NOTE

Possible occurrence of diatom cell wall-derived amino acids in Okhotsk Sea sediments

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Total amino acids in marine sediments was determined by the combined use of hydrochloric acid hydrolysis (A fraction) and hydrofluoric acid treatment (B fraction). The B fraction comprised 5 to 10% of the total amino acids. The amino acid composition of B fraction indicated a characteristic feature that threonine and serine were relatively abundant. The amino acid composition of B fraction resembles that of diatom cell walls as pointed out by HECKEY *et al.* From this fact, it is interpreted that the B fraction originated from the diatom cell walls, not from the amino acid associated with aluminosilicate such as clay in sediments.

INTRODUCTION

The aspects of the forms of amino acids in marine sediments have been investigated by several authors (ERDMAN *et al.*, 1956; CLARKE, 1967; BROWN *et al.*, 1972; MITTERER, 1972). DEGENS *et al.* (1961) have reported in their early studies in this field that the amino acid contents of sediment samples collected in the Santa Barbara Basin, showed vertically irregular changes. They have presumed that the changes depend upon the redox conditions of surface sediments immediately after accumulation on sea bed. STEVENSON and CHENG (1969, 1970) have reported the presence of a fraction extractable with HF. Therefore, the total amino acid in sediments is determined as the sum of the fraction hydrolyzable with HCl and the fraction originated from HF pretreatment.

Although the determination of total amino acids was recently made with the STEVENSON and CHENG's method (e.g. DUNGWORTH *et al.*, 1977), satisfactory results have not been obtained with respect to the estimation of total amino acid and the proportion of non-hydrolyzable fraction to the total amino acid.

We have studied on the early diagenesis of

organic nitrogen compounds in core sediments of the Okhotsk Sea (MONTANI *et al.*, 1979; MAITA *et al.*, 1982). Particularly, the forms of amino acids have been examined in a sample taken at the central part of the Okhotsk Sea.

The objective of this report is to explain the origin of the fraction which shows the resistivity against acid hydrolysis from a point view of amino acid composition.

MATERIALS AND METHODS

Sediment core samples submitted to this study were collected at the central part of the Okhotsk Sea (53°53'N, 149°00'E, 1,070m depth) in the 13 Cruise of R/V D.-Mendeleev, from August to September, 1974.

Several grams of a dried sediment sample sieved through 0.25mm mesh was weighed, put into a glass ampoule with a 10 times volume (w/v) of 6M-HCl and aerated with nitrogen gas. The sealed samples were hydrolyzed in an oil bath at 105°C for 24hr. Hydrolyzed samples were filtered through glass fiber filter (Whatman GF/C). The filtrates were passed through a cation exchange column (Dowex 50W X8, H form, 1 × 25cm) after adjusting pH to 2.0 - 2.2

with a dilute NaOH solution. The column was washed with 0.01M-HCl and eluted with 2M-NH₄OH. An effluent was vacuum evaporated and an aliquot of concentrates was injected to a Liquid Chromatograph (Hitachi Co. Model 034). This fraction is expressed as A fraction. The solid phase removed by filtration was sufficiently washed with re-distilled water and dried in an oven. One gram of the dried samples (removing A fraction) was treated with a few ml of the equivalent mixture of 5M-HF and 0.1M-HCl in a teflon beaker with occasional shaking. Most of the mineral lattice or biogenic opal was destroyed by this operation. The wet sample was dried in a desiccator in vacuum to remove HF. The dried sample was hydrolyzed with 6M-HCl. The concentration and composition of amino acids were examined in the similar fashion described above. This fraction was expressed as B fraction.

On the other hand, the concentrations of biogenic opal were determined by the method of HURD (1973). Twenty to 50mg of a dried sediment was suspended in 125ml of 5% Na₂CO₃ solution and allowed to stand for at least 5hr at 85°C with occasional shaking. After centrifuga-

Fig. 1. Vertical distribution of total carbon, nitrogen, amino acid (B fraction) and biogenic opal in the sediment core sample.

tion, 1 ml of the supernatant solution was diluted with re-distilled water to 25ml. Reactive

