PE1.12 Comparison of whole, soluble and mitochondrial protein profiles between fast and slow bovine muscles 109.00

Mika Oe (1) mooe@affrc.go.jp, K Takeda(1), M Ohnishi-Kameyama 2, I Nakajima (1), S Muroya (1) M Shibata (3), K Ojima (1), K Chikuni (1)
(1)National Institute of Livestock and Grassland Science, Japan
(2)National Food Research Institute, Japan
(3)National Agricultural Research Center for Western Region, Japan

Abstract- Meat quality is affected by the composition of muscle fiber types. However, it is not clear what kind of molecule(s) leads to distinct meat quality. In this study, we applied two-dimensional difference gel electrophoresis (2D DIGE) to assess quantitative differences in the proteome of masseter (slow) and semitendinosus (fast) muscles from Holstein cows. Our results showed that 2D DIGE analysis allowed the detection of 100 spots in a whole protein of muscles. The number of up-regulated spots was 8 in fast muscles, and 5 in slow muscles. An identical trend was obtained in soluble proteins. The expression levels of some mitochondrial proteins were also different between the two muscles. The difference of whole, soluble and mitochondrial proteins may contribute to the variation of meat quality in fast and slow type muscles.

M. Oe, K. Takeda, I. Nakajima, S. Muroya, K. Ojima and K. Chikuni are with the National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki 305-0901, Japan (corresponding author to provide phone: +81-29-838-8686; fax: +81-29-838-8683; e-mail:mooe@affrc.go.jp).

M. Ohnishi-Kameyama is with the National Food Research Institute, Tsukuba, Ibaraki, 305-8642, Japan (e-mail: kameyama@affrc.go.jp)

M. Shibata is with National Agricultural Research Center for Western Region, Ohda, Shimane, 694-0013, Japan (e-mail:shibatam@affrc.go.jp)

Index Terms—fiber types, bovine, proteome.

I. INTRODUCTION

Quality of the meat is largely related with muscle fiber type. Beef tenderness was positively correlated with the frequency of slow twitch oxidative fibers and negatively correlated with the frequency of fast twitch glycolytic fibers [1]. The aging rate is faster in fast type muscle than in slow type muscle [2].

The relationships between meat quality and muscle fiber types were discussed in terms of myofibrillar proteins, especially myosin heavy chain (MyHC). MyHC isoforms were the major proteins responsible for the different muscle fiber types [3, 4, 5], while there are many other proteins in muscles. These proteins may also influence to quality characteristics of the meat. Thus, it is important to study the difference of protein profiles between fast and slow type fibers to elucidate the mechanism by which muscle proteins influence to meat quality.

Modern proteomic technologies have provided a more detailed characterization of the protein expression profiles of muscles [6, 7]. However, the majority of studies were performed on the whole proteins. Therefore, proteins expressed at low level were not detected. Fractionating methods were suitable for the detection of less-expressed proteins [8].

To clarify the difference of proteins expressed in fast and slow muscles, in this study, we compared the whole, soluble and mitochondrial proteins by two-dimensional difference gel electrophoresis (2D DIGE).

II. MATERIALS AND METHODS

A. Animal and Muscle samples

The animals were cared for as outlined in the Guide for the Care and Use of Experimental Animals (Animal Care Committee of the National Institute of Livestock and Grassland Science). Holstein cows were slaughtered after a brain concussion induced by captive-bolt gun stunning. Within 1 h postmortem, approximately 1 cm^3 pieces of masseter and semitendinosus were excised from hanging carcasses and frozen in liquid nitrogen.

B. Preparation of whole proteins

The frozen samples were crushed into a fine powder in a liquid nitrogen cold crusher (NRK R-8, Nihon-Rikagakukikai, Tokyo, Japan), and 200 μg were homogenized with homogenizing buffer, 8 M urea, 30 mM Tris-HCl, 4 % (w/v) CHAPS, pH 8.5. The homogenate was shaken for 30 min. Then the homogenate was centrifuged at 12000 x g, 4 ºC for 30 min to pellet insoluble material. The protein concentration was determined with BCA protein assay reagent (Thermo).

