Molecular understanding of tenderness: a proteomics approach

Kristin Hollung and Eva Veiseth-Kent

Nofima AS, PO Box 210, NO-1431 Ås, Norway

Abstract – An increasing number of studies over the last years using different proteomics tools have led to a better understanding of postmortem proteolysis and its' relation with meat tenderization. Examples of this include the discovery of degradation products from proteins thought to be unaltered during post mortem storage (e.g. actin and myosin heavy chain), and the involvement of heat shock proteins during the conversion of muscle to meat. Furthermore, a wide range of metabolic proteins including glycolytic enzymes and stress proteins change in abundance post mortem. Proteomics has also been used to identify potential protein markers for tenderness, and several candidates have been proposed to explain the variation in tenderness being observed. The power of these candidate markers to explain variation in tenderness remains unclear, but it is certain that they will contribute to building a better picture of this complex process.

Key Words – tenderness, proteomics, protein markers.

I. INTRODUCTION

Tenderness is considered the most important quality trait of beef by consumers, and reducing the unacceptable levels of variation in tenderness remains an important goal for the meat industry. A large variation in beef tenderness has been reported, and identification of markers for meat tenderness in cattle has gained attention in recent years. Quality traits of muscle foods are influenced by a number of different factors such as genetics, environmental factors and processing conditions. The proteome is the protein complement of the genome and consists of the total amount of proteins expressed at a certain time point and may thus be viewed as the mirror image of the gene activity. While the genome contains information on which genes and alleles are present in the genome, the proteome contains information on which genes are actually being expressed and translated into proteins. In contrast to the genome, the proteome is continuously changing according to factors influencing on either protein synthesis, degradation or posttranslational modifications. In this regard, the proteome can be seen as the molecular link between the genome and the functional quality of the muscle or meat. Thus understanding the variations and different components of the proteome with regard to certain quality or processing parameters will lead to knowledge that can be used in optimizing the conversion of muscles to meat. In contrast to traditional methods studying one or a few specific genes or proteins at a time, studies can now be conducted without any a priori hypotheses on the mechanisms involved. Meat scientists have been willing to adopt a variety of proteomics approaches and an increasing number of publications have provided us with a better understanding of postmortem proteolysis and its' relation with meat tenderization [1, 2, 3].

II. RESULTS AND DISCUSSION

Proteomics is a powerful technology to study global changes of proteins occurring during postmortem storage. Examples of this include the discovery of degradation products from proteins thought to be unaltered during postmortem storage (e.g. actin and myosin heavy chain) [4].

Changes in metabolic protein levels in biopsies from bovine *longissimus thoracis* in the living animals to samples collected 1 h postmortem demonstrated that 24 proteins classified as either metabolic proteins or heat shock proteins were changed in abundance [5]. We observed a clear shift in energy metabolism in the muscle post mortem with an increase in enzymes involved in both the glycolytic pathway as well as in the TCA cycle. These findings suggest that an increased aerobic energy metabolism occurs the first hour after slaughter. The increased aerobic energy metabolism will probably affect the rate of glycolysis in muscles after slaughter and eventually lead to variation of meat quality.

To obtain more information on the metabolic changes occurring during the early postmortem period, samples collected between 1 and 24 h after slaughter were analyzed [6].The proteins found to change in abundance during this early postmortem period were part of the biochemical network cooperating to prevent muscle cells from reducing the ATP level, while others were involved in stress response and cell death. The results show that all identified metabolic enzymes are either involved in enzymatic reactions of the glycolytic and TCA pathways or associated with energy production. Several identified enzymes and proteins, namely, glycerol-3-phosphate dehydrogenase 1 (GPD1), ADP-ribosylhydrolase like 1, biliverdin reductase B, and cytochrome bc1 complex, are not directly involved in the glycolytic pathway but take part into the production of NAD+, which may further enter the glycolytic and TCA pathway to drive the synthesis of ATP. This indicates that the level of ATP is maintained for several hours after slaughter, and the energy production is still operative under the conversion of aerobic metabolism to anaerobic metabolism in muscle. Muscle cells are under stressful conditions after slaughter caused by nutrient and oxygen depletion. This is supported by the finding of stress and defense proteins, which were changed in abundance early postmortem. The protective functions of these proteins are probably to delay cell death, thus diminishing the impairment of stress. These changes could reflect important mechanisms related to development of a satisfactory meat quality.

By comparing the postmortem changes in protein composition between the soluble and insoluble protein fractions, we were able to look at the changes in solubility during postmortem storage [7]. This study indicates a connection between the stability of myofibrillar proteins and the solubility of easily soluble proteins, such as metabolic enzymes and cellular defense/stress proteins. We have identified two metabolic enzymes (2,3-bisphosphoglycerat-mutase and NADH dehydrogenase) and one protein involved in the stress responses/apoptosis of the cell (Hsp70) that have not previously been identified in the insoluble protein fraction. The occurrence of these easily soluble proteins in the insoluble protein fraction could be due to precipitation or aggregation, thereby going from a soluble to an insoluble state. Different mechanisms might be responsible for this change in the protein solubility, e.g., isoelectric precipitation caused by the pH decline and modification of proteins; however, further studies are needed to unravel the specific mechanism behind the observed changes in this study.

