EFFECT OF ULTRASTRUCTURE DAMAGE ON PROTEOLYTIC ACTIVITY OF PORK MUSCLES.

DROBISZ D.

Meat and Fat Research Institute, Warsaw, Poland

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Introduction

Postmortem tenderization of muscle is generally considered to be due to the activity of three enzymatic systems: calcium activated factor (calpains), lysosomal enzymes (cathepsins) and multicatalytic proteinase complex (MCP) (Koohmaraie,1994).

Tenderization of meat is also caused by mechanical treatment. Mechanical tenderi-zation causes significant decrease of meat product firmness and severe changes in ultrastructure including disruption of sarcolemma, damage of lisosomes, myofibrils disruption. As calpains and cathepsins activities are influenced by several factors, one of the determinant can be the extent of ultrastructure damage.

The aim of this experiment was to evaluate the effect of mechanical treatment of pork muscles on proteases activity with relation to tenderizing effect of the treatment.

Materials and methods

M. biceps femoris were excised from pork carcasses about 48 hours post mortem. One part of the muscles was mechanically treated by use of the meat activator and the other portion, untreated, was a control sample.

In all samples pH and cathepsin B+L, D and calpains activities were measured. The experiments were carried out in 5 runs.

pH measurement

About 3 g of muscle was homogenized in 20 cm^3 distilled water for 15 s. The measurement was carried out immediately using pH-meter with combined glass electrode.

Preparation of calpains

A crude CAF preparation was made of muscle according to the procedure described by Duca-staing et al.(1985) with modifications of the method. The tissue was carefully trimmed of fat and muscles were not homogenized but just cut into slices (control samples) and then suspen-ded in 10 mM Tris-HCl buffer pH 7.5 containing 0.05 M NaCl, 4 mM EDTA, 2 mM 2-mercaptoethanol. After one hour extraction under magnetic stirring, it was centrifuged at 30,000 g for 30 minutes, the supernatant was filtered and then adjusted to pH 7.5. Precipitated material was eliminated by centrifugation at 50,000 g for 50 minutes. Preparation of the tissue at all steps were carried out at 4C with pre-cooled solutions.

Assay for calpain activity

Calcium-dependend proteolytic activity was assayed according to the method described by Koohmaraie et al. in 1986, using casein (Hammerstein Quality) as substrate at 25C in 100 mM KCl, 100 mM tris-acetate buffer pH 7.5, 10 mM 2-MCE, 5 mMCa and 5 mg/cm^3 casein. Total reaction volume was 2 cm^3. Control for enzyme and substrate accompanied each assay. The reaction was initiated by addition of calpain and stopped by precipitating the protein with 2 cm^3 of 5% trichloroacetic acid (TCA). Then it was centrifuged at 1000 g for 20 minutes and the absorbancy of the supernatant was measured at 278 nm.

Preparation of B + L cathepsins

A portion of muscle tissue was used for the preparation of muscle lysosomes by using a modificated method of Spanier et al. (1982). Muscle tissue was not homogenized but just cut into slices before being

suspended into 0.25 M sucrose, pH 7.2, containing 0.02 M KCl and 1 mM EDTA and then extraction under magnetic stirring followed. The tissue was recovered by centrifugation at 2.000 g for 10 minutes, resuspended in sucrose solution. The supernatant solution was saved and the resuspended tissue was then centrifuged again. The pooled supernatant solution was centrifuged at 23.000 g for 15 minutes. The pellets (enriched lysosomal fraction) were homogenized with a teflon homogenizer in 0.03 M phosphate buffer pH 5.8, containing 0.2% (v/v) triton X-100 and centrifuged at 23,000g for 15 minutes.
The supernatant solution and lysosol served as the source of enzyme.

Assay for B+L cathepsins activity

Samples for measurement of total cathepsin B+L activity were prepared according to Barret et al. (1981). Activities were assayed with their common substrate - benzoyl-L-arginine amide (BANA) and free 2-naphtylamine was determined colori-metrically after coupling with Fast Garnet GBC (diazotized o-aminoazotoluene).

Assay for D cathepsin

The proteolytic activity of cathepsin D was detected by a modificated method of Ouali (1980) with denatured haemoglobin as substrate. The assay was terminated by adding 3 ml of 5% trichloroacetic acid. The colour due to the reaction products was read at 280 nm.

Results and discussion

The results of the measurement of the calpain activity for four enzyme fractions (lysosol and supernatant from mechanically tenderized samples as well as lysosol and supernatant control samples) are shown in theTable 1.
Slight differences were observed in the activity of CAF for the mechanically treated meat as compared to control meat. The supernatant of the mechanically treated meat had higher values than the control one, which might be due to endoplasmic and sarcoplasmic reticulum damage. Under these conditions the sarcoplasmic Ca ion concentration increases and it could then activate calpain (Whipple,1993). In the lysosol, the activity was higher in control samples than in the treated ones. A detergent (triton X-100) was added to the extration buffer resulting in lysosomes disruption and release of lysosomal enzymes into the sarcoplasm. These conditions could then cause change of the concentration of Ca ions, which might be due to the change of the level of calpastatin, a specific inhibitor of calpains (Dransfield,1993). These conditions could then activate calpain in the control sample.
Table 1 shows the results of B+L cathepsins activity evaluation. There were differences in cathepsin activities. It was found that in the mechanically treated meat the cathepsins activities were higher than in the untreated meat. The increase in the enzyme activity may result from lysosomes disruption.
Table 1 shows the results of D cathepsins activity measurements. The lower activity was noticed in the mechanically treated meat as compared to the control one. This tendency was observed irrespectively of the enzymes sources (supernatant or lysosol).
The more extensive ultrastructure damage in the mechanically treated meat, might be due to lysosomes disruption and release of lysosomal enzymes. These enzymes may cause proteolytic attack on the different protein linkages. Since the relation between the amount and activity of cathepsin D is not linear one and depends on the presence or lack of specific protein linkages, it might be possible that much more advanced damage of protein network in activated meat was responsible for lower cathepsin D activity observed.

Conclusions

According to the results of the investigation it can be assumed that ultrastructure damage of meat might influence the activity of proteases and thus take part in meat tenderization . It is only a provisional conclusion which should be supported by further studies aimed at more precise elucidation of the mechanism of meat tenderization during plasticication processes.

Literature

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