4:28

Effect of a Elastase from Alkalophilic Bacillus Strain on the Tenderization of Beef Meat

H.TAKAGI, M.KONDOU, T.HISATSUKA and *M.YAMASAKI

Central Research Laboratories, Ajinomoto Co.Inc., 1-1 Suzuki-cho, Kawasaki 210, Japan

*Department of Agricultural Chemistry, The University of Tokyo, 1-1-1 Yayoi, Tokyo 113, Ja

Tenderizing effect of a new alkaline elastase produced by alkalophilic Bacillus sp. strain B was evaluated on beef meat. First of all, we investigated its elastolytic avtivity elastin-orcein as substrate. The specific activity of the enzyme was 60-200 times higher other proteases,such as papain and bromerain. Secondly, the mode of myofibril degradation analyzed by using SDS-polyacrylamide gel electrophoresis after incubation of intact sam with or without enzymes. Elastase treatment resulted in little proteolysis against isol myofibril,in contrast to papain which degraded most of proteins, especially myosin heavy c and actin. Furthermore, in order to examine the effect of enzymes on tenderness, mechani hardness was measured by a rheometer. When each enzyme solution was injected to the beef t muscle, the force required to cut the muscle fiber was reduced in enzyme-injected sampl These data indicated that the elastase are promising as an ideal meat tenderizer.

It is well known that tenderness is the most important sensory characteristic of meat. molecular structure of collagen and elastin in connective tissues are significant fact affecting the textural characteristics of meat(CROSS et al.,1973). One approach to increa meat tenderness is to reduce significantly the amount of detectable connective tissue, wit extensive degradation of myofibrillar proteins.

For this purpose,proteolytic enzymes,such as papain,bromelain and ficin have been widely as meat tenderizers. However,they often produce undesirable textural quality, due to t broad substrate specificities (PRUSA et al.,1981). It is thought, therefore, that proteolytic enzyme for ideal meat tenderizer has a specificity for collagen and elastin connective tissues.

Although, in addition to pancreatic elastase several microbial elastolytic enzymes w digest elastin have been obtained so far as reported by SHIIO et al.(1974),these enzymes o the disadvantages of safety and effect. TSAI et al.(1983) isolated a new alkaline elas from an alkalophilic Bacillus sp. Ya-B, which was a serine protease and had very elastolytic activity. It showed a marked preference for elastin and collagen over proteins.

In this study, we showed the characteristics and evaluated the ability of a new elastase the meat tenderization in comparison with those of other proteases.

MATERIALS and METHODS:

Enzymes: Crude and purified elastase were prepared according to the method described by et al.(1988). Briefly, an alkalophilic Bacillus was aerobically cultured in a me containing 2% soymeal, which stimulates elastase production, at 37°C for 24 hours. Ammo

sulfate precipitation of culture fluid was performed to obtain the partial purified enzyme, and then this fraction was further purified using DEAE-Sephadex and CM-Sephadex column chromatography. Purified papain, bromelain and porcine elastase were purchased from Sigma Chemical Co..

Elastolytic activity: Elastolytic activity was assayed by the method of TSAI et al.(1983). Each enzyme was incubated with 20 mg of elastin-orcein in 1 ml of each buffer,with shaking for 1 hour at 37°C. Reaction was stopped by adding 2 ml of 0.7 M phosphate buffer(pH 6). The substrate was removed by centrifugation, and absorption of supernatant was determined at 590 nm. The amount of enzyme which gave half of the absorbance at 590 nm when 20 mg elastin-orcein was completely hydrolyzed was defined as 10 units.

Proteolytic activity: The proteolytic activity of elastase on myofibrillar proteins was compared to that of papain by using SDS-polyacrylamide gel electrophoresis. 10 µg of isolated myofibrillar proteins(prepared as in KIMURA et al.(1983)) was incubated with 1 ng or 10 ng of each enzyme in 30 µl of distilled water at 37°C or 4°C. At the end of the incubation time, an electrophoresis sample buffer was added, and the samples were boiled for 3 min to stop the enzymatic reaction. Electrophoresis was carried out using 4-20 % gradient gel as decribed by Laemmli(1970). The extent of proteolytic activity was assessed by comparing electrophoretic patterns. The activity of each enzyme was also determined by using casein as substrate. Caseinolytic activity was assayed by the method of TSAI et al.(1983).

