Short Paper

N-terminal amino acid sequence of trypsin from the pyloric ceca of starfish Asterias amurensis

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Trypsin (EC 3.4.21.4) is an important pancreatic serine protease synthesized as a proenzyme by pancreatic acinar cells and is secreted into the intestine of mammals. Mammalian pancreatic trypsin and its proenzyme have been extensively characterized.1 Mammalian pancreatic trypsin consists of both cationic- and anionic-type enzymes. Mammalian anionic trypsin is unstable at acidic pH, unlike cationic trypsin.2,3 Marine vertebrate trypsin, especially of fish, has also been well characterized.4,5 Fish trypsin is similar to mammalian anionic trypsin in its molecular weight, amino acid composition, Ca2+ requirement, pH stability and reaction with substrates and inhibitors. Recently, cDNAs encoding trypsin were isolated from Atlantic cod and salmon.6,7 Fish trypsin has similar distribution patterns of charged and hydrophobic amino acid residues compared with mammalian trypsin, indicating similar 3-D structure of these trypsins.

In addition, trypsin-like enzymes from some marine invertebrates have been purified and characterized.8,9 Characteristics of the enzymes from marine invertebrates resemble those of mammalian anionic and fish trypsins in molecular weight, cleavage specificities, pH stability and reaction with inhibitors. Amino acid sequence of marine invertebrate trypsin-like enzyme has not been analyzed except for the N-terminal amino acid of trypsin-like enzymes from the starfish Lysasterosa anthosticta, Glx or Asx.10 Camacho et al.11 made a structural study of the active site peptide from the enzyme was similar to that of mammalian pancreatic trypsin. However, all these trypsin-like enzymes were not activated or stabilized by Ca2+, unlike mammalian anionic and fish trypsins. These findings suggest that notable structural differences may be between vertebrate and invertebrate trypsins. Neurath et al.12 pointed out the importance of phylogenetic variations of the enzyme in tracing the process of its evolution. In this study, the N-terminal acid sequence of trypsin from the starfish Asterias amurensis was found and was compared with the N-terminal amino acid sequence of other animal sources.

The starfish A. amurensis was caught off Hakodate, Hokkaido Prefecture, Japan. The specimens were stored at –20°C for several months until analysis. Crude trypsin was extracted by stirring from the delipidated powder of pyloric ceca in 50 volumes of 50 mM Tris-HCl buffer (pH 8.0) at 5°C for 3 h. The extract was centrifuged at 10 000 ¥ g for 10 min, and then the supernatant was fractionated between 40 and 75% saturation with ammonium sulfate. The active fraction was dissolved in a minimum volume of 50 mM Tris-HCl buffer (pH 8.0) and was dialyzed against the same buffer. The dialysate was applied to a Sephacryl S-200 column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with 50 mM Tris-HCl buffer (pH 8.0) and the protein was eluted with a linear gradient of 0–1.2 M NaCl in the same
buffer. The main trypsin fraction was concentrated and applied to a Sephadex G-50 (Pharmacia Biotech) column equilibrated with 50 mM Tris-HCl buffer (pH 8.0) and the protein was eluted with the same buffer. A single trypsin fraction was obtained and was further applied to a Sephadex G-50 column under the same conditions of the above gel filtration. Trypsin from the pyloric ceca of *Asterias amurensis* was nearly homogeneous using sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE (Fig. 1). To analyze the N-terminal sequence of *A. amurensis* trypsin, the enzyme was electroblotted to polyvinylidene difluoride (PVDF) membrane after SDS-PAGE. The amino acid sequence of the enzyme was found using a protein sequencer, model 473A (Applied Biosystems, Foster City, CA, USA).

*Asterias amurensis* trypsin hydrolyzed Tos-Arg-OMe substrate effectively at alkaline pH with an optimum activity of approximately pH 8.0. The optimum pH of the *A. amurensis* trypsin was similar to that of the starfishes *Evasterias troehelli* (optimum pH: pH 8.1),9 *L. anthosticta* (pH 8.5),10 and *D. imbricata* (pH 8.0–8.5).11 *Asterias amurensis* trypsin had an optimum temperature at approximately 55°C, which was higher than that of *L. anthosticta* (= 45°C).10 *Asterias amurensis* trypsin was unstable above 50°C and below pH 5.0 and was neither activated nor stabilized by Ca²⁺. These properties of the *A. amurensis* trypsin were similar to those of other starfish trypsin-like enzymes,9–11 but were unlike mammalian cationic trypsin.1–3

The molecular weight (= 28 000) of *A. amurensis* trypsin was larger than that of starfishes *L. anthosticta* (molecular weight: 25 000)10 and *D. imbricata* (25 000–26 000)11 and of mammalian pancreatic trypsin (= 24 000)1,2 (Fig. 1).

