Changes in transcriptional activity of chronically stimulated fast twitch muscle

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mRNAs extracted from rabbit soleus, normal and 28-day, indirectly stimulated tibialis anterior muscles were translated in an in vitro system. Analysis for translation products by 2-dimensional electrophoresis showed fast myosin light chains in tibialis anterior, and slow myosin light chains in soleus muscle. The stoichiometry of the in vitro translated light chains varies from that seen in normal fast and slow twitch muscles. The stimulated muscle contained mRNA coding, both for fast and slow myosin light chains, although the pattern of slow myosin light chains appears not to be complete at this point of time of the transformation process.

Fast and slow twitch muscle *Chronic stimulation* *mRNA* *In vitro translation* *Myosin light chain*

1. INTRODUCTION

Fast twitch muscles of the rabbit can be transformed into slow twitch muscles by chronic indirect stimulation at 10 Hz in vivo [1]. This transformation affects all cellular systems so far examined and may be interpreted as an induced switch in gene expression. One of the most obvious changes is the altered myosin composition which is demonstrated by a time-dependent exchange of fast for slow myosin light chains [2,3]. We were interested in studying the appearance of the mRNA coding for fast or slow type myosin light chains during this transformation. For this reason total mRNA was extracted and purified from normal fast and slow twitch as well as from fast twitch muscles which had been electrically stimulated for 28 days. In vitro translation of the respective mRNAs revealed the coexistence of mRNAs coding both for fast and slow myosin light chains in the stimulated muscle.

2. MATERIALS AND METHODS

Male adult rabbits of the strain 'weiße Wiener' were used. Chronic stimulation was performed as follows: the left lateral popliteal nerve was stimulated 28 days at 10 Hz via implanted electrodes [4]. Stimulated lasted for 12 h/day with hourly alternating periods of stimulation and rest [5]. Total RNA was extracted from normal slow twitch soleus muscle, normal fast twitch tibialis anterior muscle, as well as from the 28-day stimulated tibialis anterior muscle by a combination of the methods of [6,7]. Poly(A)-mRNA was purified by chromatography on oligo(dT)-cellulose [8]. Translation was performed in a [^{35}S]methionine complemented reticulocyte lysate [9]. The radioactive translation products were visualized by autoradiography after 2-dimensional electrophoresis [10]. Identification of myosin light chains and other myofibrillar proteins was achieved by co-electrophoresis with a myofibrillar extract of normal muscles or purified light chains.

3. RESULTS

Chronic stimulation led to a 2-fold increase in total RNA content of the fast twitch muscle tibialis anterior (TA) which after 28 days reaches a level that is in the range of normal slow twitch soleus muscle (table 1).

RNA devoid of a poly(A) segment and therefore

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Table 1

Total RNA (means ± SD) extracted from normal and 28-day stimulated tibialis anterior (TA) and from normal soleus muscles of the rabbit

	Total RNA ($\mu g \times g^{-1}$ muscle)	Number of animals
Normal TA	259 ± 71	8
28-day stim. TA	586 ± 134	4
Soleus	567 ± 177	7

not binding to the oligo(dT)-cellulose column gave negative results in the in vitro translation assay. Poly(A) containing RNA stimulated the translational activity of the reticulocyte lysate 15–20-fold. Increasing concentrations in the range of 0.1 to 0.6 $\mu g/\mu l$ mRNA gave a linear increase in [^{35}S] incorporation by the reticulocyte lysate.

Fig.1 shows autoradiographs of 2-dimensional electrophoresis of the in vitro translation products. Translation products of soleus mRNA (fig.1A) show the typical light chain pattern of slow myosin (LC1sa, LC1sb, LC2s). Additionally, 3 faint spots could be identified by co-electrophoresis with fast myosin as the 3 fast light chains LC1f, LC2f, LC3f. On the contrary, only fast myosin light chains were translated from mRNA of normal TA (fig.1b). It is remarkable that about the same amount of radioactive label has been incorporated into the 3 fast myosin light chains. Among other unidentified spots, a protein is seen in all autoradiographs (fig.1) in the region of LC1f. It has the same app. M_r as LC1f and its isoelectric point is slightly more acidic. This protein which is translated in the in vitro assay at very low concentration from soleus mRNA, might correspond to the embryonic light chain [11]. The faint spot to the right of LC2f is considered to be its phosphorylated form, which is probably due to the presence of light chain kinase in the reticulocyte lysate. An additional difference between the translation products from slow and fast muscle mRNA is seen in the intensities of the α- and β-tropomyosins. The α-subunit predominates in soleus and the β-subunit in TA.