Mechanical texture measurement:Silver-side of imported beef from Australia was purchased from a local supermarket, and was cut into several cubes(4 x 4 x 3 cm). Enzyme treatment was done by injection of the enzyme solution (0.001 % enzyme in distilled water), amounting to 5 % of the meat on weight basis. After incubation under the various conditions, each meat was heated at 70°C for 20 min in the water bath, and was usd for mechanical texture measurement by the rheometer (NRM-2005J, Fudo Kogyo, Japan). The cores (12.5 mm diameter) were removed parallel to the grain of the muscle fibers from each meat. The force required to cut the muscle fiber was measured when pressing by a plunger with 5 kg of forces.

RESULTS and DISCUSSION:

The alkaline elastase produced by alkalophilic Bacillus Ya-B was a new type of serine protease which had a very high optimum pH and high elastolytic activity. It also had a high hydrolyzing activity against collagen. TSAI et al.(1983) showed that this enzyme did possess the two main characteristics of the elastin-hydrolyzing enzymes, high elastin binding ability and substrate specificity for aliphatic amino acid residues. Recently, KANEKO et al.(1989) cloned the structural gene, and determined the nucleotide sequence. The mature protein is deduced to have 268 amino acids, and the calculated molecular mass is 26,677 daltons.

In comparison with porcine pancreatic elastase, papain and bromelain at their optimum pH, the purified alkaline elastase had very high specific activity, 2,400 units/mg protein for elastin, and 1,900 units/mg protein for casein at pH 10.5 (Table 1). Particularly, the elastolytic activity was about 60-200 times higher than those of commercially used meat

tenderizers, such as papain and bromelain. It is also indicated from o experiments(unpublished data) that this enzyme still has higher elastin and coll hydrolyzing activity, while it decreases markedly in caseinolytic activity at lower pH distilled water). These data suggest that this enzyme showes marked preference for elastin collagen over other proteases.

To examine the mode of myofibril degradation by enzymes, the electrophoretic pattern compared. As shown in Fig.1, all of the proteins incubated with papain were highly degra especially of myosin heavy chain and actin. This action can result in extensive degradatio the meat structure and undesirable texture (CRONLUND et al.,1987). On the other hand, li difference in gel banding patterns were seen between the control and each of the elas preparations after incubation of myofibril. However, myosin heavy chain was degraded rapidly than other proteins when incubated at the increased enzyme concentration. These suggest that even partially purified elastase preparation maintain the desired specificity meat tenderization, i.e., the low specificity toward the myofibrillar proteins and the specificity toward elastin and collagen.

Finally, means for rheometer values indicate both partially purified elastase and papain significant effect on improvement the tenderness of tough meat(Table 2). The elastase favorable for meat tenderizer, since the efficiency of tenderization was almost the same the relatively low pH of meat and at the low temperature at which meat is held dur storage.

CONCLUSIONS:

The present study shows that the new elastolytic enzyme produced by alkalophilic Bacillus are promising as an ideal meat tenderizer, although further experiments, e.g., changes muscular proteins and sensory evaluation in enzyme-injected meat, should be needed.

REFERNCES:

CRONLUND,A.L. and WOYCHIK,J.H.(1987): Solubilization of collgen in restructured beef w collagenase and α-amylase.J.Food Sci.52:857-860.

CROSS,H.R.,CARPENTER,Z.L.and SMITH,G.C.(1973):Effects of intramuscular collagen and elastin bovine muscle tenderness.J.Food Sci.38:998-1003.

KANEKO,R.,KOYAMA,N.,TSAI,Y.-C.,JUANG,R.-Y.,YODA,K.and YAMASAKI,M.(1989): Molecular cloning the structural gene for alkaline elastase YaB, a new subtilisin produced by an alkalophi Bacillus strain.J.Bact.171:5232-5236.

KIMURA,S. and MARUYAMA,K.(1983): Preparation of native connectin from chicken breast musc J.Biochem.94:2083-2085.

LAEMMLI,U.K.(1970): Cleavage of structured proteins during the assembly of the head bacteriophage T_4.Nature 277:680-685.

PRUSA,K.J., CHAMBERS IV,E., BOWERS,J.A., CUNNINGHAM,F. and DAYTON,A.D.(1981): Thiamin conte texture, and sensory evaluation of postmortem papain-injected chicken.J.Food Sci.46:1684-16

SHIIO,I, NAKAMATSU,T. and OZAKI,H.(1974): Microbial production of elastolytic enzym Agr.Biol.Chem.38:1-7.

TSAI,Y.-C.,YAMASAKI,M.,YAMAMOTO-SUZUKI,Y. and TAMURA,G.(1983):A new alkaline elastase of alkalophilic Bacillus.Biochem.Int.7:577-583.

