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Myosin heavy chain composition of single fibres from normal human muscle

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Electrophoretic analysis in the presence of 33% glycerol of purified myosin from normal human muscle shows three distinct protein bands which are identified as type 1, 2B, and 2A myosin heavy chain (MHC) isoforms by affinity-purified polyclonal antibodies. Analysis of MHC of single human muscle fibres shows that human muscles contain a large population of fibres showing the coexistence of type 2A and 2B MHC.

INTRODUCTION

Human skeletal muscles are composed of fast and slow motor units which are distinguishable by their physiological, biochemical and immunological properties (see Pette & Vrbova, 1985, for review). Histochemically, four main fibre types, type 1, 2A, 2B and 2C, have been found in human muscle differing in the acid and/or alkaline lability of the myofibrillar ATPase (Brooke & Kaiser, 1970). However, the population of type 2 fibres seems to be more heterogeneous. For example, two other subgroups of type 2 fibres, type 2AB and type 2AC, have been recently described in human muscle (Ingjer, 1979*a*,*b*; Staron *et al.*, 1983).

It is well known that the histochemical reaction of the myofibrillar ATPase is correlated with the presence of different myosin heavy chain (MHC) isoforms (Billeter *et al.*, 1981; Salviati *et al.*, 1982, 1984; Danieli-Betto *et al.*, 1986). Billeter *et al.* (1981) reported that peptide maps of type 2A and 2B human MHC are different. On the other hand, other laboratories were unable to find such differences (Salviati *et al.*, 1984).

Recently we set up a simple, but very sensitive, electrophoretic system that allows the separation of rat type 1, 2A and 2B MHC isoforms (Danieli-Betto *et al.*, 1986). With this system we analysed the MHC composition of single fibres from normal human muscle. The results show that, as in the rat muscle, the three HC isoforms of human myosin can be clearly identified by our electrophoretic system. Furthermore, a large percentage of human type 2 fibres were found to contain both the 2A and 2B isoforms of MHC.

EXPERIMENTAL

Normal human muscle biopsies ($n = 4$) were obtained from orthopaedic surgery from the vastus lateralis muscle. Myosin was purified as previously described (Biral *et al.*, 1984). Chemical skinning of muscle fibres was performed as described by Salviati *et al.* (1982). SDS/polyacrylamide-gel electrophoreses, 6% (w/v), were run as described by Danieli-Betto *et al.* (1986) but including 33% (w/v) glycerol in both the separating and the stacking gel. The electrophoretic run was carried out at 50 V overnight. Immunoblotting analysis of MHC isoforms was performed by the procedure of Towbin *et al.* (1979) using the following affinity-purified polyclonal antibodies: anti-(type 2A rabbit MHC) (5 μg/ml; which cross-reacts with type 1 MHC) raised in chicken immunized with purified masseter myosin (a prevalent type 2A myosin; Biral *et al.*, 1982); anti-(type 2B rabbit myosin) (5 μg/ml), raised in chicken with purified psoas myosin (a prevalent type 2B myosin; Weeds *et al.*, 1975). The second antibody was goat anti-(chicken IgG) (1:1000) conjugated with alkaline phosphatase.

RESULTS AND DISCUSSION

Adult human skeletal muscles are mixtures of histochemically identified type 1, type 2A, and type 2B fibres. Since the histochemical reaction of the myofibrillar ATPase is correlated with the presence of different MHC isoforms (Billeter *et al.*, 1981; Salviati *et al.*, 1982, 1984; Danieli-Betto *et al.*, 1986), myosin purified from adult human muscles should contain all three MHC isoforms. However, previous work (Biral *et al.*, 1984; Salviati *et al.*, 1984) showed that human MHC can be separated into two bands only, the fastest migrating band being the type 1 MHC. In this study, by including glycerol (33%) in both stacking and separating gels, we were able to separate electrophoretically the MHC material into three distinct bands [numbered according to the migration rate, band 1, 2, and 3, in Fig. 1(*a*), lane A]. On the basis of the electrophoretic mobility, compared with that found in previous studies, we tentatively concluded that type 2 MHC was resolved into bands 2 and 3. This was checked by measuring the immunoreactivity of bands 2 and 3 against affinity-purified antibodies. Fig. 1(*a*), lane B, shows the immunostaining of the same MHC of lane A by using the anti-(type 2B) polyclonal antibody. Band 3 was the most reactive band although the amount of protein was lower than that of bands 2 and 1, as indicated by silver staining. Band 2 reactivity was intermediate, whereas a very weak reaction was shown

Abbreviation used: MHC, myosin heavy chain.

