MECHANISM OF THE INCREASE OF FREE AMINO ACIDS DURING THE STORAGE OF MEATS

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INTRODUCTION

It is well-known that meat flavor as well as texture are improved during the storage of meats at low temperature. In particular, the brothy taste of meats has been shown to be enhanced and improved during storage (1). This improvement was suggested to be caused by the increase of free amino acids and peptides during the storage of meats.

Proteins in meat mainly consist of myofibrillar and sarcoplasmic proteins which comprise about 60 and 30 % of total proteins, respectively. These proteins are degraded by the actions of various proteases during the storage of meats, which leads to the increase of free amino acids and peptides. The increase of peptides was caused by the actions of proteinases, such as cathepsins and calpains, while the increase of free amino acids was caused by the action of aminopeptidases which possess optimal pHs at a neutral pH region.

Among these aminopeptidases, aminopeptidases C and H have been suggested to be major ones contributing to the increase of free amino acids during the storage of meats on the basis of a comparison of the substrate specificity of muscle aminopeptidases with the pattern of free amino acids released during the storage of meat (2). However, the mechanism of the increase of free amino acids is not clarified completely.

This work was performed to elucidate the mechanism of the increase of free amino acids during the storage of meats.

METHODS

Action of cathepsins and calpain towards muscle proteins- Myofibrillar proteins were prepared from chicken skeletal muscle according to Yang's method (3). Sarcoplasmic proteins were extracted from chicken muscle with 2 times volumes of 0.16 M KCl/40 mM Tris-HCl (pH 7.2), after it was minced. Myofibrillar and sarcoplasmic proteins were incubated with cathepsins, prepared according to Okitani's method (4), in 50 mM phosphate buffer (pH 6.2) containing 1.2 mM EDTA and 3 mM DTT at 37 C for 12 hr., and with a calpain, prepared according to Ishiura's method (5), in 0.1 M glycerophosphate buffer (pH 7.5) containing 0.1 % 2- mercaptoethanol and 5 mM $CaCl_2$ at 37 C for 12 hr. These actions were halted by addition of methanol (final conc.; 80 %), and then the produced peptides were prepared by the centrifugation and evaporation to remove proteins and ethanol, respectively. The amount and constituent amino acids of these peptides were determined with an amino acid analyzer.

Action of aminopeptidases towards peptides- Aminopeptidases C and H were isolated from chicken skeletal muscle by the methods in previous reports (6, 7). The peptides, which were produced from muscle proteins by the actions of cathepsins and calpain, were incubated with aminopeptidases C and/or H in 67 mM Tris-HCl (pH 7.2) containing 1.3 mM DTT at 37 C for 2 hr. The released amino acids were analyzed with an amino acid analyzer.

A peptide, Tyr-Pro-Leu-Gly, was incubated with aminopeptidases C, H and P in 50 mM Tris-HCl (pH 7.2) containing 1 mM $MnCl_2$ and 1 mM DTT at 37 C. After incubation, released amino acids were also analyzed with an amino acid analyzer.

Survey of aminopeptidase P in chicken skeletal muscle- Chicken muscle extract was obtained by the homogenization of minced muscle with 3 times volumes of 40 mM Tris-HCl (pH 7.2). This extract was incubated with 0.5 mM Arg-Pro-*p*-nitroanilide in 50 mM Tris-HCl (pH 7.8) containing 1 mM $MnCl_2$ and 1 mM DTT at 37 C for 15, 30 and 60 min., and then released amino acids were determined with an amino acid analyzer.

Analysis of free amino acids and peptides- Free amino acids were analyzed with an amino acid analyzer (Hitachi model 835), after pH of sample solution was adjusted to pH 2. The amount and constituent amino acids of peptides were analyzed with the same analyzer after the sample solution was hydrolyzed in 6 N HCl at 110 C for 24 hr.

RESULTS AND DISCUSSION

The amounts of peptides produced from the chicken myofibrillar and sarcoplasmic proteins by the treatment of cathepsins and calpain were determined. The amounts of peptides (MF-cath- and SP-cath-peptides) from myofibrillar and sarcoplasmic proteins by the treatment of cathepsins were 2.68 and 2.68 μmol amino acids eq./g meat, respectively, while those (MF-cal- and SP-cal-peptides) from myofibrillar and sarcoplasmic proteins by the treatment of calpain were 1.45 and 1.05 μmol amino acids eq./g meat, respectively. From these results, the increase of peptides during the storage of meat was elucidated to be caused by both actions of cathepsins and calpain.