C. Preparation of water soluble proteins

The frozen samples were crushed into a fine powder in a liquid nitrogen cold crusher (NRK R-8, Nihon-Rikagakukikai), and 200 μg was homogenized in 20 x volume (v/w) distilled water. The homogenate was centrifuged at 12000 x g, 4 ºC for 30 min to pellet insoluble material. The supernatant was recovered. The protein concentration was determined with BCA protein assay reagent (Thermo).

D. Preparation of mitochondrial proteins

Muscle samples were minced and digested with collagenase-II in buffer A (100 mM KCl, 50 mM Tris-HCl, 2 mM $CaCl_2$, pH 7.4) for 15 min at 37 ºC. Large debris and nuclei were pelleted by centrifuging for 10 min at 650 x g, at 4 ºC. The supernatant was further centrifuged for 10 min at 6,000 x g, at 4 °C. The pellet was resuspended in buffer B (0.25 M sucrose, 10 mM Tris-HCl, pH 7.4) and centrifuged for 10 min at 9,000 x g, at 4 ºC. The pellet was stored at -20 ºC.

E. Two dimensional fluorescence differential gel electrophoresis (2D DIGE)

To analyze the difference of protein expression between masseter and semitendinosus, two dimensional fluorescence differential gel electrophoresis (2D DIGE) was conducted. For 2D DIGE, proteins extracted from masseter and semitendinosus were labeled with CyDye DIGE fluor Cy3 and Cy5 minimal dyes (GE Healthcare UK Ltd, Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, England), respectively, according to the manufacture's protocol. Both pre-labeled samples were mixed together. Pre-labeled proteins for 2D DIGE were loaded onto immobilized pH gradient (IPG) strips: 11 cm long, pH 3-10. IPG strips were rehydrated overnight. Isoelectric focusing (IEF) was performed in the Protean IEF cell (Bio-Rad). The voltage was increased gradually up to 8000 V for 2.5 h and then was maintained at a total voltage of 35000 V. Prior to the second dimensional SDS-PAGE, the strips were equilibrated for 20 min in a solution of 6 M urea, 20 % (v/v) glycerol, 2 % SDS, 50 mM Tris-HCl (pH 8.8) and 2 %(w/v) DTT. The SDS-PAGE was performed on 12.5 % polyacrylamide gel at 200 V for 50 min. After SDS-PAGE, the fluorescence of labeled proteins on the gel was scanned at Cy3 and Cy5 detection modes with the Ettan DIGE Primo system (GE Healthcare).

F. Protein identification by Mass Spectrometry

Protein spots of interest were manually excised from the gel in a clean air cabinet. Proteins in gel pieces were subjected to reductive alkylation by iodoacetamide and digested with trypsin. Peptides were desalted using ZipTip C18 pipette tips (Millipore, MA, USA.) and eluted with a matrix solution (α-cyano-4-hydroxycinnamic acid) onto the MALDI (matrix-assisted laser desorption /ionization) target. Mass spectra were recorded in reflectron mode of MALDI-TOF (time of flight) mass spectrometer (REFLEX II, Bruker Daltonics, Bremen, Germany) equipped with delayed extraction by summing 200 laser shots of a 337 nm nitrogen laser with acceleration voltage of 20 kV. The mass spectrometer was calibrated externally once every eight samples using a commercial available peptide mixture (Bruker Daltonics) covering the 700- to 3200 m/z range. Obtained mass spectra were analyzed with XMASS 5.0 or FlexAnalysis 2.2 (Bruker Daltonics) software and transferred to MASCOT peptide mass fingerprinting search engine (Matrix Science, London, UK) through the BioTools program (Bruker Daltonics) to search automatically the NCBInr (National Center for Biotechnology Information nonredundant) database. The parameters were set to allow 1 or 2 miss cleavages with the enzyme trypsin, a peptide mass tolerance of 100-200 ppm.

