Mem. Fac. Fish., Kagoshima Univ.
Vol. 22, No. 1, pp. 33～37 (1973)

Constituents of the Amino Acids Composed of the Protein in the Exoskeletons of the Crustacea.

Muneo SAMESHIMA *, Teruhisa KATAYAMA *,
and Osamu DESHIMARU **

Abstract

The constituents of the amino acids composed of the protein in the exoskeletons of the crustacea, the prawn, *Peneaus japonicus*, the crab, *Portuuns trituberculatus*, and the lobster, *Panulirus japonicus* were analyzed quantitatively. Asparatic acid, glutamic acid, alanine, glycine were in general featured by relatively high contents and the contents of proline, valine, and serine were also high, but the contents of the basic amino acids were low. The constituents of the amino acids composed of the protein in the exoskeletons of the crustacea were similar to those of amino acid patterns of elastin.

The compositions of the amino acids in the three parts of the exoskeletons of the crustacea : The amino acids in the carapaces, abdomens and pleopods, and telsons of the exoskeletons, were also analyzed, and compared each other. In the hard carapaces the contents of asparatic acid, glutamic acid and proline were high, in the soft parts of the abdomens and telsons the contents of glycine and the basic amino acids were also relatively high.

Introduction

The exoskeletons of the crustacea are basically consisted of epidermis, epicuticle and endocuticle [1]. YONGE [2] examined the differences between the epicuticle ("cuticle") and endocuticle ("chitin") and explained the method of epicuticle formation. The cuticle is composed fundamentally of a laminated chitin-protein complex and calcified layers.

The exoskeleton of the crustacea is well developed, and the crustacea repeat molting, metamorphose and grow up. Growth, reproduction and metabolism are all affected by the replacement of the integuments : molting, but molt-cycle is not clear. There are the biochemical correlation between molting and its growth in the crustacea. Each exuviation loses protein with ecdysis.

By the molting process the crustacea lose the protein and amino acids, and they should metabolize a large quantity of amino acids to give rise to the new cuticle.

Besides normal ecdysis molting is affected by the change of external conditions : light, temperature, salinity and so on. They should immediately form new exoskeleton. Though the molting of the crustacea have been studied by many researchers from the aspects of hormones [3-7], the mechanism of metabolizing amino acids to form new exoskeleton is not clear, but very important.

The present investigation was undertaken to clarify the constituents of the proteins in the exoskeleton of the crustacea. It was clarified that the main components of the amino acids

* Laboratory of Marine Biochemistry, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan.
** Kagoshima Prefectural Fisheries Experimental Station, Kagoshima, Japan.

composed of the protein in the exoskeleton of the crustacea were glutamic acid, asparatic acid, alanine, and glycine, and the contents of proline, valine, and serine were relatively high, but the contents of basic amino acids were low. The constituents of the amino acids composed of the protein in the exoskeleton of the crustacea were similar to those of elastin [8]. The amino acids composed of the protein in the carapaces, abdomens and pleopods, and telsons of the exoskeletons were quantitatively analyzed. In the carapaces the contents of asparatic acid, glutamic acid and proline were high, in the relatively soft parts of the abdomens and telsons, the contents of glycine and the basic amino acids were high.

Materials and Methods

The crustacea used as the samples were obtained at the sea shore of Izumi city, Kagoshima Prefecture. Species of the samples, length, or width of the shells are as follows :

The crab	two each	width of the carapaces : 14 – 15 cm
The prawn	ten each	length of the carapaces : 12 – 13 cm
The lobster	four each	length of the carapaces : 24 – 25 cm

Authentic amino acids and the reagents were purchased from the Wako Pure Chemicals Ltd. Alive crustacea were sacrificed and collected exoskeletons. The exoskeletons were washed with water to remove muscles or other concomitants, ground in a mortar, washed with acetone to remove the pigments or other fat soluble compounds, passed through a fourty mesh sieve and stored in a desiccator until use.

Three grams of the samples were decalcified by the addition of 6 N hydrochloric acid. Thus decalcified samples were added to an ampule made of hard glass, hydrolyzed with 6 N hydrochloric acid in a sealed evacuated ampule at 110°C for 18 hours. After having hydrolyzed, the samples were concentrated under vacuum, and analyzed. For the assay of tryptophane, hydrolysis was conducted in 6 N $Ba(OH)_2$ solution at 110°C for 18 hours in a sealed evacuated tube.

Amino acids thus hydrolyzed was dissolved in pH 2.2 citric acid buffer solution and analyzed by using Hitachi model 034 liquid chromatograph.

Results and Discussions

I. Differences of the amino acids composed of the protein of the exoskeletons of the crustacea. Differences among the amino acid compositions is the exoskeletons of the crustacea were shown in Fig. 1. In the crustacea the amounts of glucosamine are not cited.

