PE1.27 Postmortem myofibril protein degradation differs in type I and IIb porcine muscle fibers
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Abstract— Muscle fiber type is thought to be one of the factors affecting meat quality. In the present study we investigated differences in postmortem degradation of desmin and troponin T (TnT) isoforms, between different muscle fiber types. Porcine muscle fibers at day 1, 3, and 6 postmortem were sorted by immunochemical myosin heavy chain (MyHC) isoform typing. The typed muscle fibers pooled separately for each type were applied to western blotting analysis. The results showed that desmin was partly degraded at day 1 postmortem in both type I and IIb fibers, but the degradation rate was faster in type IIb than in type I fibers. Fast type TnT (fTnT) isoform was also partly degraded in type IIb fibers at day 1 but not in type I fibers, indicating rapid degradation in type IIb fibers. On the other hand, slow type TnT (sTnT) isoforms appeared to be degraded slower than fTnT in type I fibers and degradation were not detected in type IIb fibers. These results indicate that protein degradation is more pronounced in type IIb than in type I fibers. In conclusion, muscle fiber type certainly affects the rate and extent of postmortem proteolysis of desmin and troponin T. The fiber type specific differences could explain the difference in postmortem tenderization among muscles with different composition of type I and IIb fibers.

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***Index Terms*— muscle fiber type; isoform; proteolysis; postmortem aging**

I. INTRODUCTION

TENDERIZATION has consistently been shown to occur faster in *longissimus* muscle than in other muscles of cattle and ovine breeds [1, 2, 3]. In agreement, *Longissimus* muscle revealed a faster degradation of myofibrillar proteins compared to other muscles of rabbit, lamb, porcine, and bovine [2, 4-9]. To acquire a better understanding of intermuscular diversity in postmortem tenderization, numerous studies have focused on differences in proteolysis among different muscle types (red or white muscles). Muscle specific properties originate from the composition of mainly three types of muscle fibers, type I (slow oxidative), type IIA (fast oxidative-glycolytic), and type IIB (fast glycolytic) [10]. Tenderness of bovine and porcine *longissimus lumborum* (LL) has been found both to correlate positively and in some cases negatively with the proportion of type I fibers. These contradictory results might partly be explained by occasional inconsistence of fiber classification [11], however, the effect of muscle fiber type in postmortem protein degradation is potentially significant for eating quality of meat. A recent study showed that the breaking strength of porcine type I and type IIb single muscle fibers differed already at day 1 postmortem and the rate of changes from day 1 to 8 postmortem also differed between fiber types [12]. Using molecular typing of myosin heavy chain (MyHC) isoform with in situ hybridization technique on *longissimus dorsi* (LD) in various pig breeds, results showed that a higher proportion of type IIb fibers was related to less favorable quality traits, although no consistent correlation was found within muscles and breeds [13]. On the other hand, muscles with different composition of the fiber types or contractile protein isoforms not only showed different degradation rates of myofibrillar proteins, like desmin and troponin T (TnT) [4] but also indicated difference in proteolytic susceptibility between the TnT isoform types [6]. To determine the effect of muscle fiber type on postmortem muscle proteolysis we analyzed myofibrillar protein degradation in type I and IIb fibers.

II. MATERIALS AND METHODS

Muscle samples were collected from a Danish pig with carcass weight of 80 kg, obtained from a local slaughter house. Sampling was made at 1, 3 and 6 days post-mortem from the *M. longissimus dorsi* (LD).Samples were frozen in liquid nitrogen and stored at -80ºC until further analysis. Isolation of muscle fibers was performed according to the method described by Christensen et al. [13]. Briefly

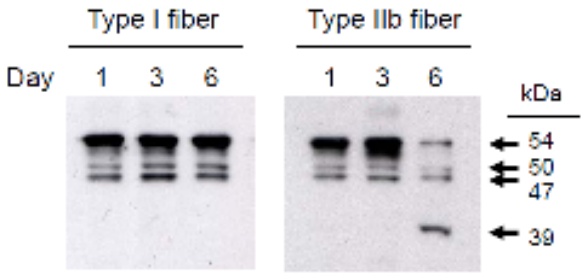

Figure 1. Difference in postmortem desmin degradation between type I and IIb muscle fibers.