III. RESULTS AND DISCUSSION

The expression levels of some proteins were clearly different between the two muscles. Our results showed that 2D DIGE analysis allowed the detection of 100 spots in a whole protein of

muscles. And nineteen proteins were identified (Fig. 1). Nine of them were myofibrillar proteins: actin-alpha1, myosin light chain-1fast (MLC-1f), myosin light chain-2slow (MLC2-s), myosin light chain-1slow-b (MLC-1sb), myosin light chain-2fast (MLC-2f), myosin light chain-3fast (MLC-3f), tropomyosin-1 (TPM1), tropomyosin-2 (TPM2) and tropomyosin-3 (TPM3). Four of them were the enzymes for glycolytic pathway: enolase-3, aldolase-A, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and triosephosphate isomerase. Aconitase-2mitochondrial was the enzyme for TCA cycle. Creatine kinase-muscle was the enzyme involved in energy homeostasis. The other 4 proteins were myoglobin, albumin, hemoglobin-alpha and hemoglobin-beta.

MLC-1f, MLC-2f, TPM-1, MLC-3f, enolase-3, aldolase-A, GAPDH and triosephosphate isomerase were expressed at higher level in semitendinosus than masseter. Myoglobin, albumin, aconitase-2mitochondrial, MLC-2s and MLC-1sb were expressed at higher level in masseter than semitendinosus.

Similar result was obtained in soluble proteins. Enolase-3, aldolase-A and triosephosphate isomerase were expressed at higher level in semitendinosus than masseter. Aconitase -2mitochondrial was expressed at higher level in masseter than semitendinosus. These soluble protein profiles reflected the metabolic property of each muscle.

The expression levels of some mitochondrial proteins were also different between the two muscles. Generally, slow type fiber contains more mitochondria than fast type fiber. These differences indicate that mitochondria change metabolic properties not only by changing their volumes but also by changing composition of mitochondrial proteins depending on the specific energy requirements of fast and slow muscles.

The difference of whole, soluble and mitochondrial proteins may contribute to the variation of meat quality in fast and slow type muscles.

IV. CONCLUSION

The expression profiles of whole, soluble and mitochondrial proteins were different between bovine masseter and semitendinosus muscles. The difference of these proteins may contribute to the variation of meat quality in fast and slow type muscles.

REFERENCES

[1] Maltin CA, Sinclair KD, Warriss PD, Grant CM, Poter AD, Delday MI & Warkup CC., (1998) The effect of age at slaughter, genotype and finishing system on the biochemical properties, muscle fiber type characteristics and eating quality of bull beef from suckled calves. Animal Science, 66, 341-348.

[2] Ouali A. (1990) Meat tenderization: Possible causes and mechanisms. A review. Journal of Muscle Food, 1, 129-165.

[3] Schiaffino S. & Reggiani C., (1994) Myosin isoforms in mammalian skeletal muscle. Journal of applied physiology, 77(2), 493-501.

[4] Schiaffino S. & Reggiani C., (1996) Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiological reviews, 76(2), 371-423.

[5] Weiss A. & Leinwand LA., (1996) The mammalian myosin heavy chain gene family. Annual review of cell and developmental biology, 12, 417-39.

[6] Talamo F., D'Ambrosio C., Arena S., Del Vecchio P., Ledda L., Zehender G., Ferrara L. & Scaloni A. (2003). Proteins from bovine tissues and biological fluids: defining a reference electrophoresis map for liver, kidney, muscle, plasma and red blood cells. Proteomics, 3(4), 440-60.

[7] Bouley, J., Chambon, C. & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. Proteomics, 4(6), 1811-24.

[8] Hamelin, M., Sayd, T., Chambon, C., Bouix, J., Bibe, B., Milenkovic, D., Leveziel, H., Georges, M., Clop, A., Marinova, P. & Laville, E. (2007). Differential expression of sarcoplasmic proteins in four heterogeneous ovine skeletal muscles. Proteomics, 7(2), 271-80.

