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Correlation between myofibrillar ATPase activity and myosin heavy chain composition in single human muscle fibers

R.S. Staron

Department of Zoological and Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH 45701, USA

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Summary. Single human muscle fibers were analysed using a combination of histochemical and biochemical techniques. Routine myofibrillar adenosine triphosphatase (mATPase) histochemistry revealed a continuum of staining intensities between the fast fiber types IIA and IIB (type IIAB fibers) after preincubation at pH 4.6. Electrophoretic analysis of single, histochemically-identified fibers demonstrated a correlation between the staining intensity and the myosin heavy chain (MHC) composition. All fibers classified as type I contained exclusively MHCI and all type IIA fibers contained only MHCIIa. Type IIAB fibers displayed variable amounts of both MHCIIa and MHCIIb; the greater the staining intensity of these fibers after preincubation at pH 4.6, the greater the percentage of MHCIIb. Those fibers histochemically classified as type IIB contained either entirely MHCIIb or, in addition to MHCIIb, a small amount of MHCIIa. These data establish a correlation between the mATPase activity and MHC content in single human muscle fibers.

Introduction

Human skeletal muscle fibers have been routinely categorized into four basic types (types I, IIA, IIB and IIC) based upon histochemical methods (Brooke and Kaiser 1970). Improved methodology has added two additional subtypes: IIAC and IIAB (Ingjer 1979). However, it has become apparent that even six fiber types represent an oversimplification of the dynamic nature of skeletal muscle fibers (Pette and Staron 1988). Indeed, it appears as if a continuum of fiber "types" exist (Pette and Staron 1990).

Early work from our laboratory suggested that the continuum of staining intensities for myofibrillar adenosine triphosphatase (mATPase) activity between fiber types IIA and IIB represented variable proportions of type IIA and IIB myosins (Staron et al. 1983b). These hybrid fibers, termed type IIAB, are also between the IIA and IIB fibers in size, capillary supply, number of subsarcolemmal mitochondrial aggregations (Ingjer 1979) and volume percent mitochondria (Staron et al. 1983a). This apparent continuum of fast fiber types suggests the possibility of gradual, ongoing transformations in response to variable usage.

Recently, the coexpression of IIa and IIb myosin heavy chain isoforms has been electrophoretically demonstrated in single human muscle fibers (Biral et al. 1988). Such fibers may represent a large population in control muscle (Biral et al. 1988). Indeed, almost half of the total fast fiber population investigated in that study contained both IIa and IIb myosin heavy chains in variable ratios. It is not known if the histochemically-determined type IIAB fiber represents these same fibers coexpressing myosin heavy chains IIa and IIb. If so, the continuum of staining intensities observed histochemically (Staron et al. 1983b) may be the result of specific ratios of IIa and IIb myosin heavy chains.

The purpose of the present investigation was to further characterize fast fiber types in human muscle by combining histochemical and biochemical analyses on the same fiber.

Materials and methods

Muscle preparation

A muscle biopsy (80 mg) was extracted from the superficial region of the vastus lateralis muscle of a control male subject using the percutaneous needle biopsy technique of Bergström (1962). The sample was removed from the needle, oriented in tragacanth gum, immediately frozen in isopentane cooled by liquid nitrogen to −159° C, and stored at −70° C.

Histochemical analysis

The muscle sample was thawed to −20° C and serially sectioned (12 μm thick) for histochemical analysis. Groups of these thin serial sections were alternated with thick sections (60–80 μm) for bio-

