CHANGES IN IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CATHEPSIN D IN MUSCLE INDUCED BY CONDITIONING AND HIGH-PRESSURE TREATMENT

Tomohito Kubo*, Atsushi Suzuki, Toshie Sugiyama*** and Tadayuki Nishiumi****

*Master's Program in Biosphere Science, Graduate School of Science and Technology, **Department of Applied Biological Chemistry, and ***Department of Agricultural Science, Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan

Key words: cathepsin D, localization of cathepsin D, meat tenderization, meat conditioning, high-pressure treatment

Background

It is well known that two kinds of proteinases , cathepsin and calpain, in the muscle have important roles on meat tenderization during postmortem conditioning. Among the catheptic proteinases, especially cathepsin D seems to be the most important to tenderize meat and to produce flavor-related compounds during conditioning because of its acidic optimum pH and the absence of its specific inhibitor in the muscle. However the cathepsin is entrapped in the lysosome and their participation in meat conditioning requires the liberation into cytosol. This release takes place during postmortem conditioning with the pH fall.

To reduce the conditioning period and improve meat tenderness, several processes including electrical stimulation, infusion of calcium chloride, acid marination etc. have been studied. High hydrostatic pressurization has been proposed as one of new technology to meat tenderization (Suzuki *et al.*,1992, 1998). This treatment accelerates the release of the catheptic enzymes from lysosome (Homma *et al.*, 1994; Jung *et al.*), resulting with the improvement of meat tenderness. The changes in the histochemical localization of cathepsin D during conditioning or high pressure treatment were not fully elucidated as compared with the biochemical works.

This paper describes the high-pressure effects on the localization of cathepsin D in the muscle by using immunoelectron microscopy, in comparison with those naturally observed in the conditioned muscle.

Materials and methods

Rabbit *L. dorsi* muscle was excised immediately after death and divided into two parts, one for high pressure treatment (100~400 MPa, 5min) and the other for conditioning at 2~4℃ for 168 hr. Pressurization of muscle was performed as described previously (Kim *et al.*, 1992). Antibody labeling was carried out according to the procedure described by Maruyama *et al.* (1985) with slight modifications. Muscle samples (1 x 1 x 2 mm^3) prepared from the pressurized and conditioned muscles fixed with 10% formaldehyde-PBS (20 mM phosphate, 150 mM KCl, pH.7.4) for 10 min at room temperature, washed with PBS and then treated with anti-cathepsin D mouse monoclonal antibody in PBS at 2 ℃ for 48 h. The unbound primary antibody was removed by extensive washing in PBS. The samples were then exposed to HRP-conjugated anti-mouse IgG 1:100 diluted with PBS at 4℃ for 48 h. After removing unbound secondary antibody by washing in PBS, the location of the cathepsin D was visualized by the reaction with HRP and H_2O_2 in deaminobenzidine solution, washed by PBS, and then fixed in 3% glutaraldehyde-0.12 M cacodylate, pH.7.4 at room temperature for 1.5 h. The samples were postfixed for 1.5 hr at 2℃ with 1.0% osmium tetraoxide-0.12 M cacodylate, pH.7.4, dehydrated with grading alcohol and embedded in Epon. The specimens were examined with Philips EM 280 S electron microscope operated at 70kV.

Results and discussion

Immunoelectron micrographs showing the localization of cathepsin D in muscle during conditioning are shown in Fig.1. The anti-cathepsin D monoclonal antibody strongly labeled the granules between the myofibrils, which supposed to be lysosome. The liberation of cathepsin D entrapped in the lysosome in the muscle prepared immediately after death proceeded with the progress of the conditioning. The release of almost all cathepsin D from the lysosome and its absorption on the myofibrils were observed in the muscle conditioned for14days. And the accumulation of cathepsin D accompanied with the disruption of myofibrillar structure was also observed. Changes in the immunoelectron micrographs showing the cathepsin D in the muscle caused by high-pressure treatment are shown in Fig. 2. With the increase of the pressure up to 300 MPa applied to the muscle, the release of cathepsin D was

accelerated, and absorption of the released cathepsin D on the myofibrils was observed as observed in the conditioned muscles. However further change was not observed in the muscle pressurized at 400 MPa. As compared with the conditioned muscles, remarkable disruption in the regular structure of the myofibrils was observed in the pressurized muscles.

Conclusion

It was cleared that the brief exposure of muscle to high-pressure induced the almost same changes in the release of cathepsin D from lysosomes as observed in the conditioned muscles. The release of cathepsin D from the lysosome is one of the causes of meat tenderization and flavor enhancement.