The peptide pattern resulting from in vitro translation of mRNA from a 28-day stimulated TA (fig.1c) is more complex and resembles that of a slow (fig.1a), as well as that of a fast twitch (fig.1b) muscle. The 3 fast myosin light chains are predominant. However, also slow myosin light chains

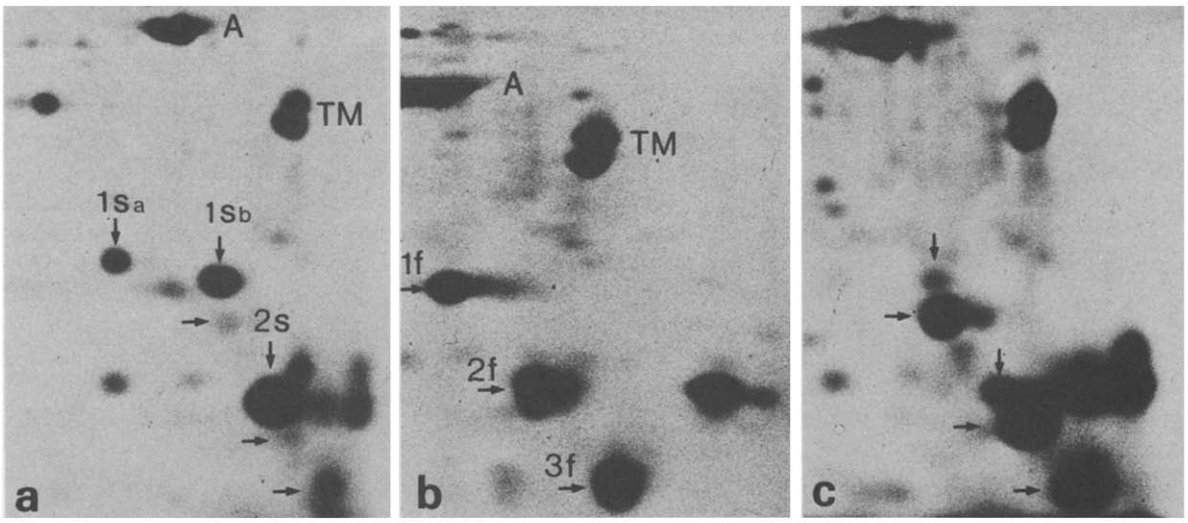

Fig.1. Autoradiographs of 2-dimensional electrophoreses of in vitro translation products from muscle mRNA. Total poly(A) containing RNA was purified from adult rabbit muscle and translated in a [^{35}S]methionine containing reticulocyte lysate. Translation assays contained 0.3 μg mRNA. Only the myosin light chain region is shown. Abbreviations: A, actin; TM, tropomyosin; 1f, 2f, 3f, fast myosin light chains; 1sa, 1sb, 2s, slow myosin light chains. (a) normal slow twitch soleus muscle; (b) normal fast twitch tibialis anterior muscle; (c) 28-day stimulated tibialis anterior muscle.

can be seen, especially LC2s and LC1s$_b$. Of these, LC2s appears to be more prominent. Only a very faint spot can be seen at the location of LC1sa. A pronounced change concerns translation products in the acidic region adjacent to LC2s. The nature of these peptides which are seen in a similar pattern in normal slow twitch muscle (fig.1a) is uncertain. One of these peptides is also found as a translation product of normal TA mRNA (fig.1b) and may be identified tentatively as troponin C. No differences are seen in the distribution of α- and β-tropomyosins between the in vitro translations of mRNA, from normal and stimulated TA which both show a predominance of the β-subunit.

4. DISCUSSION

Fast and slow twitch muscle fibres are characterized by type-specific patterns of their myosin light chains. These differences in phenotype are believed to result from transcription of different genes. The present results confirm the existence of different mRNA populations in these muscles. As visualized by in vitro translation, soleus muscle contains mainly mRNA coding for slow, and TA mRNA coding for fast myosin light chains. It remains open whether the small amounts of mRNA coding for fast myosin light chains in soleus muscle (fig.1a) stem from a small percentage of fast fibres in this muscle, or represent mRNA which is not translated in vivo. Differences between in vitro and in vivo translation are suggested by the observation (not shown) that LC3f is present in fast muscle at a lower concentration than LC1f. These two alkali light chains seem to be translated in similar amounts in vitro (fig.1b). As is evident from fig.1a, LC1sa is translated in vitro to a lesser degree than LC1s$_b$, whereas both peptides are found at similar concentrations in soleus muscle (not shown). Variations between in vitro and in vivo translation appear to exist also for the tropomyosin subunits. A predominance of the α-subunit is characteristic of tibialis muscle, whereas α- and β-tropomyosins are found at nearly identical concentrations in soleus muscle [13]. On the contrary, β-tropomyosin is the major in vitro translation product of TA mRNA, whereas α-tropomyosin is that of soleus mRNA. Since no differences exist in the methionine content of α- and β-tropomyosin [14], these results suggest a control step either in the in vitro or in the in vivo translation. The same conclusion is valid for the above stated differences between in vitro and in vivo translation of the myosin light chains.

It is quite evident that chronic stimulation of fast twitch muscle induces quantitative and qualitative changes in transcriptional activity. In addition to the increase in total RNA, new mRNA coding for slow myosin light chains is transcribed. It appears that the mRNA for the various slow myosin light chains is differentially transcribed. After 28-day stimulation LC2s predominates over LC1s$_b$. Further examination of the autoradiograph (fig.1c) shows only an extremely faint spot in the position expected for LC1sa. From this result it is probable that this light chain is not significantly translated. These differences may reflect a sequential order in the changes of transcription. An untypical pattern of slow and fast myosin light chains in the same fibre has been demonstrated in [15]. Studies on the time course of the transformation of the light chain pattern in vivo also indicate that the increase in LC2s precedes that of LC1s (K. Seedorf and D. Pette, unpublished). These results extend earlier observations on the appearance of slow myosin light chains in stimulated fast twitch muscle [2,3] to the transcriptional level and therefore suggest that the induced transformation of the myosin light chain pattern is due to a switch in gene transcription.

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