TWO-DIMENSIONAL PEPTIDE ANALYSIS OF MYOSIN HEAVY CHAINS AND ACTIN FROM SINGLE-TYPED HUMAN SKELETAL MUSCLE FIBERS

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1. Introduction

The different fiber types in various vertebrate skeletal muscles each have individual sets of contractile proteins [1–3]. A major difficulty in studying human muscles is the lack of uniform muscles, making it difficult to obtain pure fast or slow proteins for biochemical analysis. Nevertheless, different forms of troponin [1], tropomyosin [4] and myosin [5–7] have been found in type I (slow twitch) and type II (fast twitch) fibers. To detect minute differences of muscle proteins within fiber types I, IIA and IIB, single fiber analysis in combination with a two-dimensional peptide mapping technique [4] was applied. The two-dimensional peptide patterns of muscle α-actin from fiber types I, IIA and IIB were identical, whereas the peptide patterns obtained after digestion of the myosin heavy chains of the same 3 fiber types were different.

2. Materials and methods

2.1. *Isolation and ^{14}C-labeling of muscle fiber proteins*

Single muscle fibers (~1 μg dry wt) were dissected out of freeze-dried biopsies and typed by aid of the myofibrillar ATPase staining method [6,8]. Fiber proteins were ^{14}C-labeled by reductive methylation [9] as in [5]. This ^{14}C-labeling method, which labels the free amino residues of lysine as well as the NH_2-terminus of the protein, permits the detection of ng levels of proteins. Labeled proteins and peptides separated on gels were visualized by fluorography [10,11].

Abbreviations: 2D, two-dimensional; SDS, sodium dodecyl sulfate; M_r relative molecular mass

2.2. *Micro gel electrophoresis for the isolation of actin and myosin*

For myosin heavy chains, the proteins from the typed fibers were separated by size on an SDS microslab gel and the band corresponding to the myosin heavy chains was cut out as in [6].

For actin, the fiber proteins were separated on a micro-2D-gel as in [4], and the α-actin spot was cut out. The gel pieces containing α-actin or myosin heavy chain were equilibrated for 0.5 h in 50 μl 0.124 M Tris–HCl, 0.1% SDS, 1 mM EDTA, 1 mM DTT (pH 6.8), and then either frozen for storage or directly used for peptide mapping.

2.3. *Two-dimensional peptide mapping*

This was done as in [4] and is an extension of the one-dimensional method in [12]. The proteins are partially digested during the run through the stacking gel. In our case, chymotrypsin (0.2 μg/sample well) and *Staphylococcus* V_8 protease (0.08 μg/sample well) were used. Digestion was for 50 min. Instead of separating the individual peptides on the separating gel as in [12], the entire protein digest which migrates close to the bromophenol blue dye front [13] was cut out just before it entered the separating gel. These gel pieces were transferred into 40 μl lysis buffer [14] and the resulting slurry was frozen in liquid nitrogen for easier handling. The frozen pieces were transferred to an isoelectric focussing gel (20% ampholines pH 3.5–10 and 80% ampholines pH 5–7) and the peptides separated by 2D-gel electrophoresis [14]. The peptide M_r-values were determined by comparison with a mixture of ^{14}C-labeled proteins (The Radiochemical Center) containing (M_r): phosphorylase *b* (100 000), bovine serum albumin (80 000), ovalbumin (46 000), carbonic anhydrase (30 000) and lysozyme (14 300).

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3. Results

3.1. *Myosin heavy chains*

The fluorographs of the 2D peptide maps of the myosin heavy chains of single human type I, IIA and IIB muscle fibers are shown in fig.1. Over 70, ^{14}C-labeled peptides could be resolved by this technique after digestion with *Staphylococcus aureus* V_8 protease. The peptide pattern of the myosin heavy chain from each fiber type was characteristic and reproducible over a range of protease concentrations. The differences in the peptide pattern were less pronounced when chymotrypsin (not shown) instead of V_8 protease was used. When myosin heavy chains of type I (fig.1A) and II (fig.1B,C) were digested with V_8 protease, the patterns were clearly distinct, a result in agreement with the immunological differences between human fast and slow myosins [15].

Closer inspection of the myosin heavy chains of the types IIA and IIB (fig.1B,C) also revealed several differences between them, e.g., in peptides 1–5 with app. M_r 44 000, 40 000, 26 000, 25 000 and 22 000, respectively. Peptides 1 and 2 are, for example, only present in the myosin heavy chain digest of type IIA fibers whereas peptides 3, 4 and 5 are unique to type IIB fibers.