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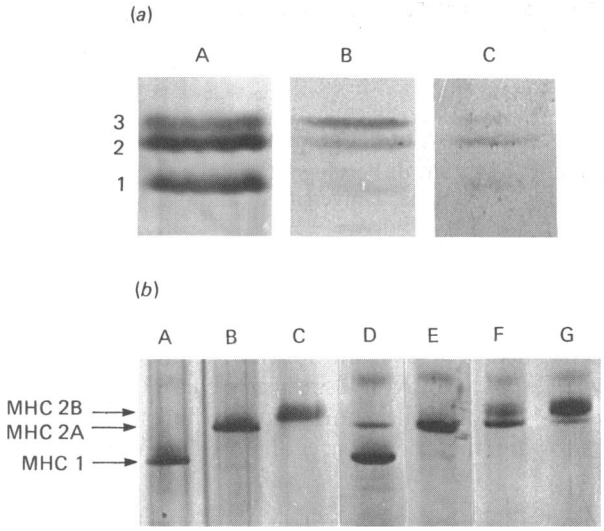

Fig. 1. Analysis of human myosin heavy chains by SDS/polyacrylamide-gel electrophoresis and immunoblotting

Panel (*a*): lane A, purified human myosin after SDS/polyacrylamide-gel electrophoresis and silver staining; lane B, purified human myosin after transfer to nitrocellulose and staining with anti-(type 2B MHC) polyclonal antibodies; lane C, purified human myosin after transfer to nitrocellulose and staining with anti-(type 2A MHC) polyclonal antibodies. Only the MHC region is shown. Panel (*b*): lanes A–G, single human muscle fibres after SDS/polyacrylamide-gel electrophoresis and silver staining. Only the MHC region is shown.

by band 1. On the other hand, when the same preparation of MHC was treated with the anti-(type 2A MHC) antibody, only band 2 was reactive. Band 1 gave the same very weak reactivity found with the anti-2B antibody. Staining of band 3 was weak also. These results demonstrate that bands 1, 2, and 3 show differential immunoreactivity with anti-(2A MHC) and -(2B MHC) antibodies, suggesting that they carry unique epitopes, different from each other. On the basis of the immunoreactivity we could therefore identify bands 1, 2, and 3 as type 1, type 2A, and type 2B MHC isoforms, respectively.

The electrophoretic analysis of MHC from single muscle fibres ($n = 192$) from four normal adult human muscles showed that about 37% of fibres contained type 1 MHC isoform [Fig. 1(*b*), lane A], 16% contained type 2A MHC isoform [Fig. 1(*b*), lane B], and 10% of fibres contained type 2B MHC isoform [Fig. 1(*b*), lane C]. The residual population of fibres (37%) showed coexistence of two types of MHC. About 10% of fibres contained type 1 and type 2A MHC isoforms [Fig. 1(*b*), lane D], although the large majority of fibres contained only trace amounts of type 2A MHC. From the histochemical point of view, these fibres would therefore give the reaction characteristic of type 1 fibres. Very few fibres were found to contain type 1 and type 2A MHC in a 1:1 ratio. Histochemically, these fibres should be type 2C fibres. The variability in the ratio of type 1 and type 2A MHC isoforms could explain the existence of type 2C′ and 2C″ fibres shown by Jansson *et al.* (1978) and of type 2AC fibres shown by Staron *et al.* (1983). No one fibre was found to contain type 1 and type 2B MHC. A large population of fibres (about 23% of total) showed the coexistence of type 2A and type 2B MHC isoforms, again in a variable ratio [Fig. 1(*b*), lanes F and G]. These fibres can account for the existence of the histochemical type 2AB and type 2AC fibres reported by Ingjer (1979*a*,*b*). Since this population represents about 50% of all type 2 fibres, the chance to isolate a pure type 2A or 2B fibre from a bundle of human fibres is very much reduced. This may explain why the identification of human type 2A and 2B isoforms of MHC by the proteolytic peptide pattern could not be obtained previously (Salviati *et al.*, 1984).

Fibres showing co-expression of different isoforms of MHC are interpreted as intermediate stages in the transition process from one type of fibre to another (Pette & Vrbova, 1985). In agreement with Billeter *et al.* (1981) our results suggest that in human muscle the sequence of transition is: type 1 → type 2C → type 2A → type 2B, and vice versa. The same conclusion has been shown in the case of rat muscles (Pierobon-Bormioli *et al.*, 1981; Danieli-Betto *et al.*, 1986). The large population of intermediate fibres containing both types of fast MHC seems to be a characteristic feature of human muscle and suggests that in these muscles type 2A and type 2B fibres are in a very dynamic equilibrium and they may easily undergo transitions from one type to the other in response to changes of muscle activity.

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