PURIFICATION AND PROPERTIES OF AN AMINOPEPTIDASE FROM BOVINE SKELETAL MUSCLE

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Backgound :

Free amino acids have been found to increase during the storage of skeletal muscle. 1) Such an increment of free amino acids is considered to contribute to the improvement of meat flavor during postmortem conditioning of meat. The increase of free amino acids was caused by the action of aminopeptidases active at neutral pH regions. Some kinds of neutral aminopeptidases in skeletal muscle have been already reported. Among them, aminopeptidases C, H and P are thought to be play an important roles in the increase of free amino acids during the storage of meat.2) However, there is little information on aminopeptidases except for aminopeptidases C, H and P, and the mechanisms involved in increase of free amino acids during posrmortem conditioning of meat have not been completely elucidated.

Objective

In the present work, we purified an aminopeptidase from bovine skeletal muscle, and investigated its properties as follows: pH optimum, effect of inhibitors, metal ions, and anions, heat stability, and substrate specificity.

Methods

Purification of the aminopeptidase from bovine skeletal muscle

All steps were carried out at about 4 degrees C. Minced muscle (600g) was homogenized with three volumes of 40 mM tris-HCl (pH 7.2) containing 0.1% 2-mercaptoethanol, and aminopeptidases were extracted. This extract was fractionated by ammonium sulfate fractionation. The precipitate obtained by 35 % to 65% ammonium sulfate saturation was dissolved with buffer. Then, this fraction was dialyzed against 10 mM tris-HCl (pH 7.2). This dialyzate was put on DEAE-cellulose column. An aminopeptidase on DEAE-cellulose column was further purified by successive chromatografies of Sephacryl S-200, Hydroxyapatite, Phenylsepharose, and HiTrap affinity chromatography. On the last chromatography, HiTrap affinity column, aminopeptidase was purified. The fractions containing the enzyme activity on this column were collected and used to examine some of its properties.

Enzyme assay

The enzyme activity against Ala-beta-naphtylamide (Ala-NA) and Xaa (amino acid)-NA was measured by the method of Matsutani *et al.*3) After the enzyme had been incubated with 0.5 mM substrate in 0.1 M tris-HCl (pH 7.2) containing 0.5 M NaCl at 37 degrees C for 60 min, 0.4 ml 0.23 M HCl in ethanol and 0.4 ml 0.06% *p*-dimethylaminocinnamaldehyde in ethanol were added to the reaction mixture, in order to stop the enzyme reaction. The red color that developed was measured at 540 nm and theβ-naphtylamine released from Xaa-NA was determined.

Protein determination

The absorbance at 280 nm was used to monitor the protein peak on the column chromatographies. The concentrations of proteins were also determined by the method of Lowry *et al.* using bovine serum albumin as the standard.

Gel electrophoresis

Gel electrophoresis in the presence of sodium dodecyl sulfate was carried out by the method of Laemmli using 12.5 % gels and bromophenol blue as the tracking dye. The proteins were stained with Coomassie brilliant blue R-250.

Results and discussion

Purification of the aminopeptidase from bovine skeletal muscle

An aminopeptidase was purified by an ammonium sulfate fractionation and five chromatographies. A SDS-PAGE profile of enzyme obtained by the HiTrap affinity column chromatography was shown in Fig. 1. The aminopeptidase was thus observed as a single band, and estimated 58 kDa on SDS-PAGE (Fig. 2).

pH optimum

The enzyme activities against Ala-NA were measured at various pH in 0.1 M acetic acid, potassium phosphate or ammonium buffer. As shown in Fig. 3, the optimum pH of this enzyme against Ala-NA was around 7.0-8.0.

Effect of inhibitors

The effect of various protease inhibitors on aminopeptidase activity was examined. The enzyme was strongly inhibited by bestatin, PMSF, and puromycin, indicating that this aminopeptidase is classified as a serine protease.

Effect of metal ions

The enzyme was incubated with Ala-NA in 0.1 M Tris-HCl (pH 7.2) containing various metal ions at 37 degrees C. The activity was remarkably inhibited by 0.2 mM Cu^{2+}, Zn^{2+}, Mn^{2+}, and Co^{2+}.