These results suggest that not only the myosin heavy chains of human type I and II fibers but also those of the subtypes IIA and IIB have different primary sequences and are presumably the products of different genes.

3.2. *Actin*

In contrast to myosin heavy chain, no differences in the 2D peptide patterns could be found in the α-actin of human muscle from different fiber types. This is shown in fig.2, where actins from 3 different fiber types (I,IIA,IIB) were partically digested with chymotrypsin (fig.2A–C) or *Staphylococcus* V_8 protease (fig.2D–F). We therefore conclude that the α-actins in the 3 human skeletal muscle fiber types are very similar, if not identical. This is in agreement with the data in [17], which show that the actins in fast and slow muscles of rabbits have identical primary amino acid sequences. However, as well as muscle actin (α), 2 forms of non-muscle actins (β,γ) have also been characterised. Muscle and non-muscle actins are substantially different proteins [17–24] and it can be concluded that these proteins are products of different genes. The identity of the peptide patterns

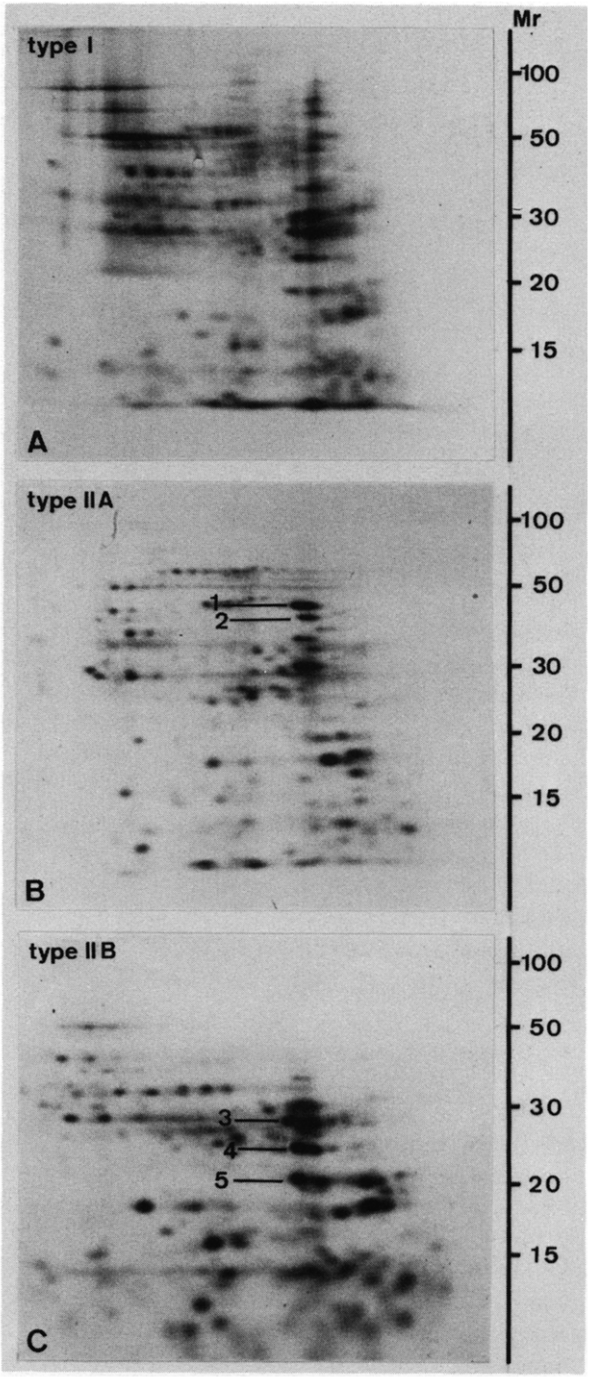

Fig.1. Two-dimensional peptide mapping of ^{14}C-labeled myosin heavy chains of type I (A), IIA (B) and IIB (C) muscle fibers after digestion with *Staphylococcus aureus* V_8 protease (0.08 μg). Major differences are indicated by numbers (1–5) (see section 3). Scale gives M_r-values (×10^{-3}). Fluorographs were exposed for 5 weeks.