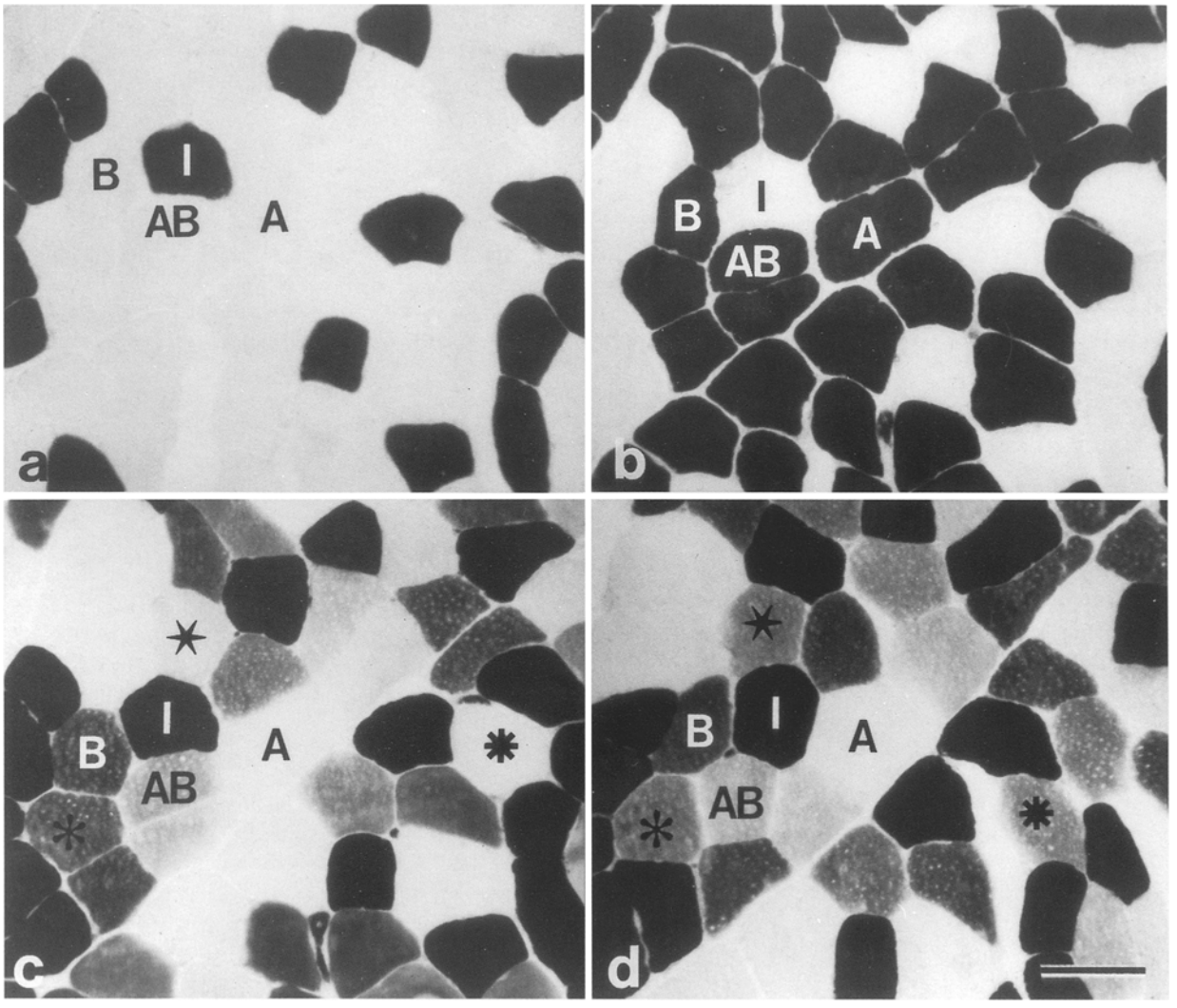

Fig. 1. Serial cross-sections of human vastus lateralis muscle assayed for mATPase activity following preincubation at pH 4.3 (**a**), 4.6 (**c, d**), and 10.4 (**b**). Sections shown in c and d were assayed simultaneously and are approximately 1.0 mm apart. Note the differences in the staining intensities of three marked fibers (*stars*) in **c** and **d**. *I*, type I; *A*, type IIA; *AB*, type IIAB; *B* type IIB. *Bar* = 100 μm

chemical analysis. The total distance sectioned and analysed was slightly more than 1 mm.

Routine myofibrillar adenosine triphosphatase (mATPase) activity was assessed following preincubation pH values of 4.3, 4.6, and 10.4 (Brooke and Kaiser 1970) with previously used modifications (Staron et al. 1983a). A total of six fiber types was delineated based on the pH sensitivity of their mATPase (Staron and Pette 1986, 1987a, b) (Fig. 1). Type I fibers were stable in the acid ranges, but labile in the alkaline. Type IIA fibers displayed a reverse pattern. All fibers stable at pH 4.6 and 10.4, but labile at pH 4.3 were classified as either type IIB or IIAB depending upon their staining intensity following preincubation at pH 4.6 (the type IIAB fibers stained intermediate between IIB and IIA fibers). Fibers classified as type IC or IIC remained stable (to varying degrees) throughout the entire pH range (Staron and Pette 1986).

