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Method for Electrophoretic Separation of Bovine Myosin Heavy Chain Isoforms

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Objectives

Myosin heavy chain (MyHC) isoform composition is a primary determinant of contractile speed of muscle fibers. Current methods for assessing bovine MyHC isoforms involve time-consuming histochemical evaluation by immunofluorescence or ATPase activity. Alternatively, electrophoretic separation of MyHC isoforms is more rapid, and this technique has been utilized in mice, pigs, and other species. Therefore, our objective was to establish a reliable procedure for separating bovine MyHC isoforms (I, IIa, and IIx) using sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by validation with Western blotting and histochemical analyses.

Materials and Methods

Muscle samples were collected from beef carcasses within 1.5 h after exsanguination; samples were processed for SDS-PAGE and immunohistochemical determination of fiber type and size. Muscles were chosen to represent a variety of fiber type compositions, including masseter, superficial pectoral, longissimus lumborum, and cutaneous trunci. For SDS-PAGE, proteins were extracted using a sodium phosphate SDS buffer. To determine appropriate conditions for MyHC isoform separation, the following parameters were evaluated: percent acrylamide (7 to 9% for separating; 4 to 5% for stacking) and acrylamide to bis-acrylamide ratios (50:1 and 37.5:1), glycerol concentrations (30 to 45%), and electrophoresis running buffers. After SDS-PAGE, proteins were stained with Coomassie to validate all 3 isoforms were separated. Once conditions were established, MyHC composition was calculated using band intensity for each isoform relative to total intensity of all 3 types. In addition, Western blotting was used to confirm identity of MyHC isoforms. Primary antibodies (Developmental Studies Hybridoma Bank; Iowa City, IA) were of unique isotypes to detect a combination of MHC using 2 color detection. A primary antibody for all MHC types (MF 20; IgG2b) was used in conjunction with BF-32 (MHC I and IIa; IgM), A4.840 (MHC I; IgM), SC-71 (MHC IIa; IgG1), or 6H1 (MHC IIx; IgM). Primary antibody BA-F8 (MHC I; IgG2b) alone was also used. In conjunction, relative area of muscle fiber types was calculated using immunohistochemical determination of MyHC composition and cross-sectional area. Muscle cross-sections were incubated with primary antibodies (BA-F8 and BF-32), followed by AlexaFluor conjugated secondary antibodies; AlexaFluor 488 conjugate to wheat germ agglutinin was used to visualize muscle cell membranes. Fiber CSA and area were determined using ImageJ software.

Results

All 3 bovine MyHC isoforms were separated using a discontinuous gel system. The separating gel consisted of 37% glycerol, 8% acrylamide-bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS, and the separating gel was composed of 37% glycerol, 4% acrylamide-bis (50:1), 70 mM Tris, 4 mM EDTA, and 0.4% SDS. Ammonium persulfate and TEMED were used to initiate polymerization of separating and stacking gels. Electrophoresis was performed at 80V for approximately 40 h at 4 C. Identity of isoforms was confirmed with Western blotting, and percent MyHC composition evaluated by SDS-PAGE was consistent with relative area determined by immunohistochemistry ($P < 0.05$).

Conclusion

Modification to SDS-PAGE parameters results in clear and consistent separation of bovine MyHC isoforms, thereby providing a more rapid means for determining MyHC composition compared to histochemical methods.

