Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms

ROBERT J. TALMADGE AND ROLAND R. ROY
Department of Physiological Science and Brain Research Institute, University of California, Los Angeles, California 90024-1527

TALMADGE, ROBERT J., AND ROLAND R. ROY. Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. J. Appl. Physiol. 75(5): 2337-2340, 1993.—A new technique for the sodium dodecyl sulfate-polyacrylamide gel electrophoretic separation of rat skeletal muscle myosin heavy-chain (MHC) 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 and Coomasie Blue staining is similar to that previously reported. This new technique provides a novel and easy-to-perform method for the separation of rat skeletal muscle MHC isoforms.

MHC is the primary component of the thick filament and is the predominant protein in skeletal muscle. MHC is encoded by a multigene family consisting of several members (22), which are expressed in a tissue-specific and developmentally regulated manner (22). To date, four MHC proteins have been identified in the limb, jaw, and respiratory muscles of adult rats (1, 29, 33). These are identified as types I, IIA, IIX or IID, and IIB. The unique expression of any one of these MHC protein isoforms in a single muscle fiber is primarily responsible for the distinct fiber types observed in skeletal muscle after staining for qualitative myofibrillar adenosinetriphosphatase (ATPase) after acid and alkaline preincubations (33). The coexpression of multiple MHC isoforms within a given muscle fiber can occur (3, 4, 16, 28, 30), but this coexpression is thought to occur only during MHC transitions. The MHC protein expressed in a single muscle fiber is correlated to its maximum contraction velocity (5, 28, 32), which is related to the maximum myosin ATPase activity of a muscle (2). The myofibrillar and myosin ATPase activities of a muscle are related to the isoforms of myosin expressed in the muscle (13). Ranatunga and Thomas (27) demonstrated that the MHC-based histochemical fiber type composition of a muscle can be used to predict the contractile properties of the whole muscle. Thus the MHC expressed in a single muscle fiber, to a large degree, determines the histochemical type, myosin and myofibrillar ATPase activities, and contractile properties of the muscle fiber.

One of the responses of skeletal muscle to chronic changes in the amount and/or pattern of neuromuscular activity is an adaptation at the level of the MHC (26). Similarly, changes in the MHC expressed by a muscle are observed during development (8, 19) and aging (17, 21) in response to endogenous and exogenous endocrine factors (9, 13, 15), with changes in innervation status (23, 24), and in response to neuromuscular diseases (14). Therefore electrophoretic techniques that allow for the separation of MHC isoforms are critical to the analysis of skeletal muscle adaptation to 1) alterations in neuromuscular activity, 2) neuromuscular disease, 3) alterations in circulating levels of endogenous and exogenous hormones, and 4) signals related to development and aging.

In an attempt to obtain complete electrophoretic resolution of MHCs, the method of Doucet and Trifaro (12) was modified by 1) reducing the acrylamide content of the separating gel to 8%, 2) increasing the glycrol content of separating and stacking gels to 30%, 3) decreasing the gel thickness to 0.75 mm, 4) increasing the running time to 24 h, 5) decreasing the applied current to 6 mA/gel for a minigel system and 12 mA/gel for a large-gel system, and 6) omitting urea from the sample buffer. Thus a new technique is described for the electrophoretic separation of adult rat muscle MHCs. This technique has distinct advantages over previously published protocols (1, 9, 10, 20).

MATERIALS AND METHODS

Muscles from adult male and female Sprague-Dawley rats were removed while the animal was anesthetized with pentobarbital sodium. Soleus, medial gastroc-
justed. Upper and lower buffers were cooled to 4°C in a refrigerator before use, and the entire gel unit was placed in a refrigerator prior to electrophoresis. The running conditions for the electrophoretic run were appropriate. A 10-space comb was used for the minigel system, and a 15-space comb was used for the large gel. Separate stock solutions of 12) were prepared from stock solutions, and the pH values of the stacking and separating gels were not adjusted after the stock solutions were mixed. The stacking gels were composed of 30% glycerol, 4% acrylamide-\(N,N'\)-methylene-bis-acrylamide; TEMED, \(N,N,N',N'\)-tetramethylethylenediamine; PAGE, polyacrylamide gel electrophoresis.

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Separating Gel</th>
<th>Stacking Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Glycerol</td>
<td>3.000</td>
<td>3.000</td>
</tr>
<tr>
<td>30% Acrylamide-bis (50:1)</td>
<td>2.887</td>
<td>1.333</td>
</tr>
<tr>
<td>Tris</td>
<td>1.5 M (pH 8.8)</td>
<td>1.333</td>
</tr>
<tr>
<td>0.5 M (pH 6.7)</td>
<td>1.400</td>
<td></td>
</tr>
<tr>
<td>1 M glycine</td>
<td>1.400</td>
<td></td>
</tr>
<tr>
<td>100 mM EDTA (pH 7.0)</td>
<td>0.400</td>
<td>0.400</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.400</td>
<td>0.400</td>
</tr>
<tr>
<td>Distilled (H_2O)</td>
<td>1.495</td>
<td>3.362</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>10% Ammonium persulfate</td>
<td>0.100</td>
<td>0.100</td>
</tr>
</tbody>
</table>

Values are in ml. Gel mixtures are degassed before initiation of polymerization. All stock solutions are stored at 4°C, except 10% SDS, which is stored at room temperature. All stock chemicals are of electrophoresis purity. Acrylamide-bis, acrylamide-\(N,N'\)-methylene-bis-acrylamide; PAGE, polyacrylamide gel electrophoresis.

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 types I MHC in addition to types I and IIa MHC. The type II MHC migrated as a distinct band immediately below the type Ia band. The levator ani contained almost exclusively type IIa MHC, which migrated as a distinct band between types I and IIa MHC. The medial gastrocnemius contained all four MHC types but predominantly types IIa and IIb MHC. The overall order of migration by MHC type was I > IIb > IIX > IIa. 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-muscles, 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 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).

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of isolated myofibrils as outlined in MATERIALS AND METHODS on minigel system and stained with Coomassie Blue. Lane 1, soleus muscle myosin heavy-chain (MHC) types I and IIa; 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 IIa MHC; b, type IIb MHC; c, type I MHC; and d, type IIX MHC.
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, IIA, IIX or IID, and IIB) of rat medial gastrocnemius are clearly separated and observed as separate peaks on the densitometric tracing. The type IIA and IIX or IID 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 IIA 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 IIX (29) or type IID (1, 33). However, the electrophoretic techniques that have been used to separate the four adult rat MHCs require labor-intensive silver staining and utilize either special cooling devices, which are not readily available to

![Image](myosin-heavy-chain-electrophoresis.png)

**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.**

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

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Type I</th>
<th>Type IIA</th>
<th>Type IIX</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>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>4</td>
<td>7±1</td>
<td>10±1</td>
<td>25±2</td>
</tr>
<tr>
<td>Diaphragm (costal)</td>
<td>4</td>
<td>28±6</td>
<td>24±3</td>
<td>43±4</td>
</tr>
<tr>
<td>Levator ani</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>25±1</td>
</tr>
</tbody>
</table>

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

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 Coomasie 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 Coomasie 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.

Address for reprint requests: R. J. Talmadge, Dept. of Physiological Science, University of California at Los Angeles, 405 Hilgard Ave., 1804 Life Science Bldg., Los Angeles, CA 90024-1527.

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

References


9. Caiozzo, V. J., R. E. Herrick, and K. Baldwin. Influence of hypertrophy on maximal shortening velocity and myosin isoform...