After each peptide group (MF-cath-, MF-cal-, SP-cath- and SP-cal-peptides) was incubated with aminopeptidase C or/and H, the increase of free amino acids were determined. The amounts of free amino acids released from the MF-cath-, MF-cal-, SP-cath- and SP-cal-peptides by the treatment of aminopeptidase H were 1.42, 0.53, 1.33 and 0.68 μmoles/g meat, respectively, while those from the MF-cath-, MF-cal-, SP-cath- and SP-cal-peptides by the treatment of aminopeptidase C were 0.50, 0.16, 0.41 and 0.25 μmoles/g meat, respectively (Fig. 1). Their increment by aminopeptidase H was about three times that by aminopeptidase C. Furthermore, the treatment with both aminopeptidases C and H caused the larger increments in free amino acids than that with only aminopeptidase H. Especially, the increases of Ala, Lys, Gly, Ser, Thr, Leu, Val, Glu and Arg were large. However, no release of Pro was detected by the actions of both aminopeptidases. The pattern of the increase of free amino acid by the actions of aminopeptidases C and H was compared with that during

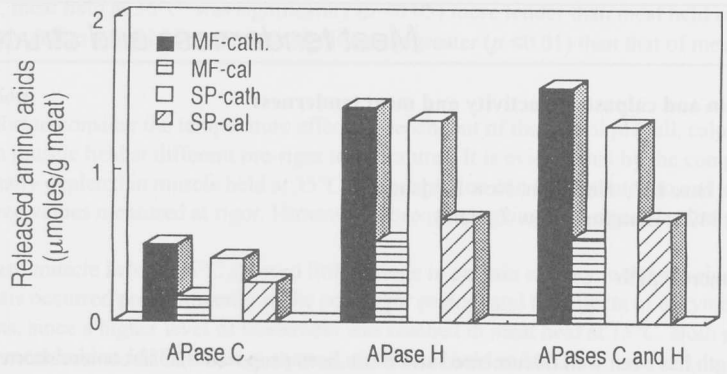

Fig. 1 Amounts of free amino acids released from peptides by the action of aminopeptidase C or/and H. MF-cath- and MF-cal-peptides indicate the peptides produced from myofibrillar proteins by cathepsins and calpain, respectively. SP-cath- and SP-cal-peptides indicate the peptides produced from sarcoplasmic proteins by cathepsins and calpain, respectively.

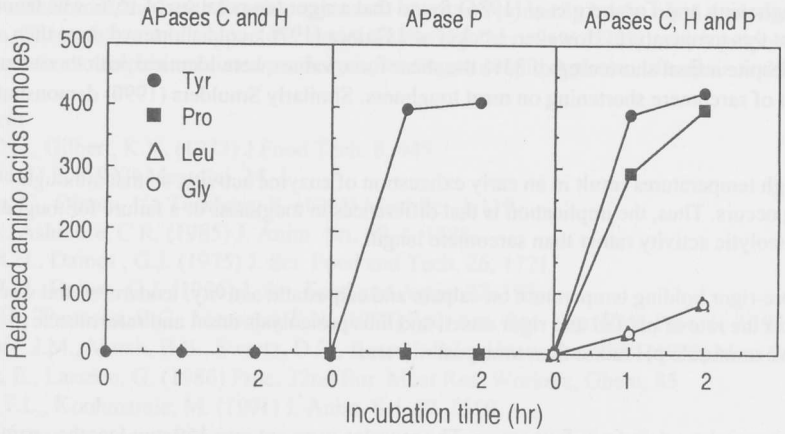

Fig. 2 Action of aminopeptidases (APases) C, H and P towards Tyr-Pro-Leu-Gly. 45.9, 21.4 and 104.1 mU of aminopeptidases C, H and P, respectively, were incubated with 1 mM substrate in 0.4 ml of 50 mM Tris-HCl (pH 7.2)/1 mM $MnCl_2$/1 mM DTT at 37 °C.

the storage of meat. The similarity of these patterns showed about 90 %, indicating that the increase of free amino acid except for Pro was mainly caused by the actions of aminopeptidases C and H.

Other aminopeptidase, which release an N-terminal amino acid from the peptide (X1-Pro-X2-X3-, Xi: amino acid) possessing Pro at the second position from its N-terminus, was suggested to contribute to the increase of Pro during the storage of meats, because aminopeptidases C and H do not show such an action as described above. So we tried to detect the aminopeptidase P-like activity in chicken muscle using Arg-Pro-*p*-nitroanilide as substrate. Arg was released in the beginning, and then Pro was released, indicating that aminopeptidase P was present in chicken skeletal muscle. Chicken muscle aminopeptidase P was purified by ammonium sulfate fractionation and successive column chromatographies of DEAE-cellulose, Ultrogel AcA34, hydroxylapatite, and Ultrogel AcA-44.

The mechanism of the increase of Pro during the storage of meats was examined using aminopeptidases C, H and P isolated from chicken skeletal muscle. The incubation of these enzymes with a peptide, Tyr-Pro-Leu-Gly, showed that Tyr was released by the action of not aminopeptidases C and H but aminopeptidase P, and Pro was released by the actions of these three enzymes (Fig. 2).

CONCLUSION

It was concluded that the increase of free amino acids during storage of meats was mainly caused by the actions of aminopeptidases C, H and P towards the peptides, which were produced from meat proteins by the actions of cathepsins and calpain (Fig. 3).

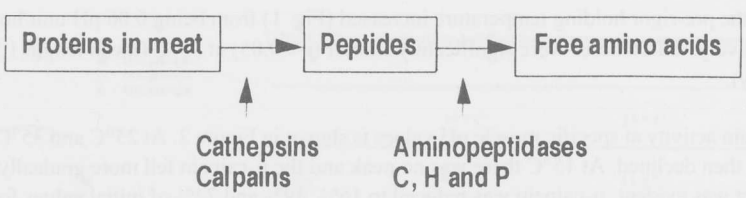

Fig. 3 Proposed mechanism of the increase of free amino acids during the storage of meats.

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