The main components of the amino acids in the exoskeletons of the crab were alanine and glutamic acid, their contents were 14.4% and 13.5% respectively. The contents of proline and valine were also relatively high, 7 – 10% respectively.

In the prawn, the main components of the amino acids were glutamic acid, glycine and asparatic acid, the contents were 12.2% – 13.9% respectively. The contents of proline, alanine, leucine and valine were 7 – 10%.

In the lobster, the main components of the amino acids in the exoskeletons was glutamic acid, its contents were 14.3%. The contents of glycine, alanine and asparatic acid were around

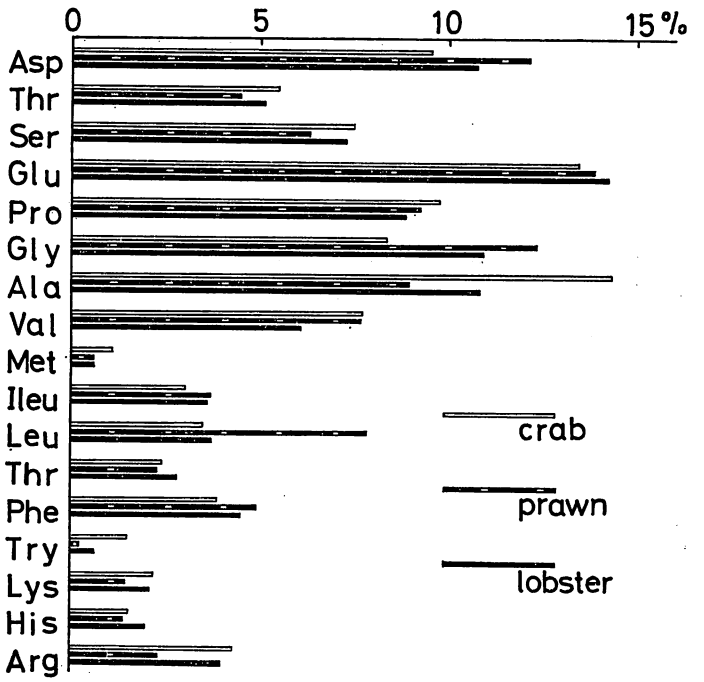

Fig. 1. The Amino Acid Composition of the Proteins of the Exoskeletons of the Crustacea.

10%, the contents of proline and serine were 7 – 9%.

There were no so much differences among the contents of the amino acids in the exoskeletons of the crustacea. As shown in Fig. 1, the amino acid composition of the exoskeletons of the crustacea is featured by relatively high contents of glutamic acid, alanine, glycine and asparatic acid. The contents of cystine, methionine, tryptophane and the basic amino acids were rather low. It would be very interesting to note that the main componet of the amino acids in the exoskeletons was alanine in the crab. In the lobster the contents of glycine and leucine were high. In the crab and lobster the amounts of arginine were higher than those in other species.

II. Differences among the compositions of the amino acids in the parts of the exoskeletons of the crustacea. The amino acids in the carapaces, abdomens and pleopods, and telsons of the exoskeletons of the prawn were also quantitativey analyzed. the results obtained are shown in Fig. 2. As the amino acid composed of the protein in the exoskletons of the prawn is featured by relatively low content of tryptophan, the content of tryptophan is not cited. In the carapaces the main component of the amino acid was proline, its contents were 21.1%. Generally speaking, the contents of glutamic acid, asparatic acid, alanine, glycine and valine were relatively high and also the acidic and neutral amino acids were featured by relatively high contents, but the contents of the basic amino acids were rather low.

In the abdomens and pleopods proline was the main component of the amino acids, its content was 20.4%. The contents of glutamic acid, glycine, valine and alanine were high as in the carapaces. In the telsons glycine was the main component of the amino acids and its content was 21.9%. The contents of proline, alanine, glutamic acid and valine were also high. The