Effect of anions

The effect of anions on the aminoeptidase activity was examined. This activity towards Ala-NA was activated by some halides

and other monovalent anions. Among all anions tested, thiocyanate anion was shown to be the most effective to activate activity. Chloride and bromide ions also activated this enzyme. This activation by anions seems to be caused by the conformational changes of aminopeptidase when anions bind to its active site. The activation of several enzymes such as alpha-amylase 4), cathepsin C 5), aminopeptidase B 6) and angiotensin converting enzyme 7) by anions has previously been reported. However, the mechanism of activation by anions has not elucidated.

Effect of heat on stability of this enzyme

After the enzyme had been kept at various temperatures in 0.1 M tris-HCl (pH 7.2) for 10 min, its activity against Ala-NA was measured. The enzyme was stable up to about 55 degrees C, but activity was almost lost at 65 degrees C.

Substrate specificity

As shown in Fig. 4, this enzyme showed a broad substrate specificity against Xaa-NA, its action on Ala-NA being especially high. Moreover, the aminopeptidase also showed low activity against Arg, Pro, Leu, Met and Lys-NA.

References

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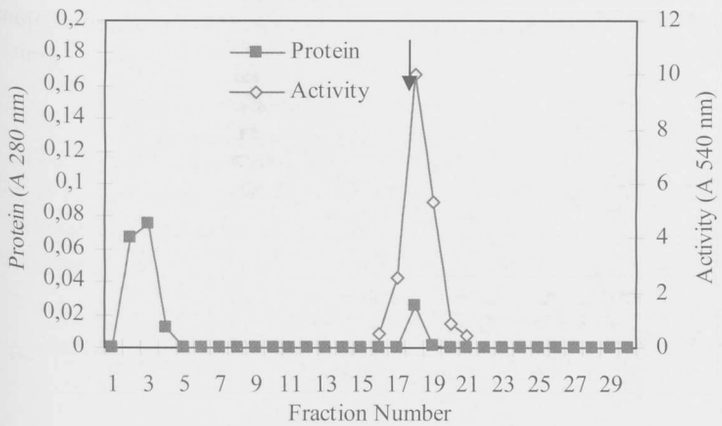

Fig. 1 HiTrap affinity column chromatography

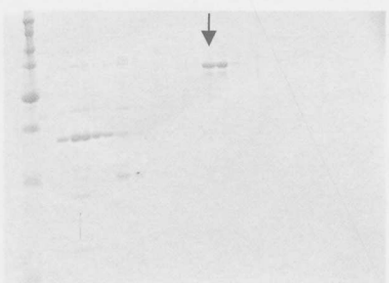

Fig. 2 SDS-PAGE of HiTrap affinity column

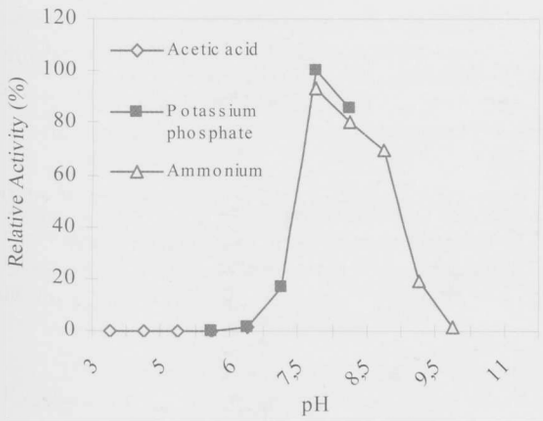

Fig. 3 Effect of pH on the activity of the aminopeptidase

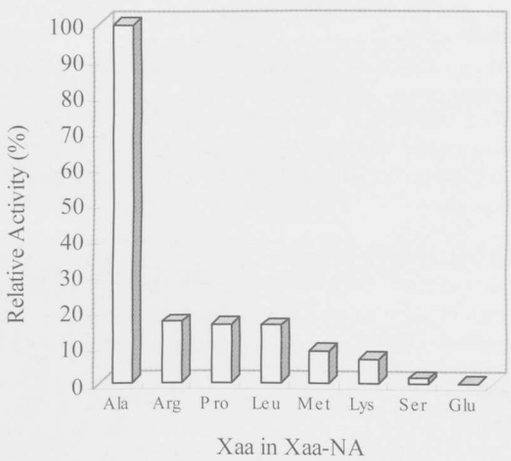

Fig. 4 Hydrolytic activity of the aminopeptidase toward peptides