α- and β-TROPOMYOSIN IN TYPED SINGLE FIBERS OF HUMAN SKELETAL MUSCLE

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1. Introduction

Tropomyosin, in association with the troponin, is involved in the calcium-dependent regulation of actin–myosin interaction [1].

In skeletal muscles two major forms have been detected. In the rabbit, both of them possess 284 amino acid residues, 39 of which are different [2]. In spite of this similarity, their mobilities on SDS–polyacrylamide gels clearly differ. By convention, the faster migrating form is called α-tropomyosin, and the slower form β-tropomyosin [3].

Minor forms have been described for α- and for β-tropomyosin, differing in their primary sequences [2,4]. In cat [5] and chicken [6] skeletal muscle, different α-forms associated with fast and slow skeletal muscle have been found. Furthermore, skeletal muscle tropomyosins can be phosphorylated, resulting in different electrophoretic mobilities [6]. It has been suggested that in mammals (and in particular in man), α-tropomyosin is confined to type II-fibers and β-tropomyosin to type I-fibers [7,8]. This contradicts the above-mentioned results from cat muscle [5]. Furthermore, in rabbit soleus muscle containing only few type II fibers [9], both α- and β-forms are present in roughly equal amounts [10].

Taking advantage of recently developed techniques for the analysis of the protein composition of typed single muscle fibers [11], we demonstrate in this study that in human skeletal muscle α- and β-tropomyosins are present in about equal amounts in type I, IIA and IIB fibers. We also present evidence that the α- and β-forms display fiber type-specific characteristics. For this purpose, a new, two-dimensional peptide mapping method was developed and this is described here for the first time.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; SDS, sodium dodecyl sulfate; M_r, relative molecular mass

2. Materials and methods

2.1. *Muscle biopsies*

Open biopsies were obtained from the rectus femoris and the triceps muscles of a healthy 36 year-old woman and a healthy 28 year-old man.

2.2. *Myofibrils*

These were prepared according to [12]. Approximately 100 μg were suspended in 20 μl Hepes (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid) (pH 7.5) and 20 μl SDS (3%)/β-mercaptoethanol (1%), boiled for 10 min and finally applied to the two dimensional gel [13].

2.3. *Muscle fiber typing*

Single muscle fibers were dissected out of freeze dried biopsies [14] and the fiber type determined as described [11,14].

2.4. *In vitro labeling of muscle fiber proteins*

Proteins within single fibers (~1 μg dry wt) were ^{14}C-labeled by reductive methylation [15] as in [11]. This ^{14}C-labeling technique allows the detection of nanogram quantities of protein. Labeled proteins and peptides were visualised by fluorography [16,17].

2.5. *Two-dimensional gel electrophoresis*

This was performed according to [13]. A mixture of 20% ampholines (LKB) (pH 3.5–10) and 80% ampholines (pH 5–7) was used. The relative subunit M_r-values of human tropomyosins were estimated by comparison with a mixture of ^{14}C-labeled proteins

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(The Radiochemical Center) containing phosphorylase *b* (M_r 100 000), bovine serum albumin (68 000), ovalbumin (46 000), carbonic anhydrase (30 000), lysozyme (14 300) and with rabbit tropomyosin (α-subunit, 34 000; β-subunit, 36 000).

2.6. *Analysis of tropomyosin from single fibers*

Typed muscle fibers were labeled with [^{14}C]formaldehyde (NEN, 52 mCi/mmol) as in [11]. Then, 3 μl 0.1 M Hepes, 3% SDS, 1% β-mercaptoethanol were added and the mixture boiled (5 min). After addition of 6 μl lysis buffer [14], separation was performed on a micro-isoelectric focussing gel (size 4 × 0.17 cm, first-dimension) at 400 V for 90 min. The gel was equilibrated (10 min) in SDS-sample buffer [13] and subsequently transferred to a microslab gel (size 2.9 cm × 4.5 cm × 0.5 mm, second-dimension). Acrylamide in the stacking and separating gels was 3% and 15%, respectively. The gel was run for 1.5 h at 60 V. After brief staining and destaining (each step 10 min), the protein spots corresponding to α- and β-tropomyosins (identified by their M_r- and pI-values and by direct comparison to a myofibrillar extract) were cut out of this micro two-dimensional gel, the pieces (~1.5 mm diam.) equilibrated in 50 μl 0.124 M Tris HCl, 0.1% SDS, 1 mM EDTA, 1 mM DTT (pH 6.8) for 30 min and either frozen or directly used for one- and two-dimensional peptide mapping.

2.7. *One-dimensional peptide mapping*

This was done as in [18,19]. Acrylamide in the separating gel was 15% and in the stacking gel 3%. The tropomyosins in the gel fragments cut out from the microgels were digested with either chymotrypsin (Boehringer, 0.04 μg/sample well) or with *Staphylococcus* V_8-protease (Miles, 0.08 μg/sample well). Electrophoresis was run at 12.5 mA for 60 min.

