P-10-11

Plectin Degradation In Postmortem Pork (#464)

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Introduction

Previous studies have shown that degradations of key muscle proteins could contribute to the quality improvement of fresh meat [1-3]. Plectin, a well-known member of plakin family, has been reported as an intermediate filaments (IFs) based versatile cytolinker protein [4]. Plectin is competent to link many cytoskeletal elements especially IFs via the universal IF binding properties [5] and anchor them to specific cytoskeletal structures and cytoplasmic organelles by a unique isoform-specific targeting ability [4]. Therefore, plectin has been empowered a great influence in sustaining and strengthening the integrity of cytoarchitecture [5-7]. However, no studies have been published about the postmortem changes of plectin in meat. Thus, the current study focused on investigating the degradation of pork plectin during postmortem aging.

Methods

The longissimus muscle (LM) from a total of 12 pig carcasses was aged at 4°C for 0 h, 6 h, 12 h, 1 d, 3 d, 7 d and 13 d under vacuum package. Samples subjected to inhibitor were cut into small pieces (0.2 g/chop), submerged in working/control solutions at the ratio of 1:1 (wt/vol), shipped on ice and stored at 4°C for 1 d, 3 d, and 7 d. Application of calpain I and II inhibitor MDL-28170 was performed as described by Walko et al [8] with slight modification. The inhibitor was dissolved in 100% dimethyl sulfoxide (DMSO) to prepare a 25 mg/ml stocking solution following by the dilution with 0.01M PBS to obtain a 100 μM working solution. The control solution was prepared similarly without inhibitor reagent. Statistical analysis for comparison in each group was performed by a one-way analysis of variance (ANOVA) and differences among individual means were assessed by Duncan's multiple-range tests.

Results

In the present study, plectin was found to be significantly degraded during postmortem aging. As shown in Fig. 1, the amount of intact plectin was rapidly reduced at the early postmortem aging ($P < 0.05$) and almost disappeared at d 3. Meanwhile, the degraded plectin under the intact band emerged at 0 h and accumulated fast during the first 3 d aging ($P < 0.05$). However, the amount of this degraded product reduced between d 3 and d 13 ($P < 0.05$) indicating a further degradation of plectin during later stage of postmortem aging.

Comparing the control group (CG) and the calpain inhibitor treated group (CIG), immunoblotting showed pronounced larger content of intact plectin in CIG samples (Fig. 2) indicating that the degradation was inhibited in great degree by MDL-28170. The intact plectin intensity still reduced in CIG suggesting that MDL-28170 decreased the rate of plectin degradation without completely stopping the plectin degradation. In terms of the degraded plectin, significant differences were also detected ($P < 0.05$). CIG had more degraded plectin at d 1 and d 3, while less than CG at d 7 ($P < 0.05$). In addition, the degraded plectin in CIG turned to be greater from d 1 to d 7, while decreased in the control group, corresponding to the expression of degraded plectin before and after d 3. Double immunostaining of μ-calpain and plectin was performed which exhibited a highly consistent distribution along with Z-discs at both 0 h (Fig. 3 A and B) and 3 d (Fig. 3 C and D) of postmortem aging indicating the possible interactions of μ-calpain with both intact plectin and the degraded plectin product.

Conclusion

The current study showed that plectin experienced a prominent and prompt degradation in pork during postmortem aging. The degradated plectin product could be further cleaved after 3 d aging. The degradation of plectin was remarkably inhibited by calpain inhibitor. The colocalization of plectin and μ-calpain further supported the potential role of μ-calpain in the degradation of pork plectin.

