MYOFIBRILLAR PROTEIN PATTERNS OF SINGLE FIBRES FROM HUMAN MUSCLE

Dirk PETTE, Jan HENRIKSSON+ and Margreth EMMERICH
Fachbereich Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, FRG

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1. Introduction

Unlike a few domesticated mammals and birds, in which certain muscles are composed of one or predominantly one fibre type, human skeletal muscle is invariably mixed of several fibre types. These may be distinguished qualitatively by histochemical staining for myofibrillar actomyosin ATPase, or for enzymes of distinct metabolic functions, such as anaerobic carbohydrate catabolism or aerobic substrate end oxidation. Preparations of myofibrillar proteins from human muscles, therefore contain variable mixtures of molecular species derived from at least two distinct populations, i.e., fast and slow fibres. Differences in the myosin light chain pattern were suggested from comparative analysis of myosin prepared from normal or pathological muscles with varying percentages of type I (slow) and II (fast) fibres. Controversial results were, however, reported from different laboratories [1–3]. This applies also to a recent study on the distribution of troponin I in human muscles. Although in analogy to rabbit slow- and fast-twitch muscles, different forms of troponin I could be distinguished, the distribution of these forms did not follow the fractional distribution of type I and II fibres in various muscles [4]. An additional uncertain factor is that the type II fraction can be further subclassified into IIA and IIB fibres, which are interconvertible in response to training or inactivity [5,6]. It is not known to what extent these subgroups differ with regard to contractile protein characteristics. The question may be raised, therefore, whether studies on whole, mixed human muscles represent a valuable approach for investigating the properties of slow- and fast-twitch myofibrillar proteins.

This study is based on a more direct procedure. Fragments of single muscle fibres were dissected from freeze-dried human muscle biopsies. They were typed by staining small pieces for myofibrillar actomyosin ATPase after preincubations at three different pH-conditions, revealing type I, IIA and IIB fibres. Capillary electrophoreses were performed and patterns of myofibrillar proteins could thus be correlated with at least two different fibre types.

2. Materials and methods

Needle biopsies were taken from the quadriceps femoris muscle (vastus lateralis) of healthy young volunteers of an average activity level. Biopsies were immediately frozen in liquid nitrogen and freeze-dried as described [7,8]. Fibre fragments were microdissected and small pieces were mounted on microscopic slides [9] and stained for myofibrillar actomyosin ATPase after preincubations either at pH 10.4, 4.6 or 4.3 [10,11] for classification as type I, IIA and IIB. For electrophoretic studies of myofibrillar proteins, fibre samples weighing between 0.3–0.5 μg were pre-extracted by mild sonication in a medium (pH 7.0) containing 80 mM KCl, 1 mM TES, 0.1 mM dithiothreitol, 0.1 mM phenylmethane sulfonylfluoride (PMSF). Pre-extraction was performed in capillaries immersed in ice water. After 30 min centrifugation at 15 000 × *g* (Beckman centrifuge, model L265B, rotor 50 Ti with special adaptors for capillaries according to [12]), supernatant was decanted quantitatively. Myofibrillar proteins of the pre-extracted fibre were solubilized at 95°C in 3 μl of a medium (pH 6.8)

+ Present address: Department of Physiology III, Karolinska Institutet, Stockholm, Sweden

containing 125 mM Tris–HCl, 2% sodium dodecylsulphate (SDS), 5% (v/v) β-mercaptoethanol. After mixing with an equal volume of 80% sucrose with pyronin G, samples were applied to the stacking gels. Electrophoresis in capillaries (80 × 0.4 mm) was performed as described with the exception that a higher polyacrylamide concentration of 15% was used in the separating gel [8]. For macroscale analyses, slab gel electrophoresis was performed as described [13].