TSAI,Y.-C.,JUANG,R.-Y.,LIN,S.-F.,CHEN,S.-W.,YAMASAKI,M. and TAMURA,G.(1988): Production further characterization of an alkaline elastase produced by alkalophilic Bacillus strain B. Appl.Environ.Microbiol.54:3156-3161.

Table 1. Hydrolysis of elastin and casein

Enzyme	Elastolytic*1 (unit/mg)	Caseinolytic*2 (unit/mg)
Alkaline elastase	2400	1900
Pancreatic elastase	500	670
Bromelain	37	360
Papain	12	270

*1 Unit definition was described in MATERIALS and METHODS.
*2 Unit was expressed as mg of tyrosine released per minute.

Table 2. Mechanical texture measurement score

Treatment	Forces (kg)	Tenderness (cm/100g)
Control	3.41(0.49)*a	0.056(0.014)a
Alkaline elastase		
(4℃,17hr)	2.49(0.42)b	0.074(0.013)b
(37℃,1hr)	2.45(0.43)b	0.082(0.014)b
Papain		
(4℃,17hr)	1.79(0.66)b	0.094(0.016)b
(37℃,1hr)	2.01(0.20)b	0.073(0.005)b

a-b Values within the same column with no superscript in common are signficantly different($p<0.05$).
* Mean(SD) of 4 samples.

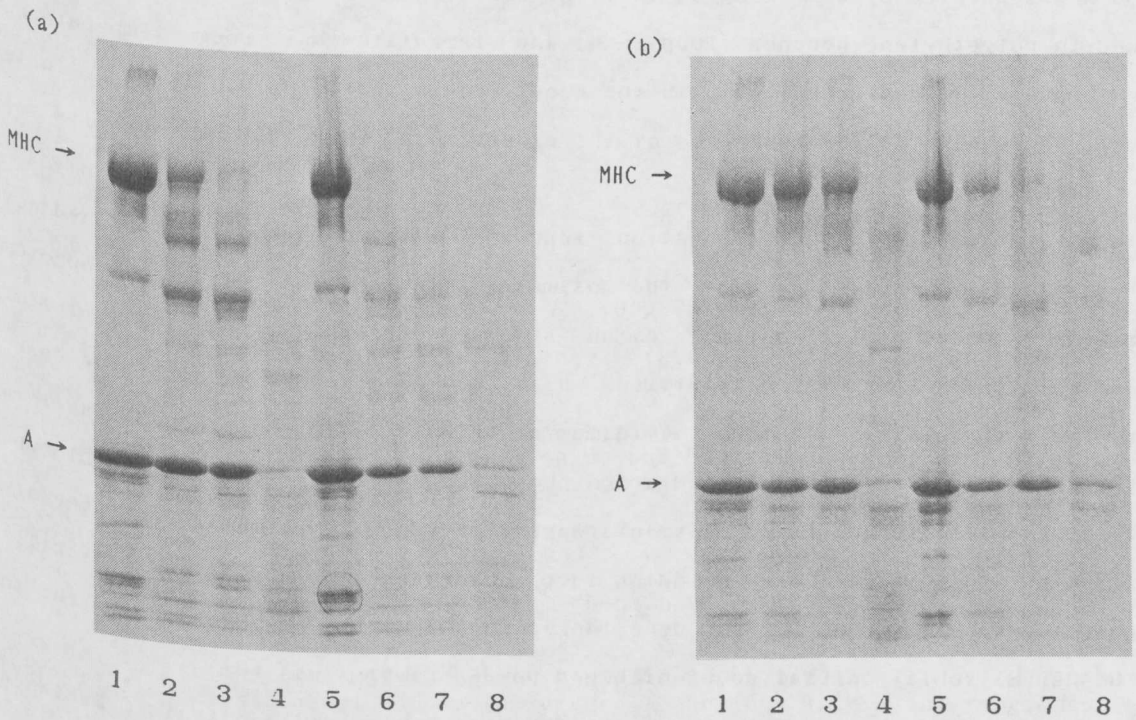

Fig.1 SDS-polyacrylamide gel electrophoresis of myofibrillar proteins incubated with enzymes in distilled water. (a)substrate:enzyme=1,000:1,(b)substrate:enzyme=10,000:1.(1)-(4)incubated at 4℃ for 15h (5)-(8)incubated at 37℃ for 1hr; (1)(5)myofibrillar proteins incubated in the absence of enzyme; (2)(6)incubated with purified elastase; (3)(7)incubated with partial purified elastase; (4)(8)incubated with papain. MHC,myosin heavy chain; A,actin.