains.

The composition of total cellular extracts of muscles during the recovery period after stimulation had been stopped at 10 weeks (fig.2) showed a loss of light chains of the slow type and an increase in those of the fast type. After 6 weeks of recovery, slow light chains were still present and the subunit transitions were proceeding considerably more slowly than during stimulation. This is true of other recovery phenomena (see [14]) and is probably attributable to lower overall rates of protein synthesis and degradation accompanying the reduction in contractile activity. In this slow-to-fast myosin transition the change-over point of the P light chains occurred marginally later than that of the A light chains, i.e. the reverse of the order of transition observed during stimulation.

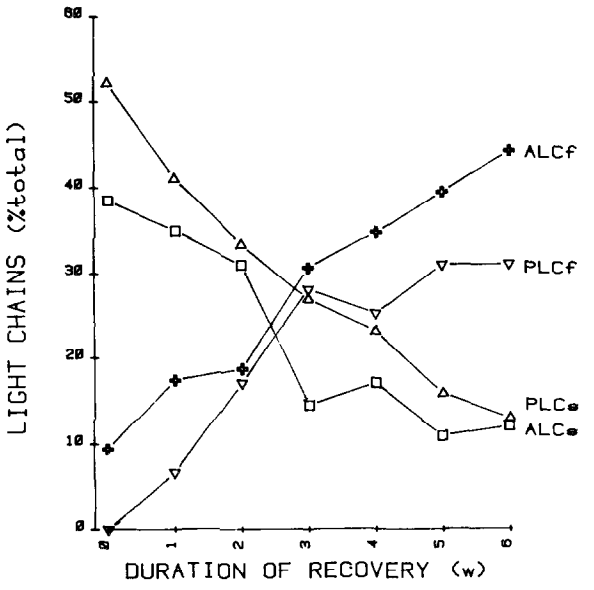


Fig.2. Proportions of individual myosin light chains as a percentage of the total light chains in the cellular pool of TA muscles recovering their original, fast characteristics after 10 weeks of stimulation.

4. DISCUSSION

During the fast-to-slow fibre type transformation, changes in the light chain composition of native myosin mirrored to a large extent those in the total cellular pool. Evidently the myosin subunit composition is determined predominantly by the local availability of each subunit for assembly. The small differences that were observed between these 2 light chain pools indicate the existence of significant amounts of free light chains within the sarcoplasm. The size of this population is presumably determined by the rate of turnover of free light chains and the rate of incorporation and removal of light chains from native myosin. In general, the decline of the fast components was more rapid in the total cellular pool than in the myosin-bound compartment; this would be expected if there were some delay in the removal of light chains which were already bound into myosin. The fact that the molar excess of PLCf disappeared during the first week of stimulation may also indicate that free light chains were turned over more rapidly than myosin-bound light chains in stimulated muscle.

In addition, there were differences between the 2 compartments in terms of the change-over points of the 2 light chain classes; change-over of the PLC isoforms in the myosin pool occurred slightly in advance of that in the total cellular pool and change-over of the ALC isoforms was slightly retarded. While the delay in replacement of the ALC isoforms could be attributed to the slower turnover of bound light chains, such a mechanism would not explain the discrepancy with respect to the PLC isoforms. A comparison of fig.1a with b reveals a difference between the proportions of PLCf and PLCs in the total cellular pool and in the myosin-bound light chains after 3 weeks of stimulation: less PLCf was incorporated into myosin than would be expected from its relative abundance in the cell. It appears that some positive selection of the slow PLC may have taken place from the cellular pool. During fast-to-slow fibre transformation a corresponding transition in the myosin heavy chains is known to occur, the slow heavy chain first appearing after 2 weeks and

becoming the predominant species after 4 weeks of stimulation [7]. The influx of the slow heavy chain into the cellular pool may therefore have been responsible for selection of PLCs from the cellular pool by exercising a specific affinity. Since quantitative measurements of the myosin heavy chain were not undertaken in this study it would be difficult to subject this suggestion to more stringent test.

Asynchrony in the replacement of the A and P light chains in the cellular pool during transformation eliminates the possibility, raised in our discussion of the earlier results [9], that the sequence of subunit transitions observed in myosin-bound light chains is determined entirely at the level of assembly. We are left with 2 alternatives: either a temporal difference in switching between expression of fast and slow genes for the different classes of light chain, or simultaneous switching, with the asynchrony then attributed to differences in the rates at which the A and P light chains are accumulated and eliminated. Examination of this switch under different conditions enabled us to distinguish between these mechanisms. When electrical stimulation of TA is discontinued, the muscle reverts to its original fast-twitch characteristics, providing and opportunity for us to examine the switching of expression of the myosin light chains in the slow-to-fast direction. We do not suppose that myosin light chains turn over at the same rate during recovery as they do during stimulation, but it seems reasonable to assume that any such differences in turnover are not strongly selective in their effects on A and P light chains. Had the rate of change-over been intrinsically faster for P than A light chains, the transitions in the forward and reverse directions would have been expected to occur, if not at the same rate, then at least in the same sequence. During the slow-to-fast myosin transition, however, change-over of the P light chains occurred not before, but if anything slightly after, that of the A light chains. These results therefore indicate that the genes for the A and P light chains are in fact switched at different stages, possibly under the control of separate regulatory mechanisms.

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