3. Results and discussion

Figure 1 shows the peptide pattern of a total myofibrillar extract of human gastrocnemius muscle obtained by polyacrylamide gel electrophoresis in the presence of SDS. Several bands are readily distinguished

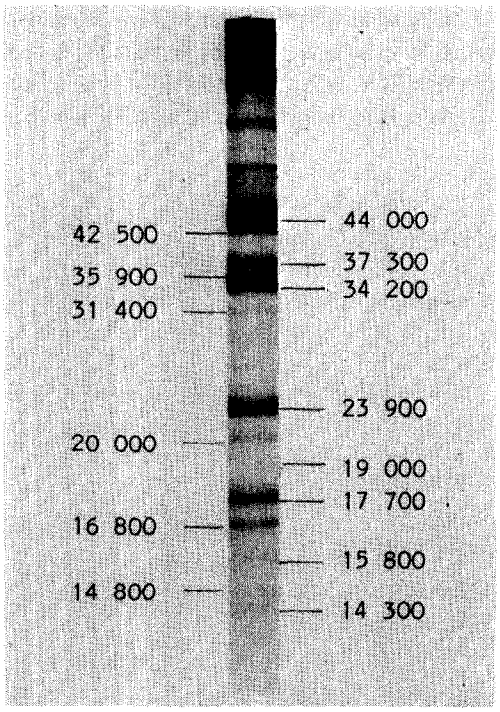

Fig.1. Polyacrylamide gel electrophoresis in the presence of SDS of a myofibrillar extract of human gastrocnemius muscle after pre-extraction of the myogen fraction. Apparent molecular weights are given according to calibration with phosphofructokinase, serum albumin, aldolase, lactate dehydrogenase, adenylate kinase and cytochrome *c* [10].

such as myosin heavy chain at the top of the gel and others according to their apparent molecular weights. Presumably the 44 000 M_r peptide is actin and the 35 900/34 200 M_r peptides are the tropomyosin subunits. The three prominent bands of 23 900, 17 700 and 16 800 M_r might represent myosin light chains of fast-twitch fibres. However, molecular weights of peptides defined mainly in rabbit muscles might not apply to the pattern shown in fig.1. Since the muscle studied contains a mixture of slow- and fast-twitch fibres, one would expect a mixed pattern of the respective myosin light chains. Such a pattern has been reported [1]. Two species of light chain LC_2, most probably corresponding to fast- and slow-type light chains LC_{2F} and LC_{2S} were distinguished in human muscle. On the other hand only a single band for the slowest migrating light chain was found [1], thus suggesting that no difference exists in the apparent molecular weights of LC_1 in different fibre types of human muscle. This latter finding is supported by similar observations [2]. However, no double band exists for LC_2 according to [2] and the myosin light chain pattern of mixed human muscles corresponds instead to that of rabbit fast-twitch muscle. It was pointed out [2] that myosin of human muscles with a high percentage of (slow) type I fibres could be distinguished from that of muscles with a higher percentage of (fast) type II fibres only by a lower content of LC_3. Our electrophoretic findings in fig.1 resemble the results reported [1] and may thus be interpreted as a pattern composed of fast- and slow-type myosin light chains.

Under the assumption that muscle fibres of a given type synthesize only one species of myosin molecules at steady state conditions, electrophoretic analyses of single human muscle fibres should display peptide patterns of either one or the other myosin type. Analyses of single fibres should therefore reveal some of the molecular characteristics related to slow- and fast-twitch fibres in human muscles. Figure 2 shows peptide patterns obtained by polyacrylamide gel electrophoresis in the presence of SDS of a single type I and a single type II fibre of human vastus lateralis muscle. Since pre-extraction of the myogen fraction was performed, the peptide patterns in fig.2 represent only myofibrillar proteins. The bands appear larger than those of fig.1. It should be taken into account that these are microgels with a total

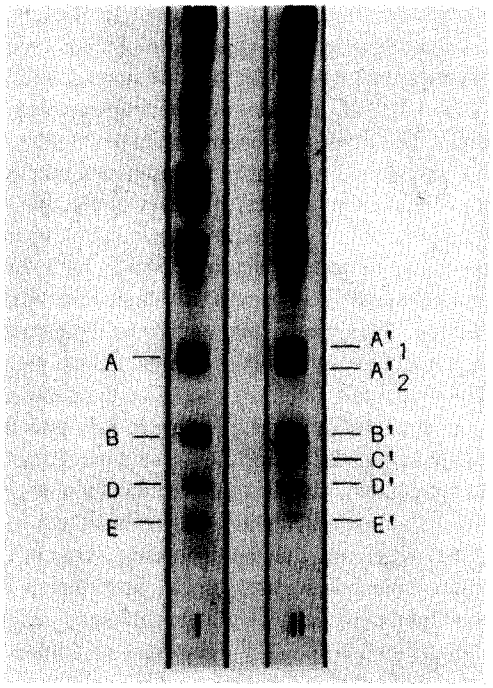

Fig.2. Polyacrylamide gel microelectrophoreses in the presence of SDS of a single type I (slow) and a single type II (fast) fibre of human vastus lateralis muscle after pre-extraction of myogen. Magnification: 6-fold.

separation distance of only 14 mm. The gels in fig.2 were photographed with 6-fold magnification, whereas the gel in fig.1 is about actual size.

