Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms

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Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms is presented. This technique allows for the separation of the four identified MHC isoforms known to be present in adult rat skeletal muscle. These types of MHC are commonly called I, IIA, IIX or IID, and IIB. The procedure can be performed using minigel electrophoresis systems and does not involve preparation of gradient separating gels or the use of special cooling devices. The procedure accommodates both silver and Coomasie Blue staining. Thus the procedure is simple to perform and highly repeatable, providing high-resolution separation of MHC protein isoforms. The percent composition of the four adult MHCs in rat soleus, medial gastrocnemius, diaphragm, and levator ani muscles by use of this procedure of the four identified MHC isoforms known to be present in adult rat skeletal muscle. These types of MHC are commonly called I, IIA, IIX or IID, and IIB. 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muss, diaphragm, and levator ani were chosen for study because of their varied MHC percent compositions (1, 33). The muscles were weighed, frozen in liquid nitrogen, and stored at −70°C. Frozen muscles were minced with scissors in 9 vol of ice-cold homogenization buffer [250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM tris(hydroxymethyl)aminomethane (Tris), pH 6.8]. The muscle minces were subsequently homogenized by hand in glass tissue grinders. The homogenates were used for the preparation of washed myofibrils (34). The washed myofibrils were boiled in sample buffer (18) for 2 min at a final protein concentration of 0.125 mg/ml. Total protein was assayed according to the method of Bradford (6).

The stacking gels were composed of 30% glycerol, 4% acrylamide-bis-acrylamide (50:1), 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% sodium dodecyl sulfate (SDS). The separating gels were composed of 30% glycerol, 8% acrylamide-bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS. The gel constituents (12) were prepared from stock solutions, and polymerization was initiated with 0.05% N,N,N',N'-tetramethylethylenediamine and 0.1% ammonium persulfate. The pH values of the stacking and separating gels were not adjusted after the stock solutions were mixed. All chemicals were of electrophoresis grade. Table 1 lists the stock concentrations and volumes used for the preparation of minigels in the Biorad Mini-Protean II Dual Slab Cell electrophoretic system utilizing a Biorad 1000/200 power supply. The gel volumes listed provide for two 0.75-mm-thick gels. For a large-gel apparatus (CBS Scientific SG-200), doubling the volumes would be appropriate. A 10-space comb was used for the minigel system, and a 15-space comb was used for the large gel. Separate upper and lower running buffers were used. The upper running buffer consisted of 0.1 M Tris (base), 150 mM glycine, and 0.1% SDS. The lower running buffer consisted of 50 mM Tris (base), 75 mM glycine, and 0.05% SDS. The pH values of the running buffers were not adjusted. Upper and lower buffers were cooled to 4°C in a refrigerator before use, and the entire gel unit was placed in a styrofoam box containing cooling packs and/or ice to maintain the temperature below 10°C for the duration of the electrophoretic run. The running conditions for the minigel were 70 V (constant voltage) for 24 h. For the large-gel apparatus, the running time was 24 h at 275 V (constant voltage). In the minigel system the blue tracking dye ran off the gel, but in the large-gel system it ran to the bottom of the gel (20 cm total length of gel). The amount of protein run on the gel was varied between 1.0 and 0.5 μg of total protein per lane.

The gels were stained with Coomasie Blue if the protein per lane was 1.0 μg or with silver (Biorad silver stain plus kit) if the total protein per lane was 0.5 μg. The stained gels were scanned with a Pharmacia LKB Ultrascan scanning densitometer and photographed.

### RESULTS

Typical MHC isoform separations of rat muscles with use of the minigel system and Coomasie Blue staining are shown in Fig. 1. Identification of the region of the gel containing MHC was accomplished with use of rabbit muscle myosin as a standard. The separation of the MHC bands is distinct. The success rate of this protocol for yielding four well-differentiated MHC bands was 100%.

Identification of the various MHC bands with this protocol was based on the previously published (1, 33) MHC compositions and migration patterns of soleus, diaphragm, and levator ani (Fig. 1). Soleus contained only types I and IIa MHC, with a predominance of type I MHC. Type I MHC was the fastest-migrating band (lower band), and type IIa MHC was the slowest-migrating band (upper band). The diaphragm contained type IIx MHC in addition to types I and IIa MHC. The type IIx MHC migrated as a distinct band immediately below the type Ia band. The levator ani contained almost exclusively type IIb MHC, which migrated as a distinct band between types I and IIx MHC. The medial gastrocnemius contained all four MHC types but predominantly types IIx and IIb MHC. The overall order of migration by MHC type was I > IIb > IIx > Ia. This migration order was identical to that in previously published protocols (20, 31, 33). The separation of the MHC isoforms was similar when the large-gel apparatus was used; however, the distance between the bands was slightly greater (data not shown). Figure 2 shows a gel similar to that in Fig. 1, displaying the four MHC bands in rat fast- and slow-

![Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of isolated myofibrils as outlined in MATERIALS AND METHODS on minigel system and stained with Coomasie Blue. Lane 1, soleus (myosin heavy-chain (MHC) types I and Ia); lane 2, midcostal diaphragm (MHC types I, IIa, and IIx); lane 3, levator ani (MHC type IIb); lane 4, medial gastrocnemius (MHC types I, IIa, IIx, and IIb). All 4 MHC bands are clearly separated using minigel system. a, Type Ia MHC; b, type IIb MHC; c, type IIx MHC; d, type I MHC. Dual arrowheads in lane 2 show separation of types IIa and IIx MHC.](http://jap.physiology.org/DownloadedFrom/)http://jap.physiology.org/DownloadedFrom/
twitch muscles, but is silver stained. A densitometric tracing of the MHC region of rat medial gastrocnemius (lane 3, Fig. 2) is shown in Fig. 3. The four MHC bands (types I, Ia, Ix or Id, and IIb) of rat medial gastrocnemius are clearly separated and observed as separate peaks on the densitometric tracing. The type Ia and Ix or Id bands are separated into two clearly resolvable peaks with the minigel system. The percent compositions of the four MHC isoforms in the various muscles are shown in Table 2 and are similar to those in previously published reports (1, 31, 33).

**DISCUSSION**

The separation of mammalian MHC isoforms was initially accomplished by Carraro and Catani (10). This procedure only allowed for the separation of two MHC bands designated as slow (type I) and fast (type II). Later, adaptations of this procedure distinguished two isoforms of fast MHC designated Ia and IIb (9, 11, 25), which appeared to correlate with the myofibrillar ATPase histochemical technique of Brooke and Kaiser (7). Subsequently, a third type II MHC was independently identified by two groups and called type Ix (29) or type IId (33). However, the electrophoretic techniques that have been used to separate the four adult rat MHCs require labor-intensive silver staining and utilize either gradient gels (1, 33), which are difficult to reproduce, or special cooling devices, which are not readily available to all laboratories (20). Therefore the need for a consistent and high-resolution SDS-polyacrylamide gel electrophoresis technique for the separation of rat MHCs that could be used in conjunction with Coomassie Blue staining was apparent. The electrophoretic method described here can differentiate the three type II rat MHC isoforms, making it useful in the analysis of muscle MHC content. The technique has advantages over previously published protocols; for example, the technique 1) allows for the identification of the four identified MHC isoforms found in adult rat skeletal muscle, 2) does not require the laborious and difficult procedure of preparing a gradient-separating gel, 3) does not require the use of a special cooling apparatus, 4) can be run on a minigel system, and 5) accommodates Coomassie Blue or silver staining of protein bands. Finally, because the method described here makes use of a single percent separating gel, diffusion gradients that may be encountered when silver staining gradient gels are not present.

We thank Dr. V. R. Edgerton for critical review of the manuscript and Dr. K. M. Baldwin and R. E. Herrick for helpful discussions. This work was supported by National Institutes of Health National Research Service Award DE-07212 to R. J. Talmadge and Grant NS-16333 to R. R. Roy.

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Received 7 July 1993; accepted in final form 16 August 1993.

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**FIG. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of isolated myofibrils as in Fig. 1, but silver stained. Muscle samples are different from those in Fig. 1. Lane 1, soleus; lane 2, diaphragm; lane 3, medial gastrocnemius.

**FIG. 3.** Densitometric tracing of MHC region of rat medial gastrocnemius (lane 3, Fig. 2) is shown in Fig. 3. The four MHC bands (types I, Ia, Ix or Id, and IIb) of rat medial gastrocnemius are clearly separated and observed as separate peaks on the densitometric tracing. The type Ia and Ix or Id bands are separated into two clearly resolvable peaks with the minigel system.

**TABLE 2.** MHC percent composition of various rat skeletal muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Type I</th>
<th>Type Ia</th>
<th>Type Ix</th>
<th>Type IIa</th>
<th>Type IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>6</td>
<td>89±3</td>
<td>11±3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>4</td>
<td>4±1</td>
<td>10±1</td>
<td>25±2</td>
<td>58±2</td>
</tr>
<tr>
<td>Diaphragm (costal)</td>
<td>4</td>
<td>25±6</td>
<td>24±3</td>
<td>43±4</td>
<td>5±3</td>
</tr>
<tr>
<td>Levator ani</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2±1</td>
<td>98±1</td>
</tr>
</tbody>
</table>

Values are means ± SE. MHC, myosin heavy chain.


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