small muscle slices were cut with a scalpel from the frozen LD muscle, and transferred to ice-cold dissection buffer (50 mM MES; pH 5.6; 100 mM KCl, 280 mM Mannitol and 0.2 mM EGTA). Single muscle fibers were isolated under a stereoscope. The isolated fibers (3-12 mm) were dissolved in a sample buffer for SDS-PAGE (4 M urea, 1 M thiourea, 25 mM Tris(hydroxymethyl)-aminomethanes, pH 6.8, 1.5% SDS). The isolated fibers were classified into the types I, IIa, and IIb, using MyHC isoform-specific antibodies (MHC-2042, MHC-2043 and MHC-2047; American Type Cell Culture, Manassas, USA) according to the method of Christensen et al. [13]. The secondary antibody (horseradish peroxidase (HRP)-conjugated anti-mouse IgG) was applied. Spots of MyHC isoforms on the polyvinylidene fluoride (PVDF) membrane were visualized using 3,3'-diaminobentizine (DAB). Myofibrillar proteins were separated by SDS-PAGE on 10% Bis-Tris gel with MOPS buffer (Invitrogen, Carlsbad, CA, USA). The proteins of pooled fibers were loaded onto the gel at a protein amount corresponding to the same summed length of fibers of approx. 7.5 mm per lane, and then the proteins were electrically transferred to PVDF membranes (Invitrogen). Desmin and its degradation products were separated by electrophoresis on 10% Bis-Tris gels, transferred to PVDF membranes and incubated in primary mouse anti-desmin antibody [4]. The membrane was then incubated with avidin-conjugated HRP (Sigma-Aldrich Corporation, MO, USA) diluted in TBS-T (1:5,000) for 30 min. After chemiluminescent reaction in the final incubation of the membrane (ECL plus kit; GE Healthcare), desmin bands were detected on HyperFilm ECL (GE Healthcare). fTnT and sTnT bands were detected using anti-fTnT (Santa Cruz Biotechnology) and -sTnT (Santa Cruz Biotechnology) polyclonal goat antibodies according to the method of Muroya et al. [6, 14]. TnT bands on the membrane were finally visualized with DAB.

III.RESULTS AND DISCUSSION

Desmin degradation was faster in type IIb fibers than in type I fibers (Figure 1)**.** At day 1 postmortem, intact desmin (54 kDa) as well as the two degradation products of desmin with molecular weight around 50- and 47-kDa were present in type IIb fibers, while the degradation bands only appeared faint in type I fibers. At day 3, both 50- and 47-kDa degradation products were detected in type I fibers. At day 6, these degradation products appeared to increase in type I fibers. Desmin was degraded faster in type IIb fibers than in type I fibers, as clearly indicated by the presence of an additional 39 kDa desmin fragment and a reduction in the content of intact desmin, and of the 50 and 47 kDa degradation products at 6 days postmortem in type IIb fibers.

fTnT isoforms were degraded faster in type IIb fibers than in type I fibers. Intact fTnT isoforms with molecular weight of 38, 37, and 34 kDa were detected in both type I and IIb fibers at day 1 postmortem. Degradation products with molecular weights of 31, 29, and 28 kDa appeared in type IIb fibers at day 1. In type I fibers these three degradation products could not be detected before day 3. Thus, fTnT took place earlier and more rapidly in type IIb fibers than in type I fibers, and the extent of fTnT degradation until day 6 was greater in type IIb fibers than in type I fibers. Total amount of fTnT-related polypeptides was apparently higher in type IIb fibers than in type I fibers.

Slow type TnT isoforms was not detected in type IIb fibers and was degraded to only a small extent in type I fibers. In type I fibers, three types of sTnT isoforms, 37, 36, and 34 kDa, were detected with the western blot analysis. The degradation products of these isoforms were not detected until day 6 postmortem when there was only a small amount of 31- and 28-kDa degradation products in type I fibers. No intact sTnT isoforms or degradation products were detected in type IIb fibers. Our results indicate that sTnT isoforms were degraded only in the late phase of aging and with a much lower extent than fTnT isoforms.

Discussion: Various factors are expected to affect the differences in proteolysis between fiber types observed in our study. Some of those factors are direct: fiber type specific activity of proteolytic systems (1); intrinsic proteolytic susceptibility of each protein isoforms (2); and some of them are