Notes

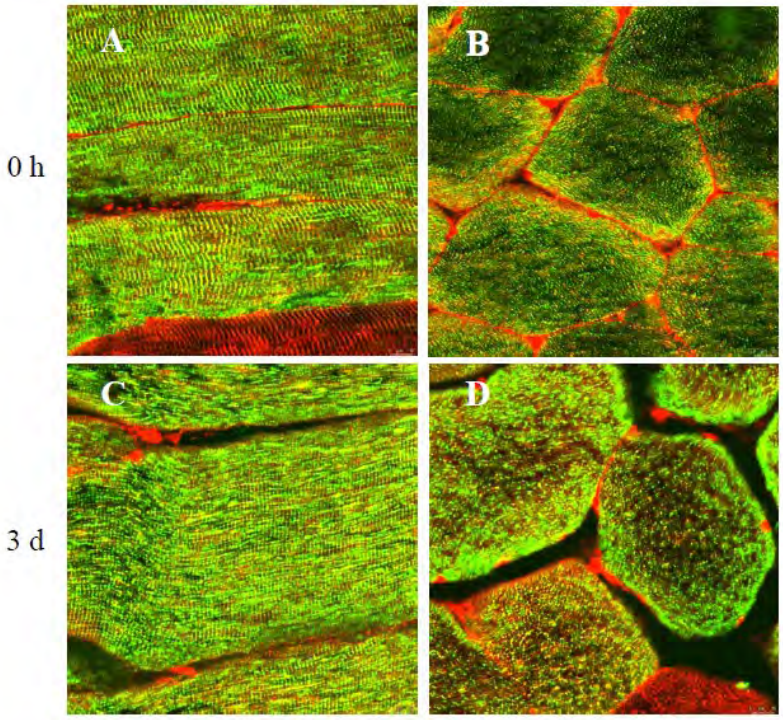

Figure 3. Double immunofluorescence labeling of plectin and μ-calpain in pork at 0 h and 3 d
Sections were stained with μ-capain (red) and plectin (green) recognizing all plectin isoforms at 0 h (A and B) and 3 d (C and D). Overlaps (yellow) of μ-capain (red) and plectin indicated the colocalization.

Notes

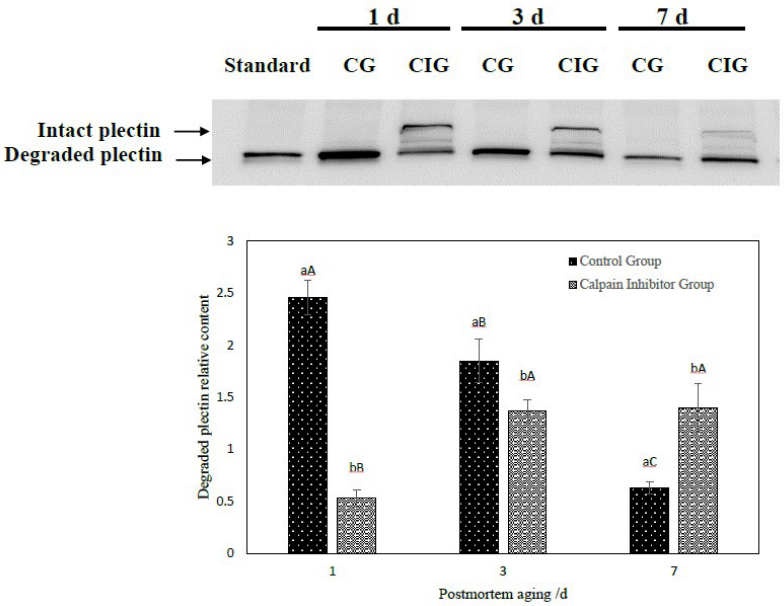

Figure 2. Western blot analysis of plectin expression after incubating with calpain inhibitor

The 50 μg total protein samples were loaded per lane. Standard was one of samples in postmortem muscle with clear and stable band and was loaded onto each gel. All measurements were expressed as the mean ± SE. Means with a, b and A, B, C superscripts differ significantly at the same storage time and the same solution ($P < 0.05$, $n = 12$).

Notes

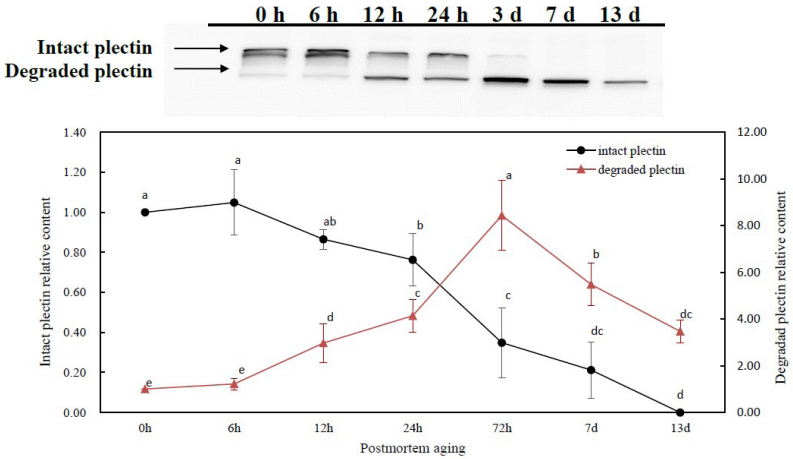

Figure 1. Western blot analysis of plectin expression in postmortem pork

The 50 μg of total protein samples were loaded per lane. The relative contents of plectin at different time points were expressed as the ratio to intact/degraded plectin at 0 h postmortem, respectively. All measurements were expressed as the mean ± SE. Means with different superscripts differ significantly ($P < 0.05$, $n = 12$).

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