2.8. *Two-dimensional peptide mapping*

This method was developed for higher resolution of peptide fragments. The first step of the procedure was essentially the same as in one-dimensional peptide mapping. However, the time of digestion within the stacking gel was reduced to 50 min only. The total protein digest (visualized by addition of bromphenol blue) was cut out from the stacking gel before entering the separating gel. These gel parts were cut out, mixed with 40 μl lysis buffer and the slurry obtained quickly frozen in liquid nitrogen. The frozen pieces, allowing easier handling of the viscous material, were transferred and separated by two-dimensional gel electrophoresis as above.

3. Results and discussion

To obtain an overall idea of the tropomyosin composition, we first analyzed a bulk preparation of myofibrils from human vastus lateralis muscle, which is usually composed of about equal numbers of type I and type II fibers. Both α- and β-forms were clearly visible (fig.1 A). The app. M_r-values of the α- and β-subunits of human tropomyosin were 34 000 and 36 000, determined by a co-migration experiment (not shown) with rabbit tropomyosin.

As in cat [5] and chicken [6] muscle, the α-subunit

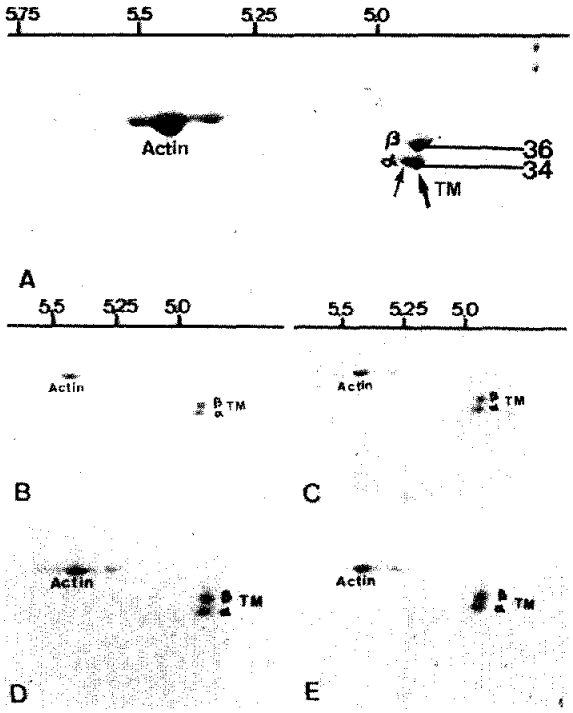

Fig.1. Enlarged sections of two-dimensional gels of: (A) Total myofibrillar proteins from a mixed human muscle, vastus lateralis, which had equal proportions of type I and type II fibers. 20 μg protein were separated on a two-dimensional polyacrylamide (15%) gel. Coomassie blue-stained proteins were identified by their pI (scale on top) and M_r-values (× 10^{-3}). (➡) Indicates position of α-tropomyosin (TM) of type II fibers; (→) α-tropomyosin of type I fibers; (B) single human type I fiber, labeled in vitro by reductive methylation; (C) type IIA fiber; (D) type IIB fiber; (E) type IIC fiber, which is rare in normal human skeletal muscle (0–2%). Proteins in B–E are visualized by fluorography (12 days exposure).

could be additionally resolved into two closely spaced protein spots, slightly differing in pI (fig.1A, arrows). Two-dimensional electrophoresis of proteins obtained from typed single fibers showed that α- and β-tropomyosin were present in about equal amounts within human type I, IIA, IIB and IIC fibers (fig.1B–E). These illustrations show fluorographies of ^{14}C-labeled proteins separated on normal-sized two-dimensional gels. Similar results were obtained when the fibers were applied onto micro two-dimensional gels and the proteins stained with Coomassie blue (not shown). This is in contrast to the suggestion that human type I fibers may contain only β-tropomyosin whereas human type II fibers may contain only α-tropomyosin [8].

As can be seen on closer inspection of fig.1B–E, the position of the α-tropomyosins in gels from type I and II fibers is slightly different, presumably giving rise to the two α-tropomyosin spots obtained when a mixture of type I and type II fibers is analyzed (fig.1A, [5,6]).

One-dimensional peptide mapping, using chymotrypsin or V_8-protease as hydrolytic enzymes, displayed the expected differences between the α- and the β-chain in type I and IIA fibers (not shown). Chymotryptic digestion led to identical patterns for the α-chains and for the β-chains within each fiber types. *Staphylococcus* V_8-protease, however, produced an additional peptide in α-tropomyosin of type I as opposed to α-tropomyosin in type IIA fibers (not shown). Combining *Staphylococcus* V_8-protease digestion with the newly developed two-dimensional peptide mapping method, we obtained the results shown in fig.2A–F. Again, differences in the peptide maps between the α-form and the β-form are clearly visible. But in addition, it also emerges that neither α- nor β-tropomyosin produces identical peptide maps in the 3 fiber types. β-Tropomyosin of type IIA fibers (fig.2B) contains an additional peptide fragment [1] compared to β-tropomyosin of type I (fig.2A) and type IIB fibers (fig.2C). β-Tropomyosin of type IIB fibers (fig.2C) is

