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Electrophoretically defined myosin heavy chain patterns of single human muscle spindles

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At least four myosin heavy chain (MHC) isoforms were separated by SDS-PAGE in extracts of intrafusal fibers isolated by microdissection from human lumbrical muscles. The fastest migrating MHC represents a slow isoform. The slowest migrating MHC was identified as the embryonic MHC_{emb}. A faint band, moving slightly faster than MHC_{emb}, most likely represents a neonatal/fetal MHC isoform. A prominent band, moving between the latter and the slow isoform is suggested to represent a hitherto unidentified, spindle-specific MHC isoform, MHC_{if}.

Human muscle; Intrafusal fiber; Muscle spindle; Myosin heavy chain isoform; Single fiber study

1. INTRODUCTION

Muscle spindles are specialized sensory receptors found in skeletal muscle. They are encapsulated bundles of small-diameter muscle fibers receiving both sensory and motor innervation. Three types of intrafusal fibers have been distinguished, i.e. nuclear bag_1, nuclear bag_2, and nuclear chain fibers, each with distinct morphological, histochemical and physiological characteristics. To date, only immunocytochemical studies exist on the myosin heavy chain (MHC) patterns of mammalian intrafusal fibers. These studies have identified several MHC isoforms, including the slow-tonic $MHCI_{ton}$ [1–8], the slow twitch MHCI (β-cardiac MHC) [1–8], an embryonic MHC_{emb} [2,5,7,9], a fetal/neonatal MHC_{fet} [2,5], and the α-cardiac MHC [8,10]. It was also shown that intrafusal fibers display type-specific, non-uniform patterns of myosin expression [3,4,9,11]. However, it has been observed that immunocytochemistry for MHC isoforms in muscle spindles may yield ambiguous results, as different antibodies against the same MHC yielded different staining patterns [4]. Also, the immunocytochemical data available do not exclude the existence of hitherto unknown MHC isoforms in muscle spindles [4,12]. Therefore, an independent, biochemical analysis of the MHC composition of intrafusal fibers appeared to be timely. For this purpose, we isolated single muscle spindles by microdissection and analysed their MHC isoform patterns by gradient polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE).

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2. MATERIALS AND METHODS

2.1. *Muscles, histochemistry, microdissection*

The first lumbrical muscle was collected from four human subjects shortly after death or undergoing surgery due to trauma. The muscles were divided into 2–4 pieces, mounted for cross-sectioning and frozen rapidly in propane chilled in liquid nitrogen. Each muscle sample was serially cross-sectioned at −20°C. Three 9 μm sections were stained for myofibrillar actomyosin ATPase (mATPase) after preincubation at pH 10.3, 4.6, or 4.3 [13]. Ten 6 μm sections were processed for immunocytochemistry [14]. Thereafter, alternating 60 μm and 8 μm thick sections were taken. The 8 μm sections were stained with eosin [13], whereas the 60 μm sections were freeze-dried at −38°C [15]. A composite photomontage of each section stained for mATPase was used to map muscle spindles and mATPase-classified extrafusal fiber types. Eosin-stained sections were used to quickly identify mapped muscle spindles and extrafusal fibers in the freeze-dried sections. Twenty muscle spindles cut through the encapsulated portion were analysed.

Individual muscle spindles, as well as single extrafusal fibers, were dissected from the 60 μm thick freeze-dried sections under a stereomicroscope. Whenever possible, individual intrafusal fibers were dissected free from the surrounding matrix and spindle capsule. An average of three (range 1–10) sections from either a single muscle spindle or, in some cases, from different muscle spindles cut through the same region, were transferred into glass capillaries and lysed during 10 min at 65°C in 5 μl of a solution containing 10% glycerol, 5% 2-mercaptoethanol and 2.3% sodium dodecyl sulfate in 62.5 mM Tris-HCl (pH 6.8).

Whole muscle extracts were prepared as previously described [16] from the biceps muscle of a human fetus (20 weeks of gestation) and from adult human pectoral and extraocular muscles.

2.2. *Immunocytochemistry*

The following monoclonal antibodies (MAbs) were used [5,14]: MAb ALD19 [17], showing strong affinity to slow-tonic $MHCI_{ton}$ when applied at high dilutions [14]; MAb 2B6 [18] being specific to MHC_{emb}; MAb My32 (Sigma) reacting with fast and neonatal MHC isoforms. In addition, MAb 9812 [19] and MAb F262D11 (Sera-Lab), specific to slow twitch MHCI (β-cardiac MHC), and the polyclonal antibody NN5, specific to human fetal MHC [20], were used.

2.3. *Gradient gel electrophoresis, immunoblotting*

The lysed samples were loaded on 7–10% gradient SDS-polyacrylamide gels with 4.5% stacking gels, electrophoresed at 120 V for 24 h, and silver-stained as previously described [16]. For immunoblotting, the separated proteins were transferred onto nitrocellulose sheets and exposed to the following MAbs: ALD19, 2B6, F262D11, and MY32.

