DIFFERENCE IN DEGRADATION OF TROPONIN-T ISOFORMS AMONG BOVINE MUSCLES DURING POSTMORTEM AGING

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Background

The beef tenderization during postmortem aging, largely affected by protein degradation, differs among the muscles (Olson *et al.*, 1976; Koohmaraie *et al.*, 1988). Though the activities of Ca^{2+}-dependent protease (calpains) and the inhibitor (calpastatin) vary among different type muscles, the ratio of calpain/calpastatin does not seem to be different among the muscles examined (Olson *et al.*, 1977; Koohmaraie *et al.*, 1988; Whipple and Koohmaraie, 1992). Each muscle has specific composition of four muscle fiber types which are determined by expression pattern of myofibrillar contractile protein isoforms, such as myosin heavy chains (MyHCs) (Schiaffino and Reggiani, 1996). The extent and rate of postmortem degradation of myofibrillar proteins are presumptively affected by muscle fiber type. Olson *et al.* (1976) and Whipple and Koohmaraie (1992) reported that degradation of myofibril proteins occurs faster in bovine white (faster type) muscles (longissimus, biceps femoris, semitendinosus, or gluteus medius) than in the red (slower type) muscles (psoas major or supraspinatus). In a detail investigation of pork aging by Christensen *et al.* (2004), the extent and rate of troponin T (TnT) and desmin degradation did not depend solely on muscle fiber type determined by MyHC ATPase histochemistry. Different type muscles have different isoform composition of myofibrillar contractile proteins. The degradation pattern may be changed by the type of the isoforms, because the cleavage pattern of the isoform proteins are thought to differ, depending on the molecular structures. Actually, four recombinant isoforms of rabbit fast type TnT (fTnT) are degraded by calpain at different rates in vitro (Gomes *et al.*, 2001). Furthermore, we found that fast and slow type TnT isoforms are differently degraded in postmortem bovine LT muscle (Muroya *et al.*, 2004).

Objectives

The purpose of this study is to clarify the relationship among protein degradation patterns of muscles and the isoforms types of the protein substrates. To this end, we investigated the difference in degradation pattern of TnT isoforms among muscles with different isoform composition during beef aging.

Materials and methods

The lingual (TN), masseter (MS), diaphragm (DP), longissimus thoracis (LT) muscles for 0 d postmortem samples were excised from a Japanese black steer within 1 hr after slaughter. After overnight hanging of the carcass at 2°C, the muscle blocks were excised from the carcass. At 1, 5, and 14 d postmortem, the muscle samples were prepared from the central part of the block bagged during aging at 2°C. For western blotting analysis, myofibrils were extracted from the muscles by 0.1 M potassium phosphate buffer (pH 7.4) with 0.6 M KCl. The myofibrils were applied to SDS-PAGE using 12.5% or 15% gel, followed by transfer to polyvinylidenefluoride membrane. In the western blotting, the TnT bands were detected by using commercially-available anti fast- or slow-type polyclonal antibodies for TnT (Santa Cruz Biotechnology, Santa Cruz, CA).

Results and discussion

According to our previous analysis investigating MyHC and TnT isoform expression in bovine skeletal muscles by RT-PCR, MS and DP expressed slower type MyHC and TnT isoforms, while TN and LT expressed the faster type isoforms (Muroya *et al.*, 2002; Muroya *et al.*, 2003). According to the MyHC isoform expression pattern, TN is characterised to be slower than LT. Especially, MS exclusively expressed the slow type isoform mRNAs, which agrees with the present results of isoform-specific TnT western blotting (Figs. 1 and 2).

Eight fast- and two slow-type TnT (sTnT) isoform mRNAs were detected by RT-PCR, and the distribution of the isoforms varies among the muscles (Muroya *et al.*, 2003). The fTnT isoforms are classified into four types in SDS-PAGE by the amino acid numbers, or approximate molecular weight. The four fTnT isoform types were detected in bovine TN, MS, DP, and LT muscles, with different distribution among the muscles (Fig. 1). The DP and TN muscles expressed two sTnT isoforms, while MS and LT muscles expressed only a higher molecular weight one (Fig. 2), as we detected by RT-PCR previously.

The degradation pattern of LT muscle revealed that fTnT and sTnT isoforms are degraded differently from each other (Figs. 1 and 2). On the other hand, even though two high molecular weight fTnT and one sTnT isoforms in DP and TN muscles were the same as those expressed in LT, all of the isoforms in DP and TN did not appear to be degraded. The sTnT isoform in MS muscle appeared to be slightly degraded.

