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Electrophoretic separation of developmental and adult rabbit skeletal muscle myosin heavy chain isoforms: example of application to muscle denervation study

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Abstract We present the separation by SDS gel-electrophoresis of the six main myosin heavy chains (MHC) present in rabbit skeletal muscle. The separation of the four adult MHC (1, 2A, 2X/2D, 2B) was compared to that of the corresponding rat MHC as described by Talmadge and Roy [J. Appl. Physiol. 99 (1993) 2337–2340]. We found that many rabbit muscles contained mainly one of the four MHC, in some cases the 2B MHC. In addition, we resolved the embryonic E and perinatal P developmental MHC, which should facilitate muscle differentiation and regeneration studies in the rabbit. An example of application to the study of muscle denervation is given.

Key words: Myosin heavy chain, MHC; SDS gel-electrophoresis; Rabbit skeletal muscle

1. Introduction

The polymorphism of myosin heavy chains (MHC) is believed to be responsible, at least in part, for the variable contraction velocity of muscles. In mammalian skeletal muscles, up to ten different MHC have been described, either at the genetic, or/and at the protein level (for reviews see [1,2]). The most abundant MHC are the developmental embryonic E and perinatal P heavy chains, the adult fast 2A, 2X/2D and 2B heavy chains, and the slow 1/β heavy chain; the α heavy chain, the slow-tonic heavy chain, and the superfast 2M and EOM heavy chains are found only in a few specialized muscles.

Muscle studies very often require the determination of the muscle MHC phenotype. Polyacrylamide gel-electrophoresis in the presence of SDS [3] has been used since 1975. Burridge and Bray [4] were the first to report a difference in the electrophoretic mobilities of MHC from skeletal and smooth muscles. The technique has constantly been improved over the years, mainly by the addition of glycerol, which has a sharpening effect on the electrophoretic bands [5–7]. Gradient gels were also developed, which allow a clear separation between the 2A and the 2X/2D heavy chains [8]. Finally, a major step in the separation of MHC was achieved by Talmadge and Roy [9], who described a new protocol to separate the skeletal muscle MHC of adult rats; this protocol presents distinct advantages over the previously published protocols, which in our hands, at least, were often not reproducible and also do not resolve rabbit muscle MHC satisfactorily.

The purpose of this paper is to present the separation of both developmental and adult myosin heavy chain isoforms of skeletal muscles of the rabbit. The 2A, 2X/2D, 2B, and 1/β heavy chains were neatly separated, in this order of increasing mobility, and the perinatal P heavy chain, which in other systems co-migrates with the 2B heavy chain, gave a distinct and independent band, which had the lowest mobility; the embryonic E heavy chain migrated very close to the 2A heavy chain, but could, however, be discriminated by adjusting the protocol.

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2. Experimental

2.1. Muscle sampling and myosin extraction

Several muscles were dissected from New Zealand rabbits, provided by CEGAV. Myosin was crudely extracted in a high ionic strength buffer, as described in [10].

2.2. Electrophoretic analysis of MHC and quantification

Electrophoresis was performed as described in [9], with a few modifications allowing the separation of the developmental MHC. Mini-gels were used in the Bio-Rad Mini-Protean II Dual Slab Cell. Electrophoresis took place in a cold cupboard, at a temperature which remained at 10°C for the whole run. To separate all the heavy chains, and in particular the embryonic and the 2A heavy chains, the duration of the run was 30–32 h. The gels were stained with Coomassie blue R-250 and the relative amounts of the different MHC were measured using a densitometer equipped with an integrator.

3. Results

Fig. 1 shows the pattern of MHC from adult rat and rabbit diaphragm. Like rat diaphragm [9], rabbit diaphragm contained the three fast-type 2A, 2X/2D, 2B (only a trace) and the slow-type 1 MHC. The MHC were less well separated in the rabbit diaphragm than in the rat diaphragm. This explains why, with the methods previously available, it has been more difficult to resolve the MHC from rabbit muscles.

