Biochemical Studies on Pearl. IX. Amino Acid Composition of Conchiolin in Pearl and Shell

By Shozo TANAKA, Hiroyuki HATANO and Osamu ITASAKA*

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An organic constituent of shells of molluscs was first named conchiolin by Frémy, and then it was recognized as a kind of protein by Schlossberger and Voit[1]. The amino acid constituents of this protein were studied by Krukenberg & Engel[1], Wetzel[2], Stary & Andratschke[3] and Friza[4]; and the presence of glycine, leucine, tyrosine, tryptophane and arginine was found in hydrolysates of conchiolin. Amino acid components of conchiolin prepared from different species of molluscs were also studied by Roche, Ranson & Eysseric-Lafon[5] and by Grégoire, Duchâteau & Florkin[6]. The composition of various kinds of conchiolin was reported to differ with the kinds of molluscs from which they were prepared. The authors have reported that about fifteen kinds of amino acids were found in hydrolysates of conchiolin prepared from the pearls and the shells of pearl oysters[7]. Halogen-containing amino acids, iodotyrosine and bromotyrosine, which are known as the peculiar components of screloproteins obtained from calciferous skeletons of marine invertebrata such as sponge and gorgonia, were not found in conchiolin of pearls and shells[7].

Electron-microscopic studies on conchiolin by Grégoire, Duchâteau & Florkin[6] and also by Grégoire[8] alone showed that the physical structure of conchiolin in the shells was reticulated unlike that of keratin or collagen which has fibrous structure. Conchiolin has been reported to be successfully fractionated into several preparations by suitable chemical reagents[6,9]. From chemical and crystallographical studies on the pearls and the shells of pearl oysters, the nacre of pearls was found to be identical with the nacreous layer of shells but quite different from the prismatic layer of shells[10]. These results may be of interest in studying the correlation between physical observations and chemical structures. This paper deals with the results of quantitative analyses of amino acids in the hydrolysates of conchiolin prepared from the pearl and the two layers of the shell of an oyster.

Experimental

Materials.—*The pearl and the shell of the pearl oyster.*—The pearls were harvested from the pearl oysters, *Pinctada martensii* (Dünker)**, which had been cultured in the pearl farm at Matoya Bay near Kashikojima during two winters after culture operations. The shells were obtained from the same pearl oysters. Both were rinsed thoroughly with tap water and with distilled water, and then dried at room temperature.

The nacreous substance of pearls.—This substance was obtained from crushed pearls, from which nuclei had been mechanically taken away.

The nacreous substance of shells.—This substance was obtained from shell valves by grinding off prismatic substances after removing the sea-weed

* Present address: School of Chemistry, Shiga University, Otsu.

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and other adhering materials from the surface.

The prismatic substance of shells.—This substance was obtained from the thin posterior shell margins. All materials were crushed and pulverized into powder of about ten mesh, washed with tap water, and dried at room temperature.

Conchiolin. — Conchiolin was prepared from the above materials by the method described in the previous paper[7]. The materials were immersed in 1 N hydrochloric acid solutions, which were frequently renewed, until no more evolution of carbon dioxide was observed in vacuo. Insoluble matter was washed several times with distilled water and was dialyzed against running water until the chloride ion in the dialyzate became undetectable. The conchiolin prepared from the nacre of pearls and from the nacreous substance of shells had a faint yellowish color, but that from the prismatic layer of shells, had a reddish-brown tint.

Methods.—*Preparation of the conchiolin hydrolysates.*—About 50 mg. of pulverized conchiolin was hydrolyzed with 5 ml. of 6 N hydrochloric acid at 100°C in a sealed tube for 30 hr. After small amounts of humin formed were filtered off, the hydrochloric acid was evaporated in vacuo repeatedly. The hydrolysate was dissolved into a mixture of *n*-butanol, *n*-propanol, and 0.1 N hydrochloric acid (1 : 2 : 1, v/v).

Amino Acid Analysis.—The analyses were carried out chromatographically by Stein and Moore's method on starch columns[11-13]. The preparatory procedure of the columns of potato starch (150 mesh), the selection of solvents and also the procedure for chromatography followed the description by Stein and Moore[11-13]. Columns of 0.9 cm. × 40 cm. of starch containing 30% of water were prepared and were washed under pressure of 150 mmHg until the washings became negative to a ninhydrin test. The hydrolysate was poured on the columns under pressure of 150 mmHg. Two kinds of solvent were used for elution. Solvent I was a mixture of *n*-butanol, *n*-propanol, and 0.1 N hydrochloric acid (1 : 2 : 1, v/v) and Solvent II was composed of *n*-propanol and 0.5 N hydrochloric acid (2 : 1, v/v). The eluting rate had been controlled as 1.5 ml. of the effluent per hour at the optimum velocity.

Each 0.5 ml. of the effluent fractions was collected by an automatic fraction collector. The amount of amino acids in each effluent fraction was estimated by the colorimetric ninhydrin method[14]. A solution of 250 ml. of ethylcellosolve containing 5 ml. of 0.01 M potassium cyanate, a solution of 2.5 g. ninhydrin in 50 ml. of ethylcellosolve and a 0.2 M citrate buffer solution adjusted to pH 5.4 with 1 N sodium hydroxide, were mixed. After each 0.5 ml. of the effluent aliquot was adjusted to pH 5.4 with 1 N sodium hydroxide in the case of Solvent II, though the pH was not controlled in the case of Solvent I, 0.5 ml. of the citrate buffer solution and 0.5 ml. of the ninhydrin reagent were added to the effluent. The mixed solution was allowed to stand for 20 min. in a boiling water bath. By heating the definite volume of the reaction mixture (5 ml.) with 60% ethanol, violet color was developed. Colorimetric determination was carried out by measuring the optical density of the coloring solution at 570 mμ by using a Beckman model DU spectrophotometer. Only for proline, the measurement was done at 440 mμ. The color yield of each amino acid was determined by comparing it with that of pure amino acid, respectively.