The results revealed that postmortem TnT degradation in beef varies depending on the muscle type. The degradation was observed only in LT muscle that has faster contractile properties than MS, DP, and TN muscles, suggesting that TnT proteins are degraded more easily in faster muscles than in slower muscles. This is consistent with the previous results reported by Olson *et al.* (1976) and Whipple and Koohmaraie (1992). There might be differences in activity of calpain or calpastatin and in some metabolic effect among the muscles examined. Though fTnT and sTnT isoforms are differently degraded, the difference in the rate and extent of TnT degradation among fTnT or sTnT isoforms during postmortem aging in beef is still unknown.

Conclusions

The results revealed that postmortem TnT degradation in beef differs depending on the muscle type. The degradation was observed only in LT muscle that has faster contractile properties than MS, DP, and TN muscles, suggesting that TnT proteins are degraded more easily in faster muscles than in slower muscles.

References

Christensen, M., Henckel, P., and Purslow, P. P. 2004. Effect of muscle type on the rate of post-mortem proteolysis in pigs. Meat Sci. 66:595-601.

Gomes, A. V., Guzman, G., and Potter, J. D. 2001. Degradation of fast skeletal troponin T. FASEB J. 15:A533.

Koohmaraie, M., Seideman, S. C., Schollmeyer, J. E., Dutson, T. R., and Babiker, A. S. 1988. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407-410.

Muroya, S., Nakajima, I., and Chikuni, K. 2002. Related expression of MyoD and Myf5 with myosin heavy chain isoform types in bovine adult skeletal muscles. Zool. Sci. 19:755-761.

Muroya, S., Nakajima, I., and Chikuni, K. 2003. Amino acid sequences of multiple fast and slow troponin T isoforms expressed in adult bovine muscles. J. Anim. Sci. 81:1185-1192.

Muroya, S., Kitamura, S., Tanabe, S., Nishimura, T., Nakajima, I., and Chikuni, K. 2004. N-terminal amino acid sequences of troponin T fragments, including 30 kDa one, produced during postmortem aging of bovine longissimus muscle. Meat Sci. 67:19-24.

Olson, D. G., Parrish, F. C. Jr., Dayton, W. R., and Goll, D. E. 1977. Effect of postmortem storage calcium activated factor on the myofibrillar proteins of bovine skeletal muscles. J. Food Sci. 42:117-124.

Olson, D. G., Parrish, F. C. Jr., and Stromer, S. H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sci. 41:1036-1041.

Schiaffino, S. and Reggiani, C. 1996. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. 76:371-423.

Whipple, G. and Koohmaraie, M. 1992. Effects of lamb age, muscle type, and 24-hour activity of endogenous proteinases on postmortem proteolysis. J. Anim. Sci. 70:798-804.

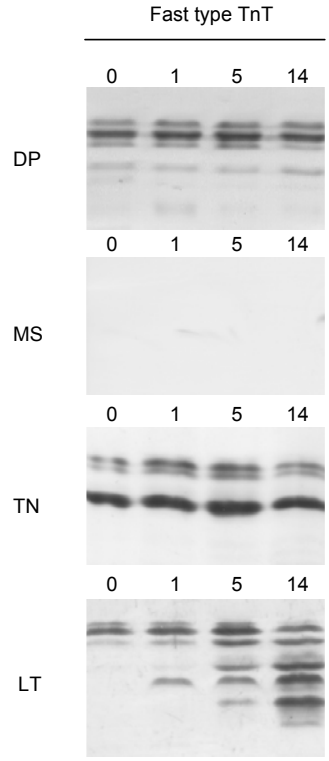

Fig. 1. Postmortem degradation pattern of fast type troponin T isoforms. Numbers indicate day postmortem. TN: lingual muscles, MS: masseter, DP: diaphragm, LT: longissimus thoracis.

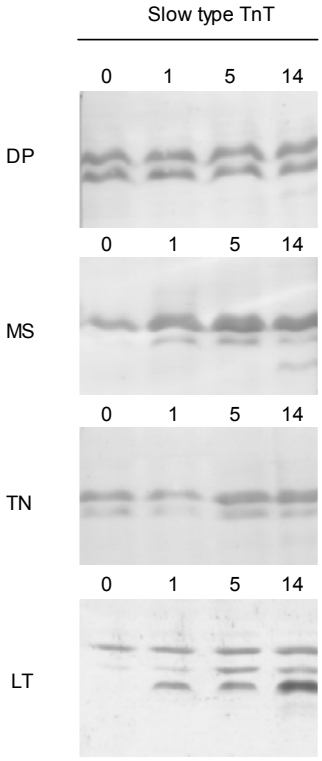

Fig. 2. Postmortem degradation pattern of slow type troponin T isoforms. Numbers indicate day postmortem. TN: lingual muscles, MS: masseter, DP: diaphragm, LT: longissimus thoracis.