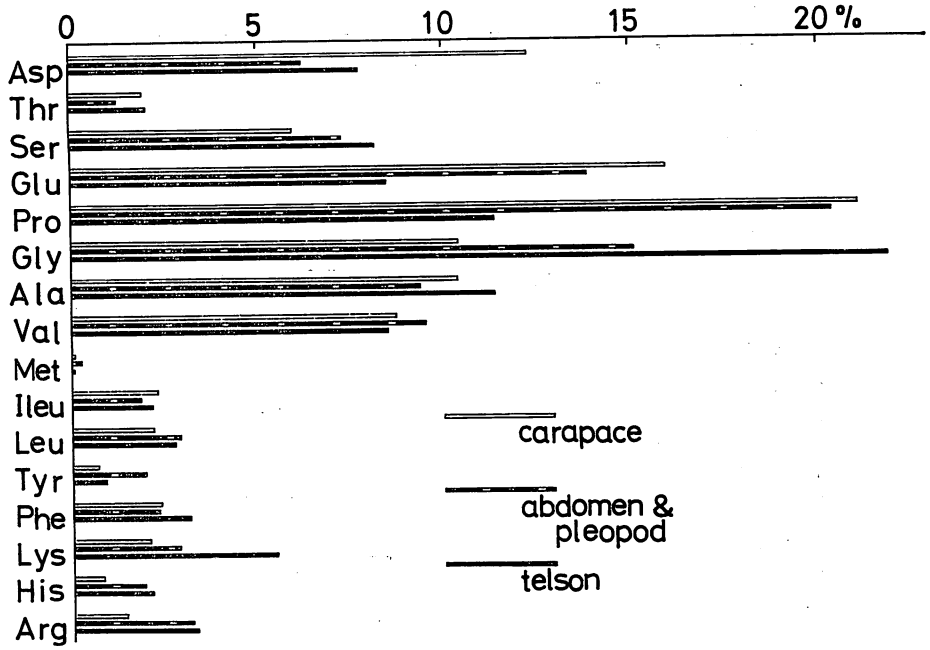

Fig. 2. The Amino Acid Composition in the Parts of the Exoskeleton of the Prawn.

contents of the basic amino acids were higher than those in the carapaces.

The carapaces are hard tissues, the contents of glutamic acid, proline and asparatic acid were relatively high, on the contrary, in the soft tissues such as the abdomens and telsons, the contents of glycine were rather high, and also the contents of the basic amino acids were higher than those in the hard tissues such as the carapaces.

Summary

The main components of the amino acids composed of the protein in the exoskeleton of the crustacea were glutamic acid, asparatic acid, alanine and glycine. The contents of proline and valine were relatively high. It was clarified that the protein of the exoskeletons of the crustacea was composed of glutamic acid, asparatic acid, alanine, glycine, proline, valine and serine in the order of the abundances of the contents. The main components of the amino acids composed of the muscle protein of the crustacea were glutamic acid and asparatic acid [9]. The existence of lysine, leucine and arginine was also confirmed in them [9]. It was conspicuous differences that the contents of basic amino acids in the muscle were higher than those in the exoskeletons.

The constituents of the amino acids composed of the protein in the exoskeletons of the crab, the prawn, and the lobster were similar to the amino acid pattern of elastin as shown in Fig. 1. The amino acid composition in the different species of the crustacea is featured by a little difference, but the ratio of those amino acids composed of the protein in the exoskeletons are almost same. The amino acids composed of the protein in the carapaces, abdomens and pleopods, and telsons of the exoskeleton were quantitatively analyzed and compared each other.

It is interesting to note that there are differences in the composition of the amino acids among the parts of the exoskeleton. Aspartic acid and proline were featured by relatively high contents in the carapaces, but the contents of glycine and basic amino acids were high in the abdomens and telsons.

Acknowledgment

The authors are indebted to Mr. M. YONEZAKI for his helpful assistance.

References

1) HIBIYA, T. (1972) : Symposium on Breeding of Crustacea. at Kochi, Jap. Soc. Sci Fish., p. 7.
2) YONGE, C. M. (1924) : Studies on the Mechanism of Feeding, Digestion and Assimilation in *Nephrops norvegicus*. *Brit. J. Exptl. Biol.*, **1**, 343–389.
3) PASSANO, L. M. (1960) : Molting and Its Contol. T. H. WATERMAN ed., The Physiology of Crustacea, Vol. **1**, Academic Press, New York & London, p. 473–536.
4) TOMBES, A. S. (1970) : An Introduction to Invertebrate Endocrinology. Acadamic Press, New York & London, p. 140–144.
5) KANAZAWA, A., TANAKA, N., and KASHIWADA, K. (1972) : Nutritional Requirement of Prawn–IV, The Dietary Effect of Ecdysones. *Bull. Jap. Soc. Sci. Fish.*, **38**, 1067–1071.
6) TAKEMOTO, T., OGAWA, S., and NISHIMOTO, N. (1967) : Isolation of the moulting hormones of insects from Achyranthis Radix. *Yakugaku Zasshi*, **87**, 325–327.
7) KRISHNAKUMARAN, A., and SCHNEIDERMAN, H. A. (1968) : Chemical control of moulting in arthropods. *Nature*, **220**, 601–603.
8) MIZUSHIMA, S., and AKABORI, S. ed. (1954) : Tanpakushitsu Kagaku, Vol. **2**, Kyoritsu, Tokyo, p. 142–143.
9) KONOSU, S., KATORI, S., AKIYAMA, T., and MORI, T. (1958) : Amino Acid Comosition of Crustacean Muscle Protein. *Bull. Jap. Soc. Sci. Fish.*, **24**, 300–304.