Myosin heavy chain analysis

Procedures for combined histochemical and biochemical analyses of single fibers have been previously published (Staron and Pette 1986). Briefly, photomontages were made of the histochemical preparations preincubated at pH 4.6 and were used to identify specific fibers in the freeze-dried sections. Pieces of specific, histochemically-defined fibers were microdissected from freeze-dried cross-sections (60–80 μm thick) and placed in glass capillary tubes. One piece of a specific fiber was sufficient for myosin heavy chain (MHC) analysis. A total of 80 single fibers was analysed (20 each of types I, IIA, IIAB, and IIB).

Each fiber fragment was lysed for 10 min at 60° C in 5 μl of a medium containing 10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 2.3% (w/v) sodium dodecylsulfate (SDS) in 62.5 m*M* *Tris*/HCl buffer (pH 6.8). The extracts were loaded for electrophoresis on 4–8% gradient SDS-polyacrylamide gels with 3% stacking gels (Bär and Pette 1988) and run at 120 V overnight. Gels were silver stained according to the procedure of Oakley et al. (1980). Protein bands were identified according to their apparent molecular masses compared with those of marker proteins.

Results

Histochemical analysis revealed a large population of fibers classified as type IIB (30.7%). Of the remaining

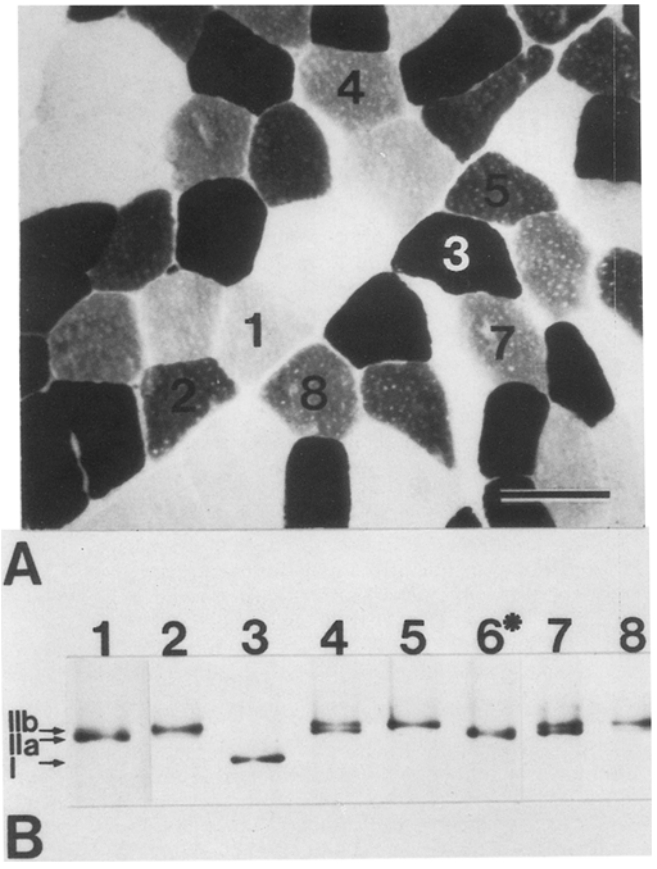

Fig. 2. Numbered, histochemically-defined fibers after preincubation at pH 4.6 (**A**) and specific myosin heavy chain analysis (**B**). 6* = myosin heavy chain analysis of fiber number 7, but in proximity to Fig. 1c where it was histochemically-identified as a type IIA fiber. Micrograph shown in **A** is the same region shown in Fig. 1d. *IIa* = myosin heavy chain IIa. *IIb* = myosin heavy chain IIb. *I* = myosin heavy chain I. *Bar* = 100 μm

fiber types, type IIAB fibers composed 13.1% of the biopsy, IIA 13.2%, IIC 0.7%, and I 42.3%. No type IC fibers were found. Biochemical analysis of specific single fibers demonstrated a correlation between the mATPase staining at preincubation pH 4.6 and the myosin heavy chain composition (Fig. 2). All fibers histochemically classified as type I contained only MHCI, and all fibers classified as type IIA contained only MHCIIa. Type IIAB fibers were composed of variable amounts of both MHCIIa and MHCIIb. The lighter staining type IIAB fibers had a greater percentage of MHCIIa (Fig. 2, fiber number 1), whereas the darker IIAB fibers contained a predominance of MHCIIb (Fig. 2, fiber number 8). Those fibers which were between fiber types IIA and IIB in staining intensity had approximately a 50-50 mixture of MHCs IIa and IIb (Fig. 2, fibers 4 and 7). Many of the fibers classified as type IIB (40% of those investigated) contained a small amount of MHCIIa (Fig. 2, fiber number 2). Therefore, the number of "pure" type IIB fibers was less than expected based upon the histochemical analysis.