No differences are seen between type I and type II fibre electrophoreses in the range of high molecular weight polypeptides and the region of actin and tropomyosin. Marked differences, however, exist in the region of the presumed myosin light chains. Due to the miniaturising of the electrophoretic method, and especially with regard to inevitable variations in migration distance from capillary to capillary, no exact calibration of the microgels was possible. Therefore, bands in fig.2 have been designated alphabetically.

A four band pattern A,B,D,E is characteristic of the type I fibre as seen in fig.2. In the type II fibre, the corresponding A band is split in two fractions A'_1 and A'_2 of similar intensity. Four other bands named B', C', D', E' are seen in the type II fibre pattern. If bands D/D' and E/E' correspond to the 14 800 and 14 300 M_r peptides shown in fig.1, band C' in the type II fibre electrophoresis of fig.2 might correspond to the 15 800 M_r peptide and presumably represent LC_3. There is no band seen at this position in the case of type I fibres. Bands B and B' would then represent LC_2. It is remarkable to find in human type II fibres a splitting of the electrophoretic band corresponding to LC_1, which is normally seen in rabbit muscle only in slow type myosin.

In order to compare the relative molecular weights of the designated bands in type I and type II fibre electrophoreses (fig.2), coelectrophoreses of samples of the two fibre types were performed (fig.3). More bands are seen in this electrophoresis than in those of the single fibres alone (fig.2). The interpretation of the combined electrophoresis in fig.3 is that bands A and A'_1 are identical, that B and B' are dissimilar, and that all the other bands can be identified individually.

It is suggested from these data that fast-twitch (type II) fibres differ from slow-twitch (type I) fibres in human muscle by an additional fraction of LC_1 (A'_2), a LC_2 of lower molecular weight (B') and an additional low molecular weight peptide C', probably representing LC_3. Coelectrophoresis of type I and type II fibres (fig.3) appears similar to the electro-

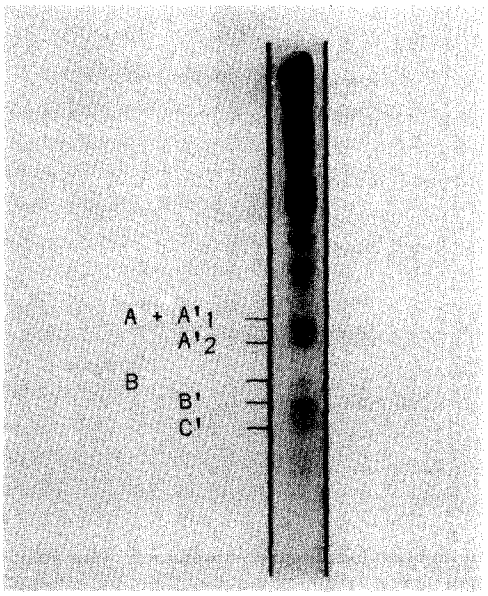

Fig.3. Coelectrophoresis of equal amounts of single type I and type II fibres of human vastus lateralis muscle after pre-extraction of myogen. Magnification: 4-fold.

phoresis of a myofibrillar extract from whole mixed muscle (fig.1). However, the intensity of the bands is not equivalent. This might be due to the fact that in the coelectrophoresis there were equal amounts of the two fibres, whereas in whole muscle the two fibre types are not proportional.

In the electrophoreses of single type II fibres we observed variations in the intensity of the band designated C'. This might be due to partial loss of this peptide during pre-extraction. Alternatively, this variation may reflect subpopulations of type II fibres. With the existing means of fibre typing, however, no reliable correlation with type IIA and type IIB fibres has yet been established by the reported micro-electrophoretic analyses.

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