Characterization of goat myoglobin

S.P. Suman[1*], P. Joseph[1], S. Li[1], G. Rentfrow[1], T. Hutchens[1], R.B. Cox [1], C.M. Beach[2], L. Steinke[3] & M. Fontaine[3]

[1]Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[2]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY 40536, USA
[3]Protein Structure Core Facility, University of Nebraska Medical Center, Omaha, NE 68198, USA.
[*]E-mail: spsuma2@email.uky.edu

Abstract

Color stability attributes of goat meat are different from those of sheep meat, possibly due to species-specific differences in myoglobin (Mb) chemistry. Information on the physico-chemical characteristics of goat Mb is scarce, and an examination of post-genomic era protein databases reveals that the primary structure of goat Mb is yet to be determined. Therefore, our objectives were to characterize the molecular mass and primary structure of goat Mb. Goat Mb was purified from cardiac muscles via ammonium sulfate precipitation. Mass spectrometry was utilized to determine exact molecular mass, whereas automated Edman degradation was employed to determine the amino acid sequence from N-terminal. Mass spectrometric analyses of intact proteins demonstrated that the molecular mass (*m/z*) of goat Mb was 16938 Da whereas that of sheep Mb was 16934 Da. Automated Edman degradation of goat Mb yielded the sequence identity of first forty amino acids, which shared 98% similarity with the corresponding segment in sheep Mb. The residue at position 8 was different in goat and sheep myoglobins. The amino acid substitution THRgoat8GLNsheep could explain, in part, the observed difference in molecular mass. Proteomic investigations are underway to determine the complete sequence of goat Mb through analyses of tryptic peptides.

Introduction

Goats are exploited as efficient meat animals under harsh environments, which generally are unfavorable to raise cattle and sheep (Alexandre & Mandonnet, 2005). Global production of goat meat increased dramatically over 400% in last four decades (FAO, 2007). Although the demand for goat meat exceeds the supply (Casey *et al.*, 2003), the benefits from scientific advances made in molecular biology have not been realized well in goats compared to other livestock species (Shrestha & Fahmy, 2005). Research over last two decades attempted to characterize goat meat, and differentiate it from sheep meat, based on quality attributes and chemical composition. Babiker *et al.* (1990) observed that goat meat was darker red in color and had higher sarcoplasmic protein content than sheep meat. Furthermore, several researchers documented that color attributes of goat meat are significantly different from sheep meat (Sheridan *et al.*, 2003; Webb *et al.*, 2005; Lee *et al.*, 2008), indicating variation in myoglobin (Mb) chemistry.

Livestock Mb is comprised of 153 amino acids (www.expasy.org); and the primary structure of Mb depends up on species. Despite the differences in the amino acid sequence, many structural and functional properties of Mb are conserved across livestock species. In the post-genomic era, protein databases have been developed for economically important livestock species across the globe. A close look at the protein databases revealed that the primary structure of goat Mb has not been determined. On the other hand, myoglobins from meat animals such as cattle, pig, horse, sheep, red deer, and water buffalo are well characterized (www.expasy.org). In this perspective, investigations were not undertaken to characterize goat Mb. Therefore, our objective was to determine the molecular mass and amino acid sequence of goat Mb.

Materials and methods

Goat Mb was isolated from hearts obtained from goats harvested at the University of Kentucky's USDA-inspected meat laboratory. Mb was purified via ammonium sulfate precipitation and gel-filtration chromatography (Faustman & Phillips, 2001). Purified goat Mb preparation, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970), was free from any hemoglobin contamination. Goat Mb appeared as 17 kDa protein band in Coomassie-stained gels. Prior to mass spectrometric analyses Mb sample were transferred to 20 mM ammonium bicarbonate buffer using a PD-10 desalting column to remove inorganic salts.

Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry ((MALDI-TOF MS) was used to determine exact molecular mass of goat Mb, and compare it with the molecular mass of sheep Mb (Suman *et al.*, 2007). Protein molecular ions were analyzed in linear, positive ion mode using 4800 MALDI TOF-TOF mass spectrometer (Applied Biosystems, Foster City, CA) using an acceleration voltage of 2.2 KV. The resulting spectra (from 1,000 laser shots) were averaged, analyzed, noise-smoothed, baseline-corrected and mass-calibrated. Purified goat Mb sample was subjected to N-terminal sequencing by Edman degradation in a Procise 494 Sequencing system (Applied Biosystems, Foster City, CA), to determine the amino acid sequence from the amino terminal. The information on N-terminal amino acid sequence was used to obtain the sequence similarity with sheep Mb (www.expasy.org).

Results and discussion

The potential of MS tools to determine exact molecular mass of globin polypeptide chain has been successfully utilized to differentiate heme pigments from farm animals (pig, cattle, sheep and horse) and for meat species identification (Taylor *et al.*, 1993). MALDI-TOF MS analyses of intact myoglobins revealed that the molecular mass of goat Mb was four Daltons greater than sheep Mb. Molecular mass (*m/z*) of goat Mb was 16938 Da, whereas that of sheep Mb was 16934 Da. The molecular mass of sheep Mb observed in the present study was close to the reported values (www.expasy.org; Ponce-Alquicira & Taylor, 2000). On their investigations to differentiate livestock myoglobins based on MS analyses, Ponce-Alquicira & Taylor (2000) reported a mass difference of five Daltons between beef and horse myoglobins, which share 88% sequence similarity. Furthermore, these researchers demonstrated that the observed difference in molecular mass was sufficient to distinguish livestock myoglobins based on MS analysis. In the present study, the observed mass difference of four Daltons between the myoglobins of two small ruminants (sheep and goat) indicated differences in amino acid sequences. In agreement, a mass difference of 86 Daltons between water buffalo and beef myoglobins, evidenced in MS analyses, accounted for variation in primary structures (Dosi *et al.*, 2006).