3. RESULTS

3.1. *Electrophoretic analyses*

SDS-PAGE revealed the presence of at least four MHC isoforms in human muscle spindles (Fig. 1, lanes 1,2). The position of the fastest migrating isoform was identical to that of the slow MHCI in the extrafusal type IC fiber (Fig. 1, lane 4) and in human fetal muscle extract (Fig. 1, lane 3). Immunoblotting with MAb F262D11 (anti-β-cardiac MHC) yielded a positive reaction, whereas no staining was seen with the MAb ALD 19 (results not shown). The slowest migrating MHC isoform of the intrafusal fibers displayed the same electrophoretic mobility as a prominent band present in fetal muscle (Fig. 1, lane 3; Fig. 2, lane 2) identified by immunoblotting with MAb 2B6 as the embryonic MHC_{emb} (results not shown). The comparison with extracts from adult and fetal human muscles revealed that MHC_{emb} displayed a slightly greater mobility than MHCIIb (Fig. 2, lanes 1,2). A faint band of slightly higher mobility than MHC_{emb} was observed in muscle spindles and in fetal muscle (Figs. 1 and 2). This band, tentatively designated as MHC_{fet}, moved faster than MHCIIa present in extrafusal fibers (Fig. 1, lane 4). The higher mobility of MHC_{fet} than that of MHCIIa was also evident from comparing adult and fetal human muscle extracts (Fig. 2, lanes 1,2,4). None of the four antibodies under study reacted with this band (results not shown). Finally, intrafusal fibers contained a MHC isoform, tentatively designated as MHC_{if}, which displayed an electrophoretic mobility intermediate between MHC_{fet} and MHCI. This prominent band was found only in intrafusal fibers, and it was not recognized by any of the four MAbs under study. MHC_{if} displayed a lower mobility than MHC_{eom} (Fig. 2, lane 3), an isoform previously identified by SDS-PAGE in extraocular muscles of rabbit [21] and rat [22].

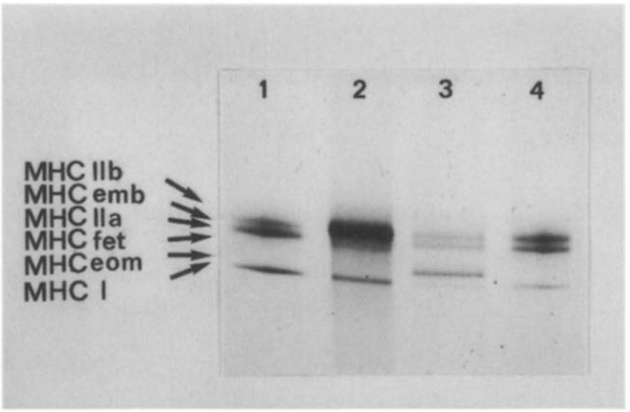

Fig. 2. Silver-stained SDS-PAGE of extracts from adult pectoral muscle (lanes 1,4), fetal muscle (lane 2), and extraocular muscle (lane 3) of the human. Note that in order to document the MHC_{fet} isoform in the fetal muscle extract, the gel had to be loaded with a high amount of protein. This overloading led to an apparently lower mobility of the MHC_{emb} band. MHC_{emb}, MHC_{fet}, MHC_{if}, MHCI, MHCIIa, MHCIIb, myosin heavy chain isoforms.

As shown by SDS-PAGE of spindles cross-sectioned at different levels, the relative concentrations of the MHC isoforms changed along the length of a given muscle spindle (Fig. 3, lanes 1–3).

3.2. *Immunocytochemistry*

All intrafusal fibers reacted with the MAb against MHC_{emb} (Fig. 4A), although the staining intensities varied considerably. Nuclear bag_2 and nuclear chain fibers

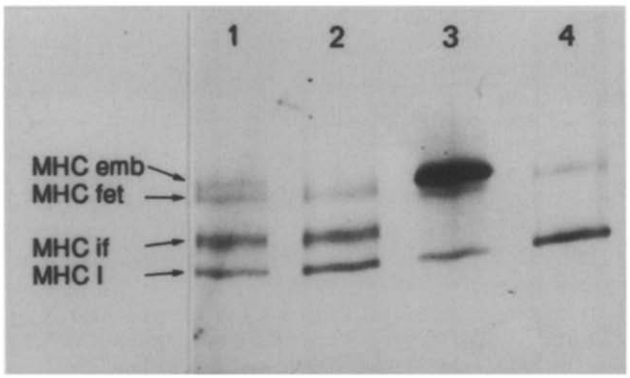

Fig. 1. Silver-stained SDS-PAGE of intrafusal fibres from adult human lumbrical muscle (lanes 1,2), run in parallel with an extract of human fetal muscle (lane 3), and an extract of an extrafusal type IC fiber (lane 4). MHC_{emb}, MHC_{fet}, MHC_{if}, MHCI, myosin heavy chain isoforms.

