Title: Identification of unique proteins in PSE pork using proteomics
NPB# 00-085

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Date Received: 9/24/2001

Abstract:

The objective of the study was to identify changes in protein profiles in the skeletal muscle of pigs that exhibit PSE characteristics. Proteomics techniques (2 dimensional electrophoresis) were used to separate the proteins to identify differences. Initial work with whole muscle extracts to identify proved to generate too many differences which most were irrelevant. Emphasis was placed on isolation of sarcoplasmic proteins because of their role in calcium regulation. Comparison of the 2-D separations of sarcoplasmic reticulum identified 20 spots that were unique. These have been excised from gels and will be identified using mass spectrometry. This preliminary work provides a base of information which may possibly help in identifying a marker to the PSE condition.

Introduction:

The goal of this project has been to identify changes in the protein profile (proteome) of skeletal muscle from pigs that are affected by the PSE condition. The rationale for this work is that identification of proteins associated with stress syndrome will provide a better understanding of its biological causes and potentially enable improved methods for diagnosis and screening. For example, information derived from these studies may contribute to development of an accurate method for identification of heterozygous (carrier) animals and be of substantial benefit to the pork industry.

These research results were submitted in fulfillment of checkoff funded research projects. This report is published directly as submitted by the project’s principal investigator. This report has not been peer reviewed

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Objective:

The initial objective of this work is to develop methods essential to the proteomic analysis.

Summary of work:

Procedures

Significant progress has been made toward the objective of optimizing a separation protocol for muscle samples. We have also been very fortunate to have acquired a large (190+) set of pig muscle samples from Dr Dave Gerrard of Purdue University. These samples have been extensively documented regarding the animal’s genetic background, slaughter data and whether the meat was indeed PSE.

The focus of proteome analysis has evolved during the past year from examination of whole tissue extracts to targeted subcellular fractions. Specifically, we have examined whole muscle extracts, fractions obtained by differential sedimentation and most recently, isolated sarcoplasmic reticulum. The later fraction is potential of greatest relevance to our studies because the sarcoplasmic reticulum and associated components are intimately involved in calcium regulation. Abnormal calcium regulation is widely held to be the underlying cause of stress syndrome and PSE, however, the proteins responsible are not known.

Differential sedimentation fractions obtained from PSE and normal muscle were initially examined by SDS-PAGE (Fig. 1a). Samples from PSE and normal muscle are shown in lanes 1-4 and 5-8, respectively. Lanes 1 and 5 contain pellets sedimented at 2,000g and are mostly composed of myofibrillar proteins. Lanes 2 and 6 contain pellets sedimented at 20,000g and are composed of proteins associated with various organelles such as mitochondria, lysosomes and membranes (e.g., sarcoplasmic reticulum). Lanes 3 and 7 contain pellets sedimented at 100,000g and are composed of cytoskeletal proteins. Lanes 4 and 8 represent the components not sedimented at 100,000g and are composed mostly of sarcoplasmic proteins.

Western blots of these fractions were also performed using a monoclonal antibody to the ryanodine receptor protein which is known to be associated with intracellular calcium regulation. The strongest staining for the ryanodine protein was found in the 20,000g and 100,000g pellets (lanes 2&3, 6&7) for both PSE (lanes 1-4) and normal (lanes 5-8) muscle samples. However, much less ryanodine staining was found in the PSE sample fractions than in the corresponding normal sample (compare lanes 2&3 with lanes 5&6).

Results:

Results strongly suggest that significant differences between normal and PSE muscle may be found in the membrane (20,000g pellet) fraction. Thus it was decided to focus our investigations using 2-dimensional gel electrophoresis on this fraction. Specifically, sarcoplasmic reticulum preparations were made from normal and PSE muscle and then
analyzed on 2-D gels (Fig 2a & b). It should be noted that a substantial amount of difficulty was encountered in optimizing the resolution of hydrophobic proteins associated with this fraction. However, the protocol we developed is working quite well and will be the subject of a forthcoming manuscript.

Comparison of 2-D sarcoplasmic reticulum separations from normal and PSE muscle by image analysis showed a number of differentially expressed proteins. A group of 20 spots corresponding to a portion of these proteins of interest were excised from the gels and sent for identification at the University of Nebraska Mass Spectrometry facility. The results of this analysis is expected shortly. It is anticipated that an additional manuscript concerning this work will be submitted for publication by December of 2001.

The sarcoplasmic reticulum fraction contains a large number of proteins as illustrated in Fig. 2. It is likely, that many proteins associated with important processes such as signal transduction, protein degradation, etc are represented in this separation. It is possible to make presumptive identifications of proteins in 2-D gels based on experimentally determined pI and molecular weight values. This information can be used to search databases (e.g., Swiss Prot Proteome) and make tentative identifications. However, it will be necessary to employ Mass Spectrometry analysis methods to make precise determinations of protein identity. It our long term goal to establish a data base of skeletal muscle proteins (i.e., its proteome) beginning with the sarcoplasmic reticulum. Data on the muscle proteome in combination with its genome will make significant contributions to the understanding of stress syndrome and other areas as well including animal growth.
Figure 1 SDS-PAGE and Ryanodine Immunoblots of Skeletal Muscle fractions

Samples from PSE and normal muscle are shown in lanes 1-4 and 5-8, respectively. Lanes 1 & 5 represent 2,000g pellets. Lanes 2 & 6 represent 20,000g pellets. Lanes 3 & 7 represent 100,000g pellets. Lanes 4 & 8 represent components not pelleted at 100,000g. The SDS-PAGE separation is shown in panel a and the immunoblot with anti-ryanodine receptor antibody (recognizes both alpha & beta forms) is shown in panel b.

Figure 2. Two-dimensional gel electrophoresis of sarcoplasmic reticulum fractions from skeletal muscle

Sarcoplasmic reticulum preparations from normal (a) and PSE affected (b) skeletal muscle were separated by two dimensional gel electrophoresis. Numerous spots representing differentially expressed proteins in panels a and b are being analyzed for identity by mass spectrometry.