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AMINO ACID COMPOSITION OF HEATED SCALLOP SHELLS

by

Masahiko Akiyama

(with 2 tables)

(Contribution from the Department of Geology and Mineralogy,
Faculty of Science, Hokkaido University, no. 1531)

Abstract

Shells of a modern scallop, *Patinopecten yessoensis* were heat-treated in order to examine diagenetic changes in amino acid compositions of conchiolin proteins. Amino acid compositions of the insoluble fractions after the heating are different from that of the original, but resemble to the compositions of the fossil shells, showing less aspartic acid and serine contents.

These changes are probably due to the presence of different conchiolin proteins of the scallop, and discussed in relation to the change of amino acid compositions of the scallop during diagenesis.

Introduction

Amino acids have been found in free and/or combined states in various fossils. Insoluble fractions of the fossil shells decalcified with 2N HCl at room temperatures release amino acids after 6N HCl hydrolysis. However, the amino acid composition in the insoluble fraction of fossil shells is different from that of the conchiolin proteins of the modern shell of the same species. Such difference of amino acid compositions has been reported in the oyster shells (Matter et al., 1969; Totten et al., 1972) and in the scallop shells (Akiyama, 1971). The difference is in the compositions of aspartic acid, glycine, and serine which are most abundant in the conchiolin proteins but recovered less from the insoluble fractions of fossil shells (Table 1).

Grégoire et al. (1955) suggested that the conchiolin was composed at least of three different kinds of proteins in the nacreous layers of the shell. If the conchiolin proteins of scallop and oyster shells both in foliated structure are composed of different kinds of proteins to the thermal degradation in sediments, the more stable conchiolin protein(s) remains in the fossils while the unstable ones undergo the degradation more readily. It may be this reason that the amino acid composition of the fossil shells is different from that of the modern shells.

In this study a simulation experiment was conducted heating modern

Table 1 Comparison of the amino acids in the recent and fossil shells

Amino acid	Recent	Pleistocene	Miocene
Lys	26	32	32
His	3	–	–
Arg	22	–	–
Asp	262	130	111
Thr	23	37	62
Ser	216	91	87
Glu	50	111	101
Pro	26	42	56
Gly	265	182	183
Ala	52	110	112
Cys/2	–	1	–
Val	13	63	57
Met	2	6	5
allo-Ile	–	6	1
Ile	7	38	38
Leu	17	86	86
Tyr	8	26	24
Phe	8	31	37
Total (μM/g)	14.53	0.17	0.22

scallop shells at an elevated temperature in order to examine a diagenetic change of the amino acid composition and to discuss the thermal stabilities of the shell proteins.

Materials and methods

The modern shells of the scallop, *Pationopecten yessoensis* (Jay) were obtained from the shore of the Uchiura Bay, southwestern Hokkaido, Japan. Several right valves of about 5 cm in height were pulverized after removing the periostracum from the valves and washing thoroughly. One gram portions of the pulverized sample were sealed in glass ampules under nitrogen atmosphere, and heated at 138 ± 3°C for periods of 25, 50, 76 and 118 hrs.

After heating the contents of the ampule were dissolved in 20 ml of 2N HCl to obtain the complete decalcification. The decalcified materials were centrifuged at 7,500 rpm for 10 min to separate a sediment containing insoluble proteins from a supernatant containing soluble products of protein decomposition. The supernatant was added by an appropriate amount of HF to remove calcium ions dissolved in the solution. Then, the soluble (S) and insoluble (I) were hydrolysed with 6N HCl in a closed ampule under nitrogen

atmosphere at 108°C for 22 hrs. The hydrolysates were dried by evaporation under reduced pressure at 50°C and were redissolved in an appropriate amount of 1/100 N HCl solution for amino acid analysis.

The amino acid analysis was performed by an automatic amino acid analyser (JLC-6AH) under the following conditions.