Automated Edman degradation of goat Mb yielded the sequence of first forty amino acids from the amino terminal (Figure 1). Comparison of this sequence with the corresponding segment in sheep Mb (Primary accession number P02190; Han *et al.*, 1972) revealed that the amino acid at position 8 was different. Sequence alignment analysis revealed that the similarity between sheep and goat Mb on the first forty amino acids is 98%.

Residue No.	10	20	30	40
Goat Mb	GLSDGEWTLV	LNAWGKVEAD	VAGHGQEVLI	RLFTGHPETL
Sheep Mb	GLSDGEWQLV	LNAWGKVEAD	VAGHGQEVLI	RLFTGHPETL

Figure 1. Comparison of first forty amino acids from the N-terminal of goat and sheep myoglobins. Difference in amino acid sequence at position 8 is underlined.

Similarly, sequence differences between buffalo and beef Mb at three positions, viz. 19, 117, and 141, which accounted for the mass difference (86 Daltons), was determined employing Edman degradation (Dosi *et al.*, 2006). Although the homology between water buffalo and beef myoglobins was 98%, these authors concluded that the presence of negatively charged residues (ALAbov117ASPbuf in helix G and ALAbov19THRbuf in helix A) could lead to destabilization of the buffalo Mb helices and subsequent rapid discoloration in buffalo meat.

The primary structure of Mb dictates its tertiary structure, which in turn influences the protein's interactions with ligands and macro-molecules, and ultimately impacts color. It is well documented that differences in Mb sequence influence redox stability as well as fresh meat color via mechanisms such as autoxidation (Gutzke & Trout, 2002) and interaction with lipid oxidation (Suman *et al.*, 2007). Observed differences in Mb sequence (THRgoat8GLNsheep) may, in part, explain the variation in color traits between sheep and goat meats.

Conclusions

This multi-disciplinary study is the first to characterize and sequence goat Mb. Analysis of partial amino acid sequence (first forty residues) indicated that goat Mb shared high similarity with sheep Mb, albeit difference at position 8 (THRgoat8GLNsheep). Currently, proteomic investigations are underway to determine the complete sequence of goat Mb through MS analyses of tryptic- and cyanogen bromide-peptides.

Acknowledgments

This work was supported by funds from the Kentucky Agricultural Experiment Station, University of Kentucky. The mass spectrometric analysis was performed at the University of Kentucky's Center for Structural Biology Protein Core Facility, supported in part by funds from National Institute of Health's National Center for Research Resources grant P20 RR020171.

References

Alexandre, G. & Mandonnet, N., 2005. Goat meat production in harsh environments. Small Rumin. Res. 60, 53-66.

Babiker, S. A., El Khider, I. A. & Shafie, S. A., 1990. Chemical composition and quality attributes of goat meat and lamb. Meat Sci. 28, 273–277.

Casey, N. H., Van Niekerk, W. A. & Webb, E. C., 2003. Goat meat. In: Encyclopedia of Food Sciences and Nutrition. Eds. Caballero, B., Trugo, L., & Finglass, P. Academic Press, London, pp. 2937–2944.

Dosi, R., Di Maro, A., Chambery, A., Colonna, G., Costantini, S., Geraci, G. & Parente, A., 2006. Characterization and kinetics studies of water buffalo (*Bubalus bubalis*) myoglobin. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 145, 230-238.

FAO., 2007. http://faostat.fao.org

Faustman, C. & Phillips, A. L., 2001. Measurement of discoloration in fresh meat. In: Current Protocol in Food Analytical Chemistry; Wiley and Sons, New York. Ch. F3 Unit F3.3.

Gutzke, D. & Trout, G. R., 2002. Temperature and pH dependence of the autoxidation rate of bovine, ovine, porcine, and cervine oxymyoglobin isolated from three different muscles-longissimus dorsi, gluteus medius, and biceps femoris. J. Agric. Food. Chem. 50, 2673-2678.

Han, K. K., Tetaert, D., Moschetto, Y., Dautrevaux, M. & Kopeyan, C., 1972. The covalent structure of sheep-heart myoglobin. Eur. J. Biochem. 27, 585-592.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature, 227, 680–685.

Lee, J. H., Kannan, G., Eega, K. R., Kouakou, B. & Getz, W. R., 2008. Nutritional and quality characteristics of meat from goats and lambs finished under identical dietary regime. Small Rumin. Res. 74, 255-259.

Ponce-Alquicira, E. & Taylor. A. J. 2000. Extraction and ESI–CID–MS/MS analysis of myoglobins from different meat species. Food Chem. 69, 81-86.

Sheridan, R., Hoffman, L. C. & Ferreira, A. V., 2003. Meat quality of Boer goat kids and mutton Merino lambs. 2. Sensory meat evaluation. Anim. Sci. 76, 73–79.

Shrestha, J. N. B. & Fahmy, M. H., 2005. Breeding goats for meat production: a review: 1. Genetic resources, management and breed evaluation. Small Rumin. Res. 58, 93-106.

Suman, S. P., Faustman, C., Stamer, S. L. & Liebler, D .C., 2007. Proteomics of lipid oxidation-induced oxidation in porcine and bovine oxymyoglobins. Proteomics. 7, 628-640.

Taylor. A. J., Linforth, R.S., Weir, O., Hutton, T. & Green, B., 1993. Potential of electrospray mass spectrometry for meat pigment identification. Meat Sci. 33, 75-83.

Webb, E. C., Casey, N. H. & Simela, L., 2005. Goat meat quality. Small Rumin. Res. 60, 153-166.