One approach to proteome profiling is to fractionate the proteome into intact proteins with subsequent identification. In this work, a multidimensional profile of bovine skeletal muscle proteome was obtained by using two-dimensional liquid chromatography for fractionation. Digested protein fractions were then concentrated, further fractionated, separated and analyzed using an automated CE-MS/MS system for protein identification.

In summary, a lysate was prepared from bovine skeletal muscle and then separated in the first dimension by chromatofocusing. In this step, the proteins were separated by their isoelectric points (pI) in the pH range of 8.5 to 4.0 and fractions were collected at pH intervals of 0.3 units. The first-dimension pI fractions were then resolved in the second dimension by high-resolution, reversed-phase chromatography with a gradient of triethylammonium acid in acetonitrile. This results in a less complex protein mixture with a large dynamic range of concentration. When the target proteins are available in trace amounts, it is difficult to digest them with trypsin because of the unfavorable enzyme kinetics. As a solution to this problem, protein fractions with trace amounts of protein were pooled and then subjected to trypsin digestion. This was followed with a two-dimensional approach that utilizes one capillary for protein identification. This simplifies the automated separation of complex mixtures with a large dynamic range of concentration. The first dimension concentrates, denatures, and fractionates the protein digest followed by a second-dimension separation and subsequent analysis by CE-MS/MS.

Preparation of Bovine Skeletal Muscle Lysate

- 100 mg sample of bovine leg muscle was minced and washed 3 times with phosphate buffered saline.
- Lysis-detergent buffer (5 M urea, 50% glycerol, 2 M thiourea, 2.5% (w/v) SB3-10, 50 mM Tris- HCl (pH 8.0), 5 mM Tris (2-carboxyethyl)phosphine hydrochloride, 2% (w/v) n-octyl-β-D-glucoside and 1 mM protease inhibitor cocktail) was added to the muscle sample to a final volume of 2.5 mL.
- The sample was frozen at -20°C, thawed at 37°C and vortexed for three cycles.
- Then it was centrifuged at 20,000g for 60 min at 18°C.
- The supernatant was carefully retained and transferred to a PD10 column in which the sample buffer was exchanged with ProteomeLab™ PD 2D Start Buffer (pH 4.3).

The sample was filtered successively through 5 micron and 0.45 micron membrane filters.

The protein concentration was determined by the bicinchoninic acid protein assay.

The sample was fractionated on the ProteomeLab™ PD 2D Protein Fractionation System which separates proteins in the first dimension by pI with chromatofocusing and then in the second dimension by hydrophobicity with reversed-phase chromatography.

The fractions were collected every 15 seconds (387.5 µL) from the second dimension for protein identification by CE-MS/MS.

Sample Digestion For Protein Identification

- Fractions from several consecutive wells were pooled as shown in the chromatogram in Figure 2.
- The pooled sample was evaporated to a volume of about 10 µL or less using a speed vacuum.
- The final volume of each protein fraction was adjusted to 10 µL with 50 mM ammonium bicarbonate solution.
- Each fraction was reduced with dithiothreitol and heated at 60°C for 1 hr.
- Finally, each fraction was digested at 37°C for 18-24 hrs with trypsin.

Chitra K. Ratnayake, Ingrid Cruzado-Park and Michael H. Simonian
Beckman Coulter, Inc., Fullerton, CA