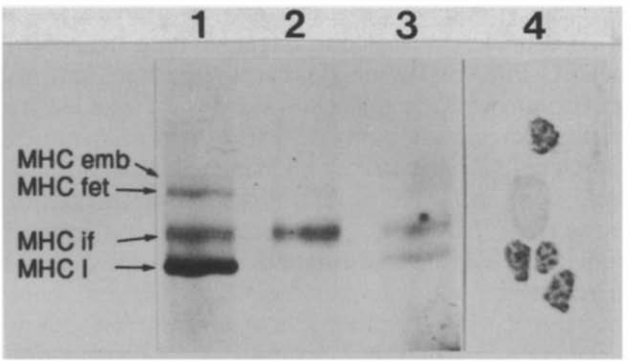

Fig. 3. Silver-stained SDS-PAGE of one single muscle spindle along the A (1), outer A (2) and inner B (3) regions. Notice the variation in relative amounts of MHC isoforms along the length of the muscle spindle. A cross-section of the same spindle stained with the MAb specific to the fetal MHC is shown in panel 4.

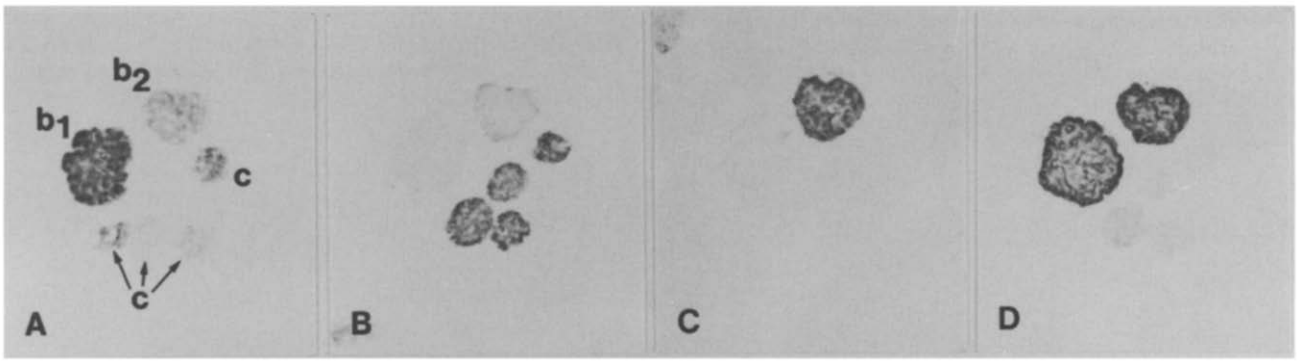

Fig. 4. Serial cross-sections through the A region of a muscle spindle containing one nuclear Bag_1 (b_1), one nuclear bag_2 (b_2), and four nuclear chain (c) fibers stained with MAbs specific to embryonic (A), fetal (B), slow-twitch (C), and slow-tonic (D) MHC isoforms.

were stained by the antibody against fetal myosin (Fig. 3, panel 4; Fig. 4B). The MAbs against MHCI (Fig. 4C) and $MHCI_{ton}$ (Fig. 4D) reacted moderately-to-strongly with the nuclear bag fibers, whereas the nuclear chain fibers were unstained.

4. DISCUSSION

The present investigation is the first biochemical analysis of the MHC composition of intrafusal fibers. It is also the first study to confirm biochemically previous immunocytochemical observations [3,4,6,9,11] on the non-uniform distribution of MHC isoforms along the length of the intrafusal fibers.

We show that adult human intrafusal fibers contain at least four electrophoretically distinct MHC isoforms. As judged by electrophoretic mobility and immunochemical reactivity; the slowest migrating isoform corresponds to MHC_{emb} and the fastest to the slow-twitch MHCI. We can not exclude the possibility that this band also contains the slow-tonic $MHCI_{ton}$ which is detected in muscle spindles by immunocytochemistry. Apparently, MHCI and $MHCI_{ton}$ have very similar electrophoretic mobilities and, therefore, may not be separated under the chosen conditions. The failure to detect $MHCI_{ton}$ by immunoblotting, although identified by immunocytochemistry, might suggest that MAb ALD19 recognizes its epitope in the native, but not in the denatured state. However, the possibility must also be considered that $MHCI_{ton}$ in intrafusal fibers is present below detectable levels by immunoblot analysis. The same applies to the α-cardiac MHC which is detected in muscle spindles by immunocytochemistry [10,14], but was undetectable by SDS-PAGE. A too low concentration might also explain the failure to identify the faint band which was tentatively designated as MHC_{fet}, by immunoblotting with MAb MY32. As to the prominent band migrating between MHC_{fet} and MHCI, its abundance and presence only in intrafusal fibers, as well as its specific electrophoretic mobility and immunochemical distinctness, suggest that it represents a hitherto unknown, spindle-specific isoform, tentatively named MHC_{if}.

Taken together, intrafusal fibers contain four electrophoretically distinct MHC isoforms, MHC_{emb}, MHC_{fet}, MHC_{if}, and MHCI. Thus, human MHC isoforms display a pattern of electrophoretic mobilities which increase in the order of MHCIIb < MHC_{emb} < MHCIIa < MHC_{fet} < MHC_{if} < MHC_{eom} < MHCI.

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