Column: 8 x 500 mm and 8 x 150 mm packed with Jeol resin LCR-2
Column temperature: 52°C
Na-citrate buffer: pH 3.22, 4.25 and 5.27; concentration 0.2N
Flow rate: Sampling pump 0.42 ml/min, ninhydrin pump 0.21 ml/min
Detection: Photometer with 10 mm cell by wavelength of 570 nm and 440 nm for ninhydrin positive compounds

Results and discussions

The total amount of amino acids in the insoluble and soluble fractions of the samples after the heating, and the amino acid composition of these fractions, are given in Table 2. Corrections for hydrolytic loss of threonine and

Table 2 Amino acid compositions of the soluble and insoluble fractions in unheated and heated scallop shells (residues/1000 residues)

Time of Heating (hours)		0		25		50		76		118	
Amino acid	Fraction	I	S	I	S	I	S	I	S	I	S
Lys		27	–	32	–	38	–	37	–	44	29
His		3	–	6	–	6	–	5	–	8	–
Arg		20	7	48	11	55	12	43	13	48	10
Asp		263	263	152	348	145	345	160	354	157	339
Thr		20	25	61	12	54	11	52	11	50	11
Ser		224	204	89	112	80	97	77	85	81	50
Glu		54	86	92	61	91	62	90	66	84	66
Pro		27	29	62	24	56	26	54	26	56	26
Gly		250	293	227	319	205	324	210	320	225	324
Ala		47	64	73	74	74	80	73	79	77	98
Cys/2		+	–	–	–	–	–	–	–	–	–
Val		13	10	46	6	50	7	46	8	44	7
Met		5	+	–	2	27	2	19	2	15	2
allo-Ile		–	–	–	+	–	+	–	+	–	1
Ile		9	5	28	3	30	2	35	4	25	4
Leu		22	15	40	17	42	17	52	22	39	23
Tyr		10	+	15	7	21	7	24	7	23	6
Phe		7	+	29	3	27	3	23	4	25	3
Total (μM/g)		25.04	2.40	1.81	22.90	2.19	25.53	3.16	24.04	2.26	19.20

serine analysed are 1.05 and 1.03, respectively. Amino acids in the insoluble (I) fraction originates from the insoluble conchiolin, and the soluble (S) fraction contains soluble proteins, polypeptides of different chain lengths and free amino acids decomposed from the original conchiolin proteins.

The heat treatment at 138°C changed more than 90% of the conchiolin proteins of the scallop shells into soluble ones by the 25 hr heating. No remarkable change in both the amount and composition of the amino acids was observed after the prolonged heatings.

Total amounts of amino acids of the sample did not change significantly after heating of the various periods. Dungworth et al. (1975) demonstrated that alanine, leucine and the other neutral amino acids might not be pyrolysed at the temperatures lower than 200°C within a period of several days, because of the high activation energies of the amino acids. This is almost true except for serine, one of the most thermally unstable amino acids in the neutral acids.

The following facts and considerations are drawn from the comparative study of the amino acid compositions between the heated and unheated samples shown in Table 2.

1) There are no significant differences in the amino acid compositions between the soluble and the insoluble fractions of the unheated scallop shells.
2) The insoluble fraction decreases drastically by the 25 hr heating and gives the different amino acid composition from the original. The large differences are observed in a decrease of aspartic acid and serine contents and in a distinct increase of valine, isoleucine and phenylalanine contents.
3) On the contrary, the soluble fraction has larger amount of aspartic acid and glycine, and smaller amount of valine, isoleucine and phenylalanine throughout the periods of heating.
4) Serine, one of the most unstable amino acids decreases in content both in the soluble and insoluble fractions, which suggests the decomposition of the acid.

From the considerations of these findings it is suggested that the majority of the conchiolin proteins become soluble rather rapidly by the heat treatment, having a high content of aspartic acid, while a minor portion of the proteins remains consistently as insoluble having less aspartic acid content. A preliminary result of partial hydrolysis of the soluble proteins with dilute acetic acid in the unheated shell shows a high content of serine as well as aspartic acid. However, the 6N HCl hydrolysis yields less amount of serine both from the soluble and insoluble fractions in the heated shells. There is no question that serine decreases after the heat treatment because of its low stability to heat.

In conclusion, the amino acid composition of the proteins of the fossil scallop shells closely resembles to those of the insoluble fractions in the heated

scallop shells, and the heating experiments can simulate the diagenetic change of the fossil organic compounds.

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