The Amino Acid Composition of the King and Hanasaki Crabs and Starfish Trypsin-like Enzymes

Journal of Hokkaido University of Education (Section II B) Vol. 28, No. 1 September 1977
北海道教育大学紀要（第2部A）第28巻 第1号 昭和52年9月

The Amino Acid Composition of The King and Hanasaki Crabs and Starfish Trypsin-like Enzymes*

Yasuzo ITO
Chemical Laboratory, Kushiro College, Hokkaido University of Education
Kushiro 085

タラバガニ，ハナサキガニ，及びヒトデトリプシン様酵素のアミノ酸組成

伊　藤　裕　三
北海道教育大学釧路分校化学教室

Trypsin and trypsin-like enzymes in invertebrates have been reported for various species and by a number of authors.

In the previous papers[1),2),3)] the author reported the separation, purificaton procedures and some enzymatic properties of these kinds of enzymes, namely king (*Paralithodes camtschaticus*) and hanasaki (*Paralithodes brevipes*) crabs trypsin-like enzymes and starfish (*Asterias amurensis Lücken*) trypsin-like enzyme. These trypsin-like enzymes were homogeneous by paper electrophoresis and polyacrylamide gel electrophoresis. And their molecular weights were approximately 20,000 for the crab enzymes and 21,000 for the starfish enzyme. The amino acid residues of the king and hanasaki crabs trypsin-like enzymes and the starfish trypsin-like enzyme were 186, 189 and 202 respectively. Some differences could be found in the constituent of amino acid.

In the present paper the author reports the amino acid composition of these purified trypsin-like enzymes, in comparison with those of mammalian trypsin 〔EC 3. 4. 4. 4〕.

Materials and Methods

Enzyme preparation

King and hanasaki crabs trypsin-like enzymes and starfish trypsin-like enzyme were purified according to the method previously reported. The obtained emzyme preparations were homogeneous as determined by paper electrophoresis and polyacrylamide gel electrophoresis.

Amino acid analysis

The amino acid analysis was performed on a Hitachi amino acid analyzer. Samples were hydrolyzed in 5. 7 N HCI, sealed vacuum at 110°C for specified periods of time.

---

* The report was presented at the 49th Annual Meeting of Japanese Biochemical Society, Sapporo, September 1976. And this investigation was supported in part by a grant from the Ministry of Education of Japan.

Results and Discussion

Amino acid composition

An amino acid analysis was carried out as described in the method. From the average and extrapolated values round to the nearest whole numbers their molecular weights were calculated to be 19,580 for king crab trypsin-like enzyme, 19,680 for hanasaki crab trypsin-like enzyme and

Amino acid	Crabs		Starfish			Shrimp (c)	Beef (d)	Human (e)	Pork (f)	Sheep (g)
	King	Hanasaki	A	B (a)	C (b)					
Lys	5	8	11	9	11	5	14	11	10	12
His	4	4	7	4	6	5	3	3	4	3
Arg	4	5	9	4	5	3	2	6	4	4
Asp	14	15	17	31	24	30	22	21	18	20
Thr	17	15	17	15	12	10	10	10	11	15
Ser	15	17	14	18	24	24	33	24	24	26
Glu	17	16	21	20	17	24	14	21	17	14
Pro	10	11	11	13	8	11	9	9	10	9
Gly	18	21	19	28	26	28	25	20	26	19
Ala	13	13	16	16	24	16	14	13	16	17
1/2Cys	10	8	10	8	10	8	12	12	8	12
Val	13	13	15	19	13	18	17	16	16	17
Met	1	1	1	2	1~2	2	2	1	2	2
Ile	11	14	9	11	11	14	15	12	15	10
Leu	16	14	14	13	15	10	14	12	16	14
Tyr	8	6	6	8	9	10	10	7	8	6
Phe	7	8	6	5	7	6	3	4	4	5
Try				5		3	6	3	4	

Table 1. Amino Acid Composition of Trypsin and Trypsin-like Enzymes.
(a) see Reference (4), (b) see Reference (5),
(c) see Reference (6), (d) see Reference (7),
(e) see Reference (8), (f) see Reference (9),
(g) see Reference (10),.

21,600 for starfish trypsin-like enzyme.