indirect, like metabolic factors affecting protease activity and conformation of proteins. The expression and activity of μ- and m-calpains and calpastatin are different among different skeletal muscles [2, 15, 16]. Muscle content of MyHC-slow (MyHC-type I), reflecting proportion of type I fiber, has been found to correlate positively with calpastatin content and activity independently of muscle type, but did not correlate with muscle tenderness [16]. Probably μ- and m-calpain and calpastatin activities are different between fiber types and those differences could explain our results, even though no clear effects on calpain proteolytic activity postmortem are evidenced in relation to fiber types. Skeletal muscles consist of various fiber types, composed of different functional protein isoforms, in relation to muscle functions and to the speed of contraction. The rate and extent of postmortem muscle proteolysis, therefore, depends not only on protease activity but also on individual susceptibility of the substrate. The present results clearly revealed that fTnT isoforms are more susceptible to proteolysis than the sTnT. Type IIb fibers seemed to have higher content of fTnT isoforms than sTnT, while type I fibers showed an opposite pattern. Therefore we could suggest that type IIb fibers are subjected to higher degradation of TnT proteins, and a possible higher protease activity. Recently Christensen et al. [12] found that breaking strain and breaking stress values of type I fibers isolated from porcine LD muscle at day 1 postmortem was higher than those of type IIb fibers. This finding indicated that initial mechanical strength is greater in type I fibers than in type IIb, suggesting that type IIb fibers could have been weakened during the first 24 h of aging. The differences in mechanical properties previously reported are consistent to the present results. It could be speculated that the earlier degradation of fTnT during 24 h after slaughter might have a significant influence on initial weakening of type IIb fibers.

IV. Conclusion

Postmortem degradation of desmin and fTnT isoforms progressed faster in type IIb than in type I fibers, indicating that muscle fiber type affects the rate and extent of degradation of myofibrillar proteins. Moreover, the results showed that fTnT isoforms are degraded faster than sTnT isoforms in postmortem type I fibers, and that these TnT isoforms have different susceptibility to proteolysis.

References

[1] Koohmaraie, M., Seideman, S. C., Shollmeyer, J. E., Dutson, T. R., & Babiker, A. S. (1988). Factors associated with the tenderness of three bovine muscles. Journal of Food Science, 53, 407–410.

[2] Ilian, M. A., Morton, J. D., Kent, M. P., Le Couteur, C. E., Hickford, J., Cowley, R., & Bickerstaffe, R. (2001). Intermuscular variation in tenderness: Association with the ubiquitous and muscle-specific calpains. Journal of Animal Science, 79, 122-132.

[3] Jurie, C., Picard, B., Hocquette, J.-F., Dransfield, E., Micol, D., & Listrat, A. (2007). Muscle and meat quality characteristics of Holstein and Salers cull cows. Meat Science, 77, 459–466.

[4] Christensen, M., Henckel, P., & Purslow, P. P. (2004). Effect of muscle type on the rate of post-mortem proteolysis in pigs. Meat Science, 66, 595–601.

[5] Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science, 82, 1195-1205.

[6] Muroya, S., Nakajima, I., Oe, M., & Chikuni, K. (2006). Difference in postmortem degradation pattern among troponin T isoforms expressed in bovine longissimus, diaphragm, and masseter muscles. Meat Science, 72, 245–251.

[7] Prates, J. A. M., Ribeiro, A. M. R., & Correia, A. A. D. (2001). Role of cysteine endopeptidases (EC 3.4.22) in rabbit meat tenderisation and some related changes. Meat Science, 57, 283-290.

[8] Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78, 958-965.

[9] Whipple, G. & Koohmaraie, M. (1992). Effects of lamb age, muscle type, and 24-hour activity of endogenous proteinases on postmortem proteolysis. Journal of Animal Science, 70, 798–804.

[10] Karlsson, A.H., Klont,R. E., & Fernandez, X. (1999). Skeletal muscle fibres as factors for pork quality. Livestock Production Science, 60, 255–269.

[11] Klont, R. E., Brocks, L., & Eikelenboom, G. (1998). Muscle fibre type and meat quality. Meat Science, 49, S219-S229.

[12] Christensen, M., Kok, C., & Ertbjerg, P. (2006). Mechanical properties of type I and type IIB single porcine muscle fibres. Meat Science, 73, 422–425.

[13] Chang, K. C., da Costa, N., Blackley, R., Southwood, O., Evans, G., Plastow, G., Wood, J. D., & Richardson, R. I. (2003). Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. Meat Science, 64, 93–103.

[14] Muroya, S., Kitamura, S., Tanabe, S., Nishimura, T., Nakajima, I., & 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 Science, 67, 19–24.

[15] Ouali, A. & Talmant, A. (1990). Calpains and calpastatin distribution in bovine , porcine, and ovine skeletal muscles. Meat Science, 28, 331-348.

[16] Sazili, A.Q., Parr, T., Sensky, P.L., Jones, S.W., Bardsley, R.G., & Buttery, P.J. (2005). The relationship between slow and fast myosin heavy chain content, calpastatin and meat tenderness in different ovine skeletal muscles. Meat Science, 69, 17-25.