Comparisons were also made along the length of single muscle fibers. Some fibers exhibited slight variations in staining intensity along their length when investigated over a distance of approximately 1.0 mm (Fig. 1c, d). These data suggest the possibility of nonuniform myosin expression along the length of normal human muscle fibers. This was subsequently confirmed by separate MHC analysis of single fragments from the same fiber at two different locations along its length (Fig. 2, numbers 6 and 7). This same phenomenon has also been observed in human soleus muscle where some fibers classified as type IIA became type IIC in subsequent sections (R. Staron, unpublished observations).

Discussion

Previous studies, combining histochemical and biochemical techniques, demonstrated a correlation between the mATPase histochemistry and myosin heavy chain content in single fibers of the rabbit soleus and tibialis anterior muscle (Staron and Pette 1986, 1987a, b). Based on peptide mapping, it appeared that the few fibers classified as type IIAB in rabbit tibialis anterior muscle coexpressed myosin heavy chains IIa and IIb (Staron and Pette 1987b). The coexpression of these fast heavy chains was confirmed in rat muscle (Danieli-Betto et al. 1986) and subsequently, in human muscle (Biral et al. 1988). It had been hypothesized that these hybrid fast fibers represent histochemically-intermediate type IIAB fibers (Staron et al. 1983b). This relationship has now been established.

It is not true, as has been implied, that fibers coexpressing two MHC isoforms will histochemically react only as the dominant isoform (Klitgaard et al. 1990). Although mATPase histochemistry is a semiqualitative technique, this method appears to be extremely sensitive with regards to the myosin heavy chain content. Fibers devoid of any mATPase activity following preincubation at pH 4.6 contain only MHCIIa. Slight staining indicates the coexpression of MHCs IIa and IIb, with a predominance of MHCIIa. As the staining intensity increases, the proportion of MHCIIb increases. However, a problem does arise with the classification of type IIB fibers. It becomes increasing difficult to distinguish "dark" staining hybrid fibers (type IIAB fibers with a predominance of MHCIIb) from the "darkest" or "pure" type IIB fibers. In the present investigation, many of the fibers classified as type IIB actually contained a small amount of MHCIIa and therefore, should have been classified as type IIAB. Such a "misclassification" may be exaggerated in trained individuals where many fibers classified as type IIB could contain a small amount of MHCIIa (R. Staron, unpublished observation).

Because the type IIAB fibers are between fiber types IIA and IIB with some fibers more like type IIA, some more like type IIB, and some a 50-50 mixture, this group can be further broken down into IIAb, IIaB, IIAB fibers, respectively. This is not unlike the C fiber population (IC, IIAC, IIC). However, it must be realized that a continuum exists with an infinite number of fiber "types". Although a multitude of fiber types is not practical for most research purposes, the histochemical

classification of type IIAB fibers as those which are between fiber types IIA and IIB in staining intensity seems justified. These type IIAB fibers are easily identifiable. Moreover, it must be realized that those light or dark staining type IIAB fibers with a predominance of either MHCIIa or MHCIIb may be "missclassified" and could, in some cases, affect the results.

Misclassification may be a more serious problem in the muscles of some small mammals. It has recently been shown that a third fast myosin heavy chain exists in rat muscle (Schiaffino et al. 1985; Bär and Pette 1988). Based on mATPase histochemistry, fibers expressing this fast heavy chain (type IID or IIX) appear similar to fibers expressing MHCIIb (type IIB fibers) (Termin et al. 1989). There is no evidence to suggest that this third fast heavy chain exists in human muscle. In addition, it must be realized that the MHCIIb in human muscle is different from the MHCIIb in the muscles of some small mammals. In rabbit (Staron and Pette 1987a) and rat (Danieli-Betto et al. 1986) muscle the MHCIIb is the fastest migrating of the fast myosin heavy chains, whereas in human muscle (Perrie and Bumford 1986) it is the slowest.

It is interesting that examples of nonuniform myosin expression have now been found in normal human muscle. This phenomenon reconfirms the concept of nuclear domains with asynchronous expression of myosin heavy chains along the length of single muscle fibers (Staron and Pette 1987c; Pavlath et al. 1989). Nonuniform expression may represent a normal, ongoing transformation process of fibers adapting from IIB⇋IIA or IIA⇋IIC or IC⇋I.

In conclusion, the correlation between mATPase activity and myosin heavy chain composition has now been established for human fast fiber types. These data illustrate the sensitivity of mATPase histochemistry and further emphasize the plasticity of skeletal muscle.

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