Acknowledgments

We wish to express our thanks to Professor Y. Watanabe, Department of Science, Niigata University, for his kind advice for electron microscopic observation.

References

1. A. Suzkuki, K. Kim, N. Homma, Y. Ikeuchi & M. Saito, In *High-Pressure Technology;* Balny, C., Hayashi, R. Heremens, K. & Masson, P., Eds, Colloque INSERM / John Libbey Eurotext, France 1992, pp. 219-227.
2. A. Suzuki, K. Kim., H., H. Tanji & Y. Ikeuchi, Recent Res. Development in Agricultural & Biological Chemistry , 2, 303-331 (1998).
3. N. Homma, Y. Ikeuchi & A. Suzuki, Meat Sci., 38, 219-228 (1994).
4. S. Jung, M. Lamballerie-Anton, R.G.Taylor & M. Ghoul, J. Agric. Food Chem., 48, 2467-2471 (2000).
5. K.Kim., Y. Ikeuchi & A. Suzuki, Meat Sci., 32, 237-243 (1992).
6. K. Maruyama, T. Yoshida, H. Higuchi, K. Ohashi, S. Kimura & R. Natori, J. Cell Biol., 101, 2167-2172 (1985).

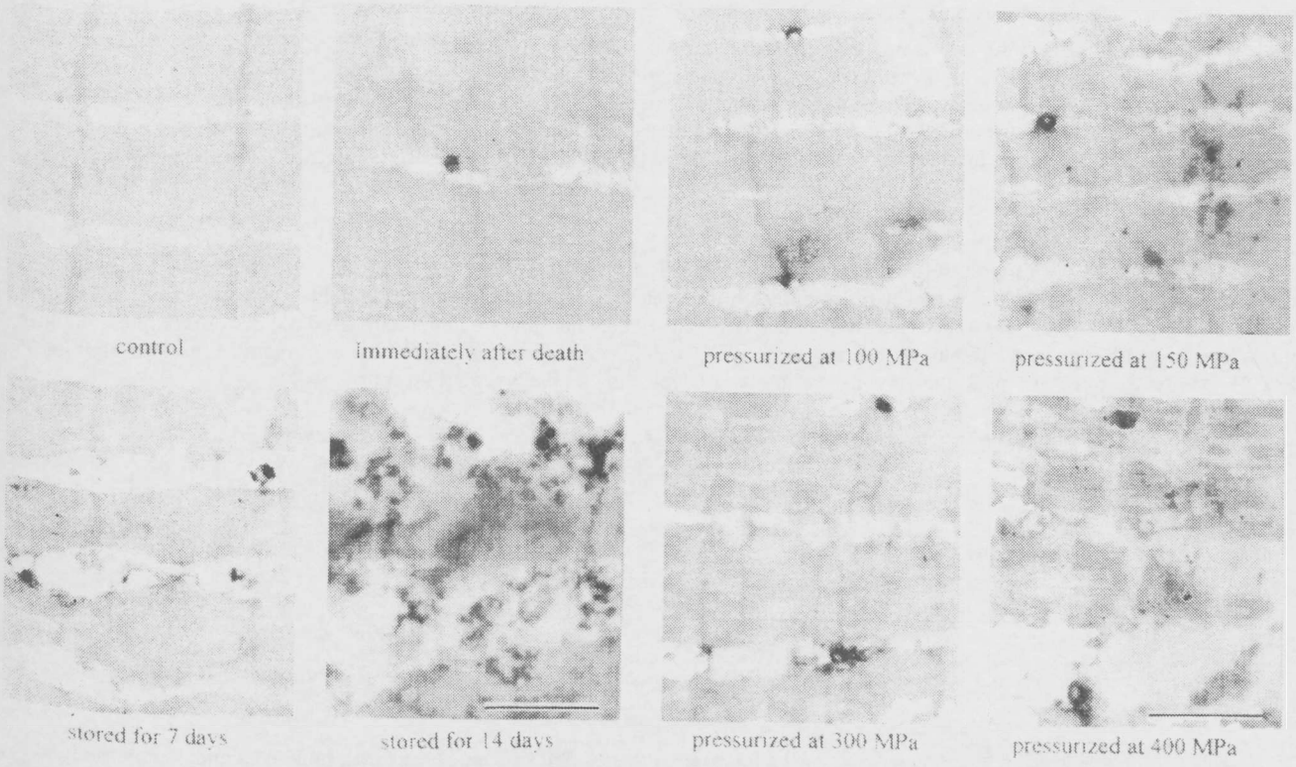

Fig.1. Immunoelectron micrographs showing localization of cathepsin D during conditioning

Fig.2. Immunoelectron micrographs showing the changes in localization of cathepsin D caused by high pressure