The pattern of MHC from several skeletal muscles of adult rabbit are shown in Fig. 2. The four adult MHC were present in different proportions in the different muscles (Table 1). In addition, a faint band with the lowest electrophoretic mobility was observed in the gastrocnemius (caput mediale), extensor digitorum longus (EDL), and tongue muscles. This MHC was identified as the perinatal P MHC from the analysis of muscles from young rabbits. The two MHC present in the cardiac ventricles of 15-day-old rabbit, the α and 1/β MHC, were not separated by this electrophoretic system.

Fig. 3 shows the MHC pattern of the soleus of a young 9-day-old rabbit, compared to the MHC pattern of an adult rabbit diaphragm. The 9-day-old soleus displayed three main bands: two corresponded to the perinatal P and to the slow-type 1 MHC; the third had the same electrophoretic mobility

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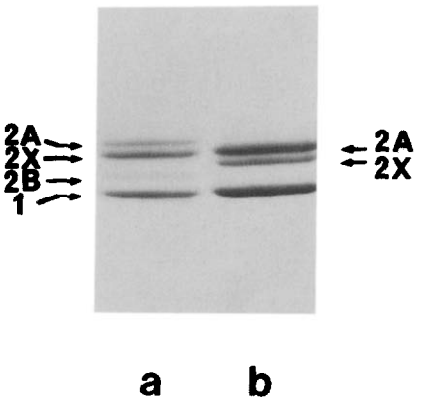

Fig. 1. MHC in adult rat (a) and rabbit (b) diaphragm.

as the fast-type 2A heavy chain from diaphragm. Increased duration of the electrophoresis revealed that this band was a doublet corresponding to the fast-type 2A and to the embryonic E MHC (Fig. 4). Longer electrophoresis did not however separate the α from the β MHC in the rabbit ventricles, although these isoforms in rat ventricle were discriminated (Fig. 4).

As an example of the application of the method as a discriminating method for the separation of MHC, Fig. 5 displays the effect on the MHC pattern of the gastrocnemius denervation performed on 8-day-old rabbits. At 60 days, the denervated muscle contained mainly the slow-type 1 MHC, some perinatal P MHC and fast-type 2X and 2A MHC, and no 2B MHC [12].

Table 1
MHC content (%) of several adult rabbit muscles

Muscle	MHC 2A	MHC 2X	MHC 2B	MHC 1
Vastus lateralis	10 (12)	33 (48)	57 (40)	0 (0)
Soleus	0 (7)	0 (0)	0 (0)	100 (93)
Gastrocnemius	18	40	16	24
Psoas	0 (3)	100 (96)	0 (1)	0 (0)
EDL	25 (41)	68 (57)	2 (0)	3 (2)
Tongue	74 (69)	17 (31)	1 (0)	7 (0)
Diaphragm	37 (54)	16 (14)	1 (1)	46 (21)

Values are percentages of total MHC. For comparison, values obtained from [11] are indicated in parentheses.

4. Discussion

The technique developed by Talmadge and Roy [9] to separate rat MHC has proven to be applicable to rabbit MHC. We obtained results for adult MHC (Table 1) comparable to those obtained with gradient gels [11]. It is clear that rabbit muscles contain a large variability in the proportions of MHC, with a predominance of either type 1 MHC (soleus), 2A MHC (tongue muscles), 2X/2D MHC (psoas, gastrocnemius, EDL), and even 2B MHC (vastus lateralis); 2B MHC was also predominant in two other muscles, the adductor magnus and the semimembranosus accessorius (not shown). The technique used proved to be easy to handle and reproducible. It did not allow us to separate the two cardiac MHC from the rabbit, although separation was possible in the rat. This shows that the separation