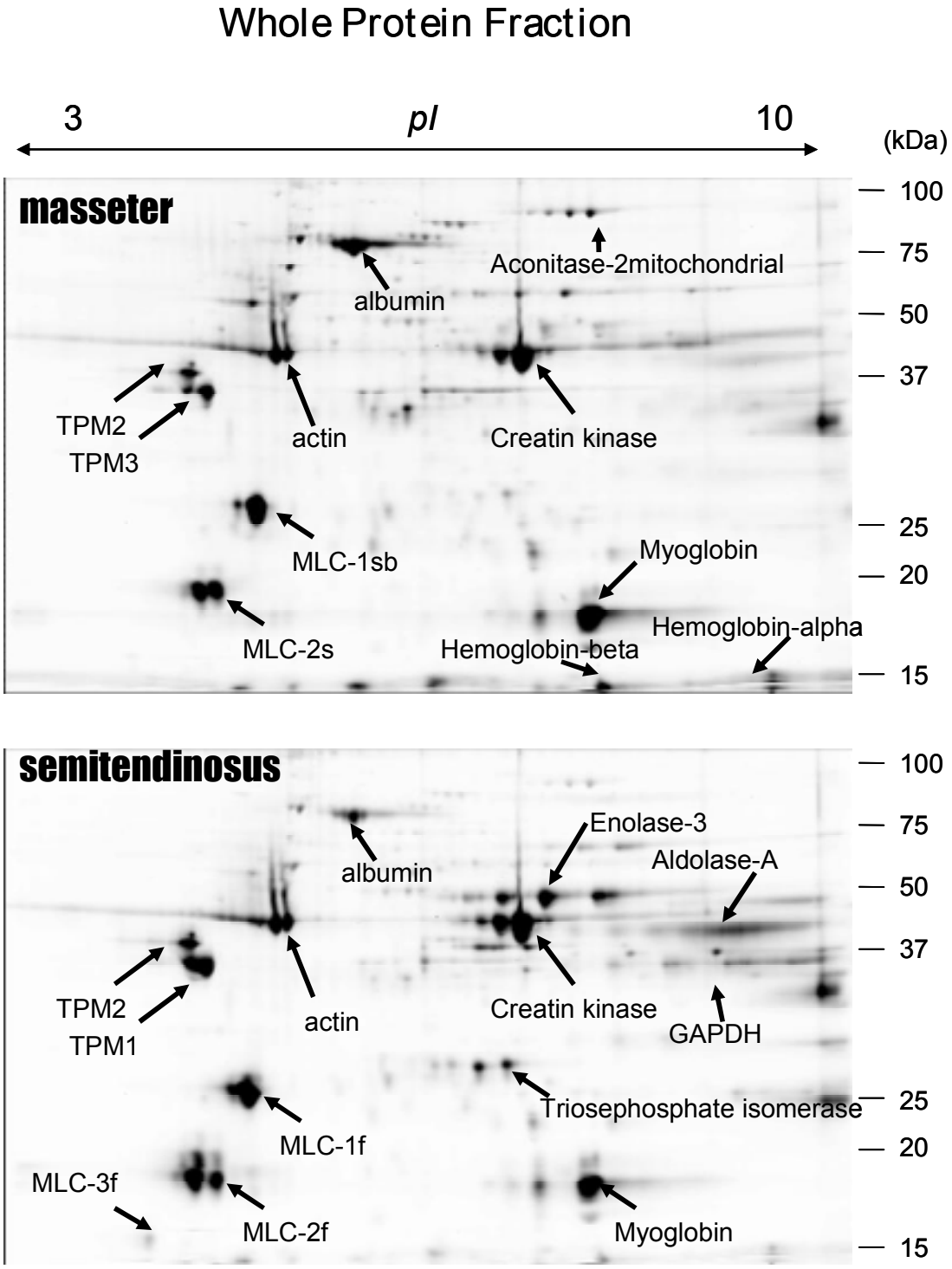

Figure 1. Representative 2D gel images of whole protein fraction.