P-10-12

Calpain-2 is proteolytic active following calcium-induced binding to myofibrils (#478)

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Introduction

The calpain system is believed to play an important role in meat tenderization, but the regulation mechanism is not very clear. It has been reported that calpain-1 associates to and binds to myofibrils postmortem in beef [1] and lamb muscle, but the activity of bound calpain measured by ^{14}C labeled casein assay was very low [2]. The binding process of calpains to myofibrils and the link to meat tenderization is currently not understood. The aim of this study was to determine the effect of calcium on calpain-2 binding to myofibrils and how this process affects calpain activity as evaluated by desmin degradation.

Methods

Myofibrils and calpain-2 were isolated from 12 h postmortem pork longissimus muscle as described by Zeng *et al.* [3]. To study the calpain-2 binding process, myofibrils were incubated with calpain-2 for 5 min, then 1 mM EGTA or 5 mM Ca^{2+} was added. Following centrifugation to sediment myofibrils, the activity of calpain-2 in supernatant was measured according to Zeng *et al.* [3]. Myofibril-bound calpain-2 activity was studied by observing desmin degradation. Myofibrils were washed to remove unbound calpain-2 and then incubated with 5 mM Ca^{2+} for 0, 0.5, 1 and 2 h. Western blot against desmin was conducted according to Liu *et al.* [4]. Each analysis was performed in triplicate. The significant differences between means (significance was defined at $P < 0.05$) were evaluated by Tukey HSD test by the IBM SPSS Statistics 24 software.

Results

Calpain-2 was isolated and mixed with myofibrils. Following addition of 5 mM Ca^{2+} a major fraction of calpain-2 became bound to myofibrils, whereas calpain-2 did not bind to myofibrils in the absence of Ca^{2+} (Fig. 1). The bound calpain-2 was not removed from myofibrils by washing. The exogenous calpain-2 was thus tightly bound to myofibrils and it was proteolytic active as all the intact desmin was degraded following incubation at 25 °C for 2 h. In addition, endogenous desmin degrading enzymes, presumed to be calpain-1 or calpain-2, were still bound to myofibrils after several washes (Fig. 2). Also Zeng *et al.* [3] reported that endogenous myofibril-bound calpain-2 degraded desmin during incubation with Ca^{2+}. These results suggest that if the Ca^{2+} concentration during postmortem storage reach the level to autolyze calpain-2 then the enzyme would not only become bound to myofibrils (Fig. 1), but also remain active long enough to degrade myofibrillar substrates such as desmin (Fig. 2). In contrast, previous report on myofibril-bound calpain-1 suggested that the bound calpain-1 only has low proteolytic activity [2].

Conclusion

In vitro, in the presence of calcium, exogenously added calpain-2 binds to myofibrils and remains proteolytic active. These results provide new insight in the role of myofibril-bound calpain-activity during meat tenderization.

Acknowledgements

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References

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Notes

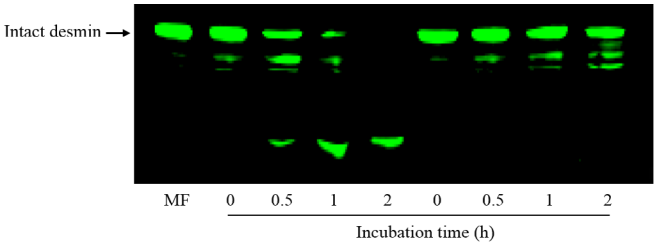

Fig. 2. Western blot of desmin after incubating myofibrils with Ca.
Myofibrils were incubated with 5 mM Ca^{2+} at 25 °C for 0, 0.5, 1, 2 h, respectively. Lane 1: Myofibrils (MF); lane 2-5: Exogenous myofibril-bound calpain-2 activity; lane 6-9: Endogenous myofibril-bound enzyme activity.

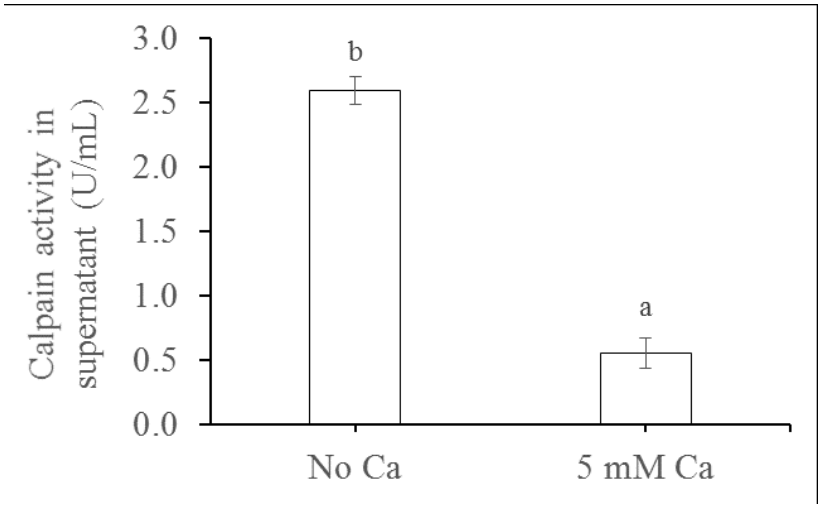

Fig. 1. Ca induces binding of calpain-2 to myofibrils.
Myofibrils and calpain-2 were mixed and 1 mM EGTA or 5 mM Ca^{2+} were added. After centrifugation, the calpain-2 activity remaining in the supernatant was determined. The value of activity was given as means ± standard deviation. Superscripts with different letters differ ($P < 0.05$).

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