A comparison of the amino acid contents of several trypsin and trypsin-like enzymes from different species is shown in Table 1. The most prominent difference between the amino acid composition of these crab enzymes and the starfish enzyme is in the content of charged amino acids. The starfish enzyme has a greater number of potentially acidic and basic residues than those of crab enzymes. The starfish enzyme has a total of 20 arginine and lysine residues, and a total of 38 aspartic acid and glutamic acid residues per molecule, in comparison to a total of 9~13 arginine and lysine residues and a total of 31 aspartic acid and glutamic acid residues for these crab enzymes. And also the basic amino acid residues per molecule of the starfish enzyme are twice as many the residues of the other starfish enzyme (Neurath, et al.) listed in Table 1. Moreover. the starfish enzyme according to the present paper, has a greater number of potentially basic residues than those of shrimp, beef, pork, sheep and human trypsins listed in Table 1.

A comparison of charged amino acid contents of several trypsins and trypsin-like enzymes from different species is shown in Table 2.

As shown in Table 1, the amino acid composision of the trypsin-like enzymes of these crabs cleary indicates close similarity. These crab trypsin-like enzymes, various starfish trypsin-like enzymes, the shrimp and human trypsins have been found to have 8~10 half-cystine residues whereas the other mammalian trypsins have 12 and cocoonase[11], 4, only 2 of which appear to be disulfide linked. It was thought that a decrease in the number of disulfide bonds was directly responsible for both differences in tertiary structure and lack of reactivity toward macromolecular inhibitors such as SBTI (Soy Bean Trypsin Inhibitor). The finding that these crab enzymes and the starfish enzyme in the present paper are partially inhibited by SBTI, is indicative of the important of other types of bonds in such interaction.

Although the difference between the amino acid composition of our enzymes and the other enzymes listed in Table 1 is in the contents of charged amino acid an a half-cystine, it is most important to note that the very fact that inhibition dose occur with both invertebrate trypsin-like enzymes and vertebrate trypsin would suggest an overall similarity in a binding site for SBTI at both levels and therefore, a common evolutionary origin for trypsin-like proteolytic enzyme.

Table 2. Acidic Amino Acid and Basic Amino Acid from Table 1.

Amino acid	Crabs		Starfishes			Shrimp	Beef	Human	Pork	Sheep
	King	Hanasaki	A	B	C					
Acidic	31	31	38	51	41	54	42	36	35	36
Basic	9	13	20	13	16	8	18	16	14	16
Ratio Acidic/ Basic (Approxi-mately)	3:1	3:1	2:1	4:1	2:1	7:1	2:1	2:1	2:1	2:1

Full details of this and other works on the specificity of trypsin-like enzymes of these crabs and the starfish will be published elsewhere.

Acknowledgments

The author would like to express his grateful acknowledgment to Dr. Teiichi Sasaki for his helpful discussion and Dr. Masahiko Fujino for his amino acid analysis.

References

(1) Ito, Y. Ozawa, S and Hobo, K., *J. Hokkaido Univ. Educ.* II A **21**,66 (1971).
(2) Ito, Y., *J. Hokkaido Univ. Educ.* II A **23**, 74 (1973).
(3) Ito, Y., *Seikagaku* **45**, 199 (1973).
(4) William, P. Winter and Neurath, H., *Biochemistry* **9**, 4673 (1970).
(5) Gilliam, E. B. And Kitto, G. B., *Comp. Biochem. Physiol.* **54** B, 221 (1976).
Camcho, Z., Brown, J. R. and Kitto, G. B., *ibid* **54** B, 27 (1976).
(6) Gates, B. J. and Travis, J., *Biochemistry* **8**, 4483 (1969).
(7) Walsh, K. and Neurath, H., *Proc.Natl. Acad. Sci.* U. S. **52**, 884 (1964).
(8) Travis, J. and Roberts, R., *Biochemistry* **8**, 2884 (1969).
(9) Travis, J. and Liener, I. E., *J.Biol. Chem.* **240**, 1962 (1965).
(10) Travis, J., *Biochem. Biophys. Res. Commun.* **30**, 730 (1968).
(11) Kafates, F. C., Tartakoff, A. M. and Law, J.H., *J. Biol. Chem.* **242**, 1477 (1967).