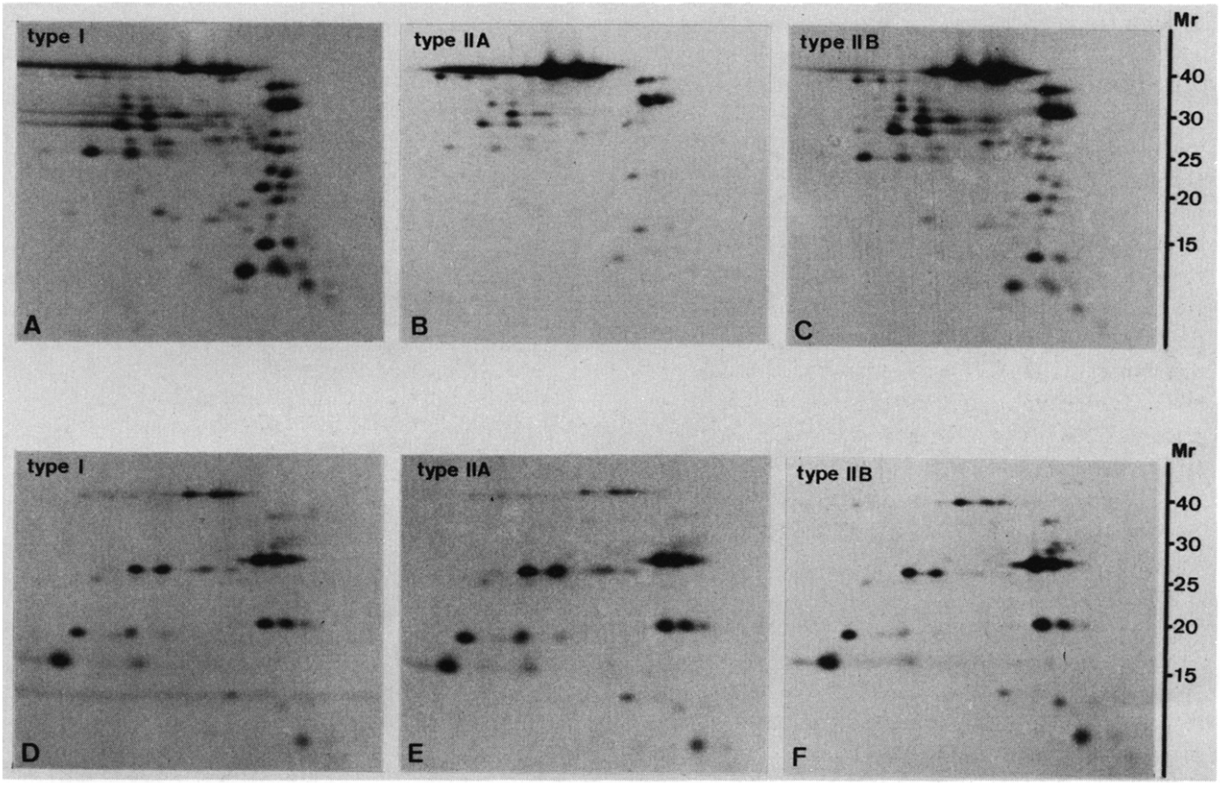

Fig.2. Two-dimensional peptide mapping of ^{14}C-labeled α-actin of type I, IIA and IIB muscle fibers after digestion with chymotrypsin (A–C) (0.2 μg) or V_8 protease (D–F) (0.08 μg). Fluorographs were exposed for 1 month.

(encompassing >30 individual spots) illustrates the discriminating power of the method employed.

4. Discussion

Our results indicate that whereas myosin occurs in clearly distinguishable fiber type-specific forms in human skeletal muscles, α-actin, the other protein directly involved in the contraction process, does not. This might reflect their different kinds of involvement in this process, since myosin acts as an enzyme during contraction (the activity of the myosin ATPase being correlated with the speed of muscle shortening [25]), but actin, on the other hand, plays a passive role in the contraction process. Actin has no enzymatic activity nor established regulatory function. The calcium regulation of muscle contraction is mediated by the troponins and tropomyosin, proteins which bind to actin and which themselves occur in muscle fiber type-specific forms [1,4]. In contrast, α-actin interacts with itself (upon polymerization) and with other proteins such as myosin, tropomyosin and with the Z-line proteins. The ability of actin to bind to many other proteins is compatible with its highly conserved nature, not only within muscle types but also between species. For example, it has been shown that rabbit and chicken skeletal muscle actins have identical primary sequences [24].

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