The N-terminal 16 amino acids of *A. amurensis* trypsin, IVGGKESSPHSRPYQV, were readily sequenced, indicating that its N-terminus was unblocked. Although the N-terminal amino acid of trypsin-like enzymes from the starfish *L. anthosticta* is Glx or Asx,10 the N-terminal amino acid of *A. amurensis* trypsin was Ile. The N-terminal 16 amino acid sequence of *A. amurensis* trypsin was aligned with those of other animal sources (Fig. 2).

Fig. 1 Electrophoresis of purified trypsin from starfish *Asterias amurensis*. (a) Electrophoresis used a 0.1% sodium dodecylsulfate (SDS)-10% polyacrylamide slab-gel.20 Lane 1 contains protein standards: cytochrome c monomer (molecular weight: 12 400), cytochrome c dimer (24 800), cytochrome c trimer (37 200), cytochrome c tetramer (49 600) and cytochrome c hexamer (74 400). Lane 2 is *A. amurensis* trypsin. (b) Electrophoresis of the starfish trypsin was performed using a 12.5% polyacrylamide slab gel at pH 8.9. Lane 1 is *A. amurensis* trypsin.

Fig. 2 Alignment of the N-terminal amino acid sequence of *Asterias amurensis* trypsin with the sequences of trypsins from other animals. Residues identical with *A. amurensis* trypsin are shaded. Starfish, *A. amurensis* trypsin (present paper); Dogfish, spiny Pacific dogfish *Squalus acanthias* pancreatic trypsin;13 Antarctic fish, antarctic fish *Paranotothenia magellanica* trypsin;14 Cod anionic I, Atlantic cod anionic trypsin I;6 Cod anionic X, Atlantic cod anionic trypsin X;6 Salmon, Atlantic salmon *Salmo salar* trypsin IA;12 Lungfish, African lungfish *Protopterus aethiopicus* pancreatic trypsin;15 Rat anionic, rat pancreatic anionic trypsin I;16 Rat cationic, rat pancreatic cationic trypsin;17 Dog anionic, dog pancreatic anionic trypsin;18 Dog cationic, dog pancreatic cationic trypsin;18 Bovine anionic, bovine pancreatic anionic trypsin;19 Bovine cationic, bovine pancreatic cationic trypsin.

### Starfish

- IVGGKESSPHSRPYQV

### Dogfish

- IVGGYECPPHAAPWTV

### Antarctic fish

- IVGGKECSSPSQHPHQQV

### Cod anionic I

- IVGGYECTHSQAHQV

### Cod anionic X

- IVGGYECRTSQAHHQV

### Salmon

- IVGGYECAYQSAHHQV

### Lungfish

- IVGGYECPLHSVPQV

### Rat anionic

- IVGGYTCEHSPQYVQ

### Rat cationic

- IVGGYTCQKNSLPYQV

### Dog anionic

- IVGGYTCSANSVPQV

### Dog cationic

- IVGGYTSCFNVESPYQV

### Bovine anionic

- IVGGYTGCANTVPQV

### Bovine cationic

- IVGGYTCAENPSPYQV
those of the other animals (Fig. 2). Similar to the trypsins from dogfish, 13 Antarctic fish, 14 cod, 6 salmon7 and lungfish, 15 A. amurensis trypsin had a charged Glu residue at position 6 where Thr is most commonly found in mammalian pancreatic trypsins 16–19 (Fig. 2). In contrast, bovine pancreatic trypsin19 had a disulfide bond between Cys-7 and Cys-142 and the other vertebrate trypsins conserved Cys-7 (Fig. 2). The Cys residue, however, was characteristically replaced by Ser in A. amurensis trypsin (Fig. 2), which implies that A. amurensis trypsin does not have the disulfide bond between Cys-7 and Cys-142 as in mammalian pancreatic trypsin.

REFERENCES