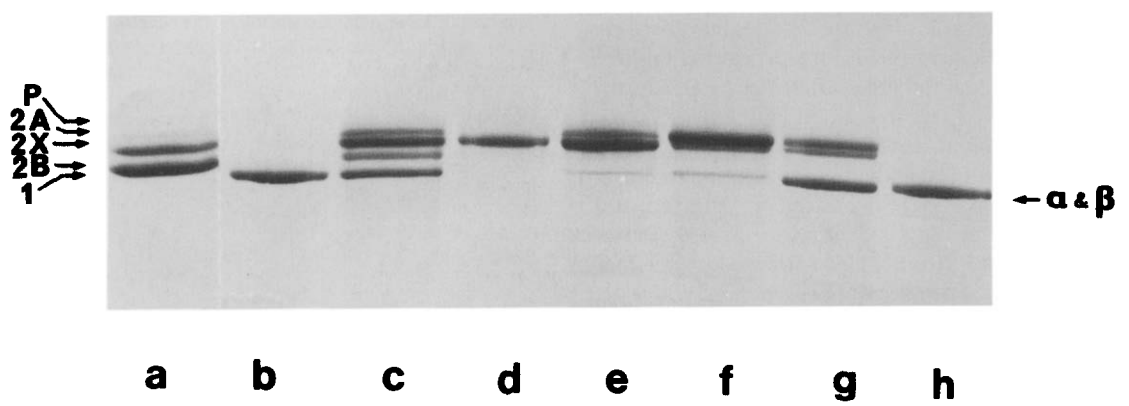

Fig. 2. MHC in various adult rabbit muscles: vastus lateralis (a), soleus (b), gastrocnemius (caput mediale)(c), psoas (d), extensor digitorum longus EDL(e), tongue (f), diaphragm (g) and cardiac ventricles (h).

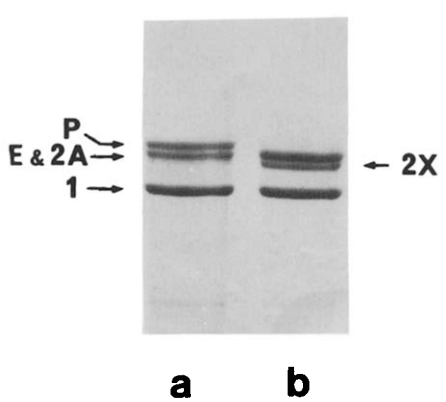

Fig. 3. MHC in 9-day-old rabbit soleus (a) and adult rabbit diaphragm (b).

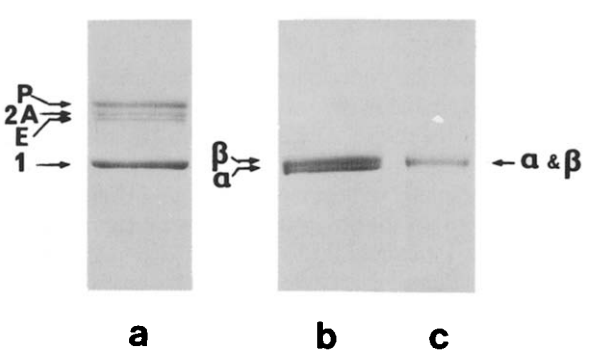

Fig. 4. MHC in 9-day-old rabbit soleus (a), adult rat (b), and rabbit (c) cardiac ventricles.

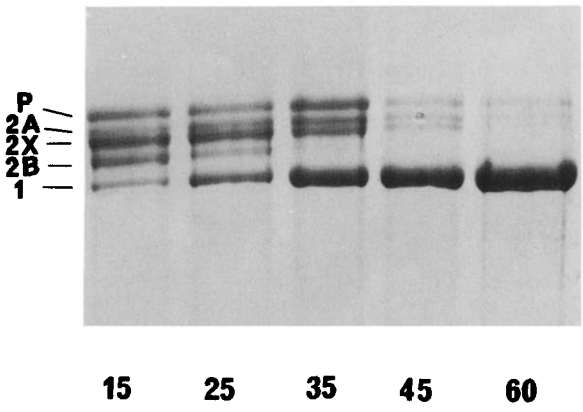

Fig. 5. MHC in 15- to 60-day-old rabbit gastrocnemius after denervation of the muscle at 8 days.

of rabbit MHC is more difficult than that of rat MHC. On the other hand, it allowed the clear separation of the perinatal P MHC, and, though less easily, that of the embryonic E MHC, which should facilitate muscle differentiation and regeneration studies in the rabbit [12].

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