Proteomics has also been used by several groups to identify potential protein markers for tenderness in beef [8, 9, 10, 11, 12, 13, 14]. The power of these candidate markers to explain variation remains unclear, but it is certain that they will contribute to building a better picture of this complex process. In one study we found that peroxiredoxin-6 was more abundant in the tender group, both in biopsies and 1 h postmortem samples from bovine M. *longissimus thoracis*. However, this was not confirmed at a satisfactory significance level in another set of animals although the tendencies were the same. So far most studies of proteome changes related to meat quality have been done using 2-dimensional gel electrophoresis for separation of proteins. In a recent study we compared results from isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and two-dimensional gel electrophoresis (2-DE) analysis [9]. A number of the proteins which have previously been related to tenderness were found to change in abundance between tender and tough samples, both in iTRAQ and 2-DE analysis. Even though the overlap in significantly changing proteins was relatively low between the iTRAQ and 2-DE analyses, certain proteins predicted to have similar function were found in both analyses and showed similar changes between the groups, like structural proteins and proteins related to apoptosis and energy metabolism. In a conclusion, most of the proteins found to change significantly between tender and tough sample groups (both in iTRAQ and 2-DE analysis) are in line with previous reports on meat tenderness. The limited number of proteins detected by both analyses can be explained by the fact that the methods are based on different principles for identification and quantification.

The fact that different results are observed in the various studies performed to unleash potential markers for tenderness reflects the complexity of a

trait such as tenderness. Many factors may influence on tenderness development in muscle and meat, thus a protein marker in one animal may not be valid in another animal due to different breeds, gender, treatments or other unknown factors. However, some general mechanisms seems to be involved including glycolysis and heat-shock proteins as well as some other candidates that should be further investigated and validated.

REFERENCES

1. Bendixen, E. (2005). The use of proteomics in meat science. Meat Science 71: 138-149.
2. Hollung, K., E. Veiseth, X. H. Jia, E. M. Faergestad, & K. I. Hildrum. (2007). Application of proteomics to understand the molecular mechanisms behind meat quality. Meat Science 77: 97-104.
3. Bendixen, E., M. Danielsen, K. Hollung, E. Gianazza, & I. Miller. (2011). Farm animal proteomics - a review. Journal of Proteomics 74: 282-293.
4. Lametsch, R., Karlsson A., Rosenvold K., Andersen H. J., Roepstorff P. & Bendixen E. (2003). Postmortem proteome changes of porcine muscle related to tenderness. Journal of Agricultural and Food Chemistry 51: 6992-6997.
5. Jia, X. Hildrum K.I., Westad F., Kummen E., Aass L. & Hollung K. (2006). Changes in enzymes associated with energy metabolism during the early post mortem period in longissimus thoracis bovine muscle analyzed by proteomics. Journal of Proteome Research 5: 1763-1769.
6. Jia, X. H., Ekman, M., Grove, H., Faergestad, E. M., Aass, L., Hildrum, K. I., & Hollung, K. (2007). Proteome changes in bovine longissimus thoracis muscle during the early postmortem storage period. Journal of Proteome Research, 6: 2720-2731.
7. Bjarnadottir, S. G., K. Hollung, E. M. Faergestad, & E. Veiseth-Kent. (2010). Proteome changes in bovine longissimus thoracis muscle during the first 48 h postmortem: Shifts in energy status and myofibrillar stability. Journal of Agricultural and Food Chemistry 58: 7408-7414.
8. Jia, X., Veiseth-Kent E., Grove H., Kuziora P., Aass L., Hildrum K.I. & Hollung K. (2009). Peroxiredoxin-6--a potential protein marker for meat tenderness in bovine longissimus thoracis muscle. J Anim Sci 87: 2391-2399.
9. Bjarnadottir S.G., Hollung K., Høy M., Bendixen E., Codrea M. & Veiseth-Kent E. 2012. Changes in protein abundance between tender and tough meat from bovine Longissimus thoracis muscle assessed by iTRAQ and 2-DE analysis. JAS *in press.*
10. Bernard, C., Cassar-Malek I., LeCunff M., Dubroeucq H., Renand G. & Hocquette J.F. (2007). New indicators of beef sensory quality revealed by expression of specific genes. J Agric Food Chem 55: 5229-5237.
11. Zapata, I., H. N. Zerby, & M. Wick. (2009). Functional proteomic analysis predicts beef tenderness and the tenderness differential. J Agric Food Chem 57: 4956-4963.
12. Kim, N. K., Cho S., Lee S.H., Park H.R., Lee C.S., Cho Y.M., Choy Y.H., Yoon D., Im S.K. & Park E.W. (2008) Proteins in longissimus muscle of korean native cattle and their relationship to meat quality. Meat Science 80: 1068-1073.
13. Laville, E., Sayd T., Morzel M., Blinet S., Chambon C. Lepetit J., Renand G. & Hocquette J.F. (2009) Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. J Agric Food Chem 57: 10755-10764.
14. Guillemin N., Bonnet M., Jurie C. & Picard B. (2011) Functional analysis of beef tenderness. J Proteomics 75: 352-365.