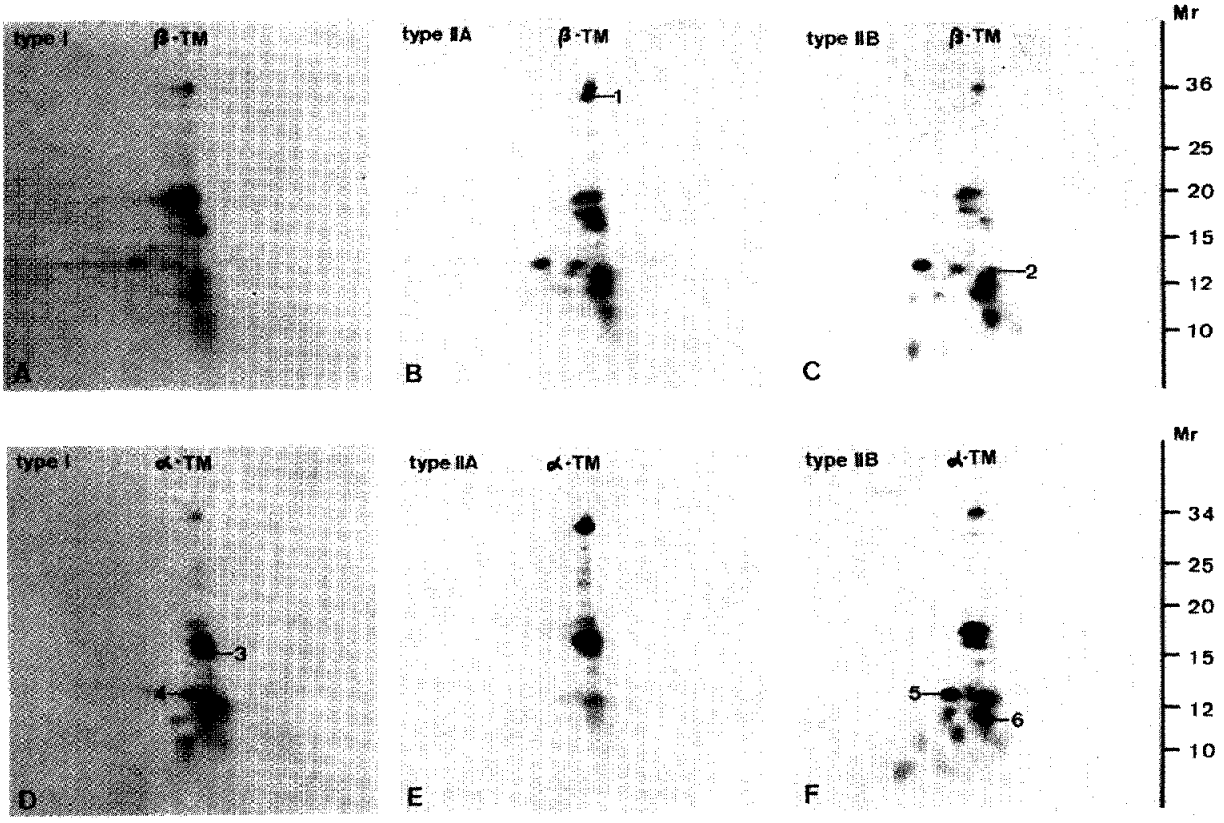

Fig.2. Two-dimensional peptide mapping of ^{14}C-labeled α- and β-tropomyosin (TM) of type I (A,D), IIA (B,E) and IIB (C,F) muscle fibers after digestion with *Staphylococcus aureus* V_8-protease (0.08 g). The experimental details are in section 2. Major differences are indicated by numbers (1–6); see section 3. Scale gives M_r-values ($\times 10^{-3}$). Fluorographs were exposed for 4 weeks.

different in the region of peptide [2] from β-tropomyosin of type I (fig.2A) and IIA fibers (fig.2B). Major differences between α-tropomyosins of type I (fig.2D) fibers and type IIA (fig.2E) fibers are the additional presence of the two peptides [3] and [4] in type I fiber tropomyosin. α-Tropomyosin of type IIB fibers (fig.2F) differs from type I (fig.2D) and type IIA (fig.2E) tropomyosin by the two peptides [5,6].

Our results show that in human muscle α- and β-tropomyosin are present in about equal amounts in all skeletal muscle fiber types. Moreover, α- and β-tropomyosins from type I, IIA and IIB fibers are all different. Whether the differences in these cleavage patterns are the result of differences in primary structure of the proteins or are (at least in part) the result of post-translational modifications (e.g., phosphorylation) is not certain. However, phosphorylation is very unlikely since it results in a much greater shift in the isoelectric point of tropomyosin [6]. Thus, our results would seem to suggest fiber-type specific primary sequences for human tropomyosins. Some additional support for this comes from a study in which the in vitro translation products of chicken muscle mRNA revealed evidence for different mRNAs coding for α-tropomyosins in slow and fast skeletal muscle [6]. Furthermore, in rabbit skeletal muscle a major and a minor form of β-tropomyosin were found, differing in 11 residues of their primary sequence [2]. The ratio of these two forms (10:1) is roughly the ratio of fast to slow fibers in the muscles used for preparation of this tropomyosin. They could therefore be fiber-type specific forms.

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