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Results and Discussion

The mensuration curves obtained with the hydrolysates of conchiolin from the nacre of pearls, the nacreous substance and the prismatic substance of shells, are shown in Fig. 1.

It was noticed that relatively large amounts of leucines, alanine, glycine, and cystine are contained in both conchiolin from the pearls and the shells. Proline was not recognized in conchiolin from the nacre of the pearl and the nacreous layer of the shell, while its existence was proved in conchiolin from the prismatic substance of the shell.

It is of interest that a relatively large amount of cystine is found in conchiolin. The fact may suggest a fibrous structure of conchiolin, like keratin in the hair and the skin which

TABLE I. AMINO ACID COMPOSITION OF CONCHIOLIN PREPARED FROM THE NACRE OF PEARLS, THE NACREOUS SUBSTANCE OF SHELLS AND THE PRISMATIC SUBSTANCE OF SHELLS*

Amino acid residue	Conchiolin**		
	Nacre of pearl	Nacreous substance of shell	Prismatic substance of shell
Leucines	9.2	13.6	9.0
Phenylalanine	1.1	0.0	16.9
Valine	2.1	0.5	1.0
Tyrosine	2.7	7.2	3.0
Methionine	0.4	0.4	0.0
Proline	0.0	0.0	7.9
Alanine	14.0	16.3	4.6
Glutamic acid	3.1	1.5	1.5
Threonine***	0.6	9.3	0.3
Aspartic acid	6.2	2.2	3.7
Serine***	5.4	2.1	3.5
Glycine	24.3	12.8	16.8
Ammonia	0.7	0.5	0.1
Arginine	7.2	15.3	10.2
Lysine	7.4	3.3	1.5
Histidine	0.5	0.0	1.0
Cystine/2	12.2	11.8	14.7

* Determined by chromatography of the acid hydrolysates on columns of starch.
** Presented as gram of amino acid residue per 100 g. of protein.
*** Corrected to the hydrolytic damage of threonine (5%) and serine (10%).

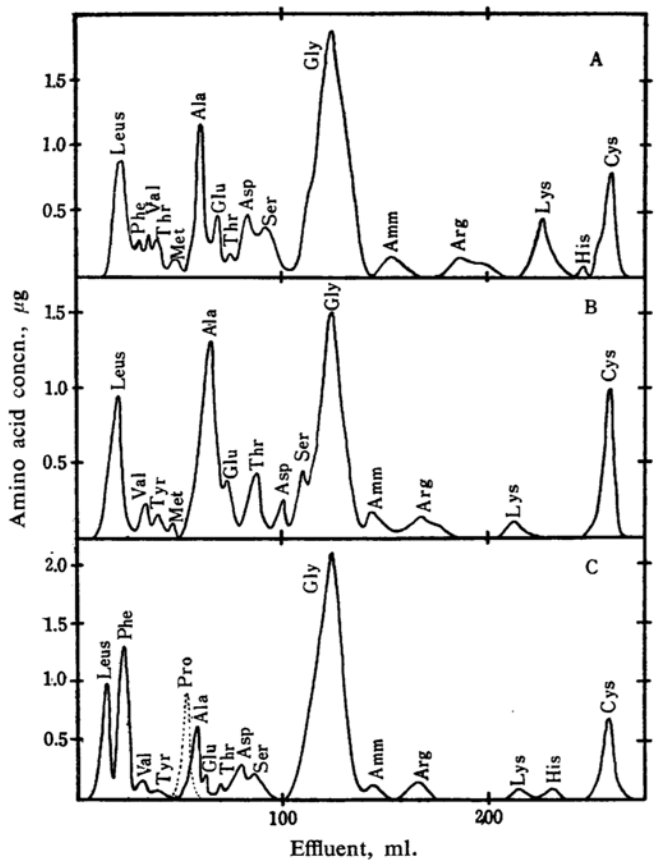

Fig. 1. Starch column chromatography of each 30 hr. hydrolysate of conchiolin prepared from the nacre of pearls (A), the nacreous substance of shells (B) and the prismatic substance of shells (C). Optical density at 570 mμ is plotted on the ordinate for the effluent fractions separated by a 0.9 cm. × 40 cm. column of starch, after treatment with the ninhydrin reagent. The values of optical densities have been corrected by baseline absorption.

causes the elasticity of the tissues. As shown in Table I, conchiolin in the nacreous layer of the shell and that in the pearl contain less phenylalanine and proline, and more alanine than that in the prismatic layer.

Summary

Conchiolin, a kind of screloprotein, was isolated from the pearl and the shell of the pearl oysters. The amino acid composition of conchiolin was determined by the chromatographic method with the starch column. Relatively large amounts of leucines, alanine, glycine and cystine were found in conchiolin.

Remarkable differences were found in the contents of phenylalanine, alanine and proline between conchiolin from the nacreous substance and that from the prismatic substance.

Department of Chemistry
Faculty of Science
Kyoto University
Sakyo-ku, Kyoto