Proteomic Analysis of Bovine Muscles during Aging

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Summary and Implications

Three structural proteins, alpha-actinin, tropomyosin, and a fragment of myosin heavy chain, changed in relative abundance with postmortem aging in the longissimus dorsi (LD), but not the adductor (AD). These results suggest that it is important to release these proteins from the myofibrillar matrix or degrade them in order to increase postmortem tenderness. Determining the process that accomplishes this release or degradation would be the next step in indentifying the vital steps in postmortem tenderization. Additionally, four enzymes involved in glycolysis were found to change in relative abundance during aging in the LD. By establishing a correlation between these proteins and postmortem tenderization there is the potential to use these proteins as indicators of tenderness that could then be used by the beef industry to identify consistently tender beef.

Introduction

During aging, enzymes found in muscle degrade specific proteins in muscle fibers that ultimately result in a more tender product. Comparing the protein profile of samples before and after aging will enable researchers to identify the proteins that are degraded during postmortem tenderization. These proteins, if identified, could be used by the beef industry as potential indicators of tenderness. A novel technology that can help identify the proteins that are changed during postmortem aging is 2-Dimensional (2D) Difference In Gel Electrophoresis (DIGE). 2D gels allow for the detection of proteins that would otherwise appear as a single band in a standard 1 dimension gel. The key to the power of the 2D gel is that it separates proteins in two directions, by ***both*** molecular weight and isoelectric point. This technique uses specially designed dyes, CyDyes. CyDyes are size-matched fluorescent dyes that attach to the lysine in proteins and are detected at different wavelengths. 2D DIGE uses CyDyes to label proteins from multiple samples individuallay. These labeled proteins can then be run on the same 2D gel and proteins detected can be assigned to a specific sample. Then a direct comparison can be made between the samples on a single 2D gel. By using 2D DIGE, a comparison can be made between samples before and after aging to determine the changes in the proteome that are linked to tenderness. The objective of this study was to determine the identity of proteins altered during the postmortem aging process that are linked to tenderness.

Materials and Methods

Previous research from our lab demonstrated that the LD had an increase in tenderness ($P < 0.01$) during aging while the AD only tended to increase in tenderness during aging ($P = 0.09$). Because of the differences seen between these two muscles, five samples from each muscle that represented the average tenderness for that muscle were chosen for 2D DIGE analysis. From these samples two protein fractions, a highly soluble sarcoplasmic fraction and a less soluble, crude myofibrillar fraction were extracted in order to improve the detection of less abundance proteins and prevent bias toward the highly soluble sarcoplasmic proteins. One and 14 day samples from the same muscle and protein fraction were run on the same gel using 2D DIGE. Samples were then labeled with CyDyes and then run on 11 cm immobilized pH gradient (IPG) strips with a pH gradient from 3-10. The 2nd dimension was run on 12.5% acrlyamide gels. Gel images were analyzed to determine differences in relative abundance of proteins between aging days in each muscle. Proteins that were found to differ between aging days were removed from a second preparative gel and then identified.

Results and Discussion

A number of proteins were found to change in relative abundance during aging in the AD, including beta enolase, glyceraldehydes-3-phosphate dehydrogenase, and troponin-T. However, these proteins may not be as important in tenderness changes because there was no significant increase in tenderness in the AD during aging. In the LD, a number of structural proteins were found to change in relative abundance during aging, including alpha-actinin, tropomyosin, and a myosin heavy chain fragment. Of these proteins alpha-actinin and tropomyosin were found to increase in relative abundance during aging while the fragment of myosin heavy chain decreased in relative abundance during aging. These proteins are likely involved in tenderization during aging because of the increase in tenderness seen during aging in the LD. Additionally, four enzymes involved in glycolysis were found to change in relative abundance during aging in the LD. Fructose-bisphosphate aldolase and phosphoglycerate kinase decreased in relative abundance during aging while malate dehydrogenase and triosephosphate isomerase increased in relative abundance during aging. Changes in abundance of these enzymes may alter beef palatability by affecting the rate or extent of postmortem glycolysis. By establishing a correlation between these proteins and postmortem tenderization there is the potential to use these proteins as

indicators of tenderness that could then be used by the beef industry to identify consistently tender beef.

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