Table 1. Composition and concentration of amino acids in A and B fractions

Depth (cm)	0-10		60-70		120-130		180-190		240-250	
Amino acid (mole %)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Asp	7.3	2.7	8.9	5.0	7.4	7.3	9.5	tr.	13.2	5.3
Glu	5.6	2.3	7.5	5.8	4.6	12.8	8.6	tr.	10.7	7.5
Thr	7.3	22.2	8.2	5.6	6.5	8.0	7.8	tr.	7.6	6.4
Ser	7.0	5.8	5.6	7.1	6.0	22.1	6.4	24.1	7.1	17.6
Gly	22.4	54.2	20.7	19.7	21.5	27.0	22.4	28.6	21.6	24.5
Ala	11.1	1.6	11.3	7.2	19.0	4.4	13.1	tr.	13.8	9.0
Val	4.5	3.7	5.3	7.5	7.0	8.0	5.8	33.2	4.2	2.7
Ileu	3.1	1.0	3.0	6.8	2.5	tr.	3.2	tr.	1.8	3.2
Leu	3.8	tr.	3.9	5.2	8.0	tr.	4.6	tr.	3.8	1.1
Pro	4.9	n.d.	6.2	n.d.	3.1	n.d.	5.2	n.d.	3.5	n.d.
Met	0.5	n.d.	0.2	tr.	2.0	n.d.	0.3	n.d.	1.2	2.2
Tyr	2.8	n.d.	6.0	n.d.	n.d.	n.d.	tr.	tr.	0.3	n.d.
Phe	8.4	3.3	2.4	8.9	1.8	n.d.	0.7	tr.	2.8	n.d.
Lys	5.6	3.2	5.5	12.8	5.2	tr.	6.0	14.1	5.8	9.6
His	1.2	tr.	1.0	2.0	1.1	n.d.	1.2	tr.	0.5	2.7
Arg	4.6	tr.	4.2	6.5	4.5	10.6	5.4	tr.	2.2	8.0
Total (μmol/g)	27.7	3.8	22.3	0.93	2.39	0.23	3.50	0.20	3.76	0.19

tr., trace; n.d., not detected.

silica was determined according to STRICKLAND and PARSONS (1968).

RESULTS AND DISCUSSION

The vertical profiles of amino acids (B fraction) and biogenic opal are shown in Fig. 1. The amino acid composition of two fractions are shown in Table 1.

As shown Table 1, the concentrations of total amino acids (A + B fractions) decreased with depth. The pronounced changes of amino acid composition were not observed vertically in A fraction, though significant changes of amino acid composition was recognized for B fraction. In the surface sediment, total amino acid was 3.65mg/g. Sediment type in this layer may be diatom ooze, since the biogenic opal content was ca. 50% of the dry sediment. In the layers of 60-70cm, composed of the mixture of diatom, silt and fine sand, amino acid contents were 2.76mg/g, while in the deeper layer below 1.2m depth composed of clayey sediment containing diatom the contents were about 0.5mg/g.

The B fraction obtained by the HF treatment ranges from 5 to 10% of total amino acids (A + B fractions). Hydroxy amino acids, i.e.

threonine and serine, were markedly concentrated in B fraction, compared with the amino acid composition of A fraction.

ABELSON (1959) has suggested that the amino acids containing hydroxyl group are more labile than the other amino acids in aquatic media. Therefore, a reasonable explanation is required for the presence of hydroxy amino acids in B fraction.

HECKEY *et al.* (1973) has analyzed 6 diatom species and suggested that threonine and serine in diatom cell wall are more abundant and amino acids are less abundant than in the cell. We compared amino acid composition of diatoms and sediment. As shown in Table 2, the amino acids of diatom cell walls and B fraction of sediments show a resemblance to each other with respect to the concentration of hydroxy amino acids. From this qualitative resemblance, it is interpreted that the hydroxy amino acids derived from diatom cell walls have been preserved in a stable form and escaped the decomposition by microorganisms. The ratio of the HF fraction to total amino acids was ca. 10% in surface sediments (0-10cm, diatom ooze) and ca. 4-5% in deeper layer sediments (clayey mud), respectively.

Table 2. Characteristics of amino acid in A and B fractions and compositions of amino acid in diatom cell walls and cell contents.

(Thr+Ser)/Total (mole %)	Depth (cm)	Fraction		Diatom*	
		(A)	(B)	cell wall	cell content
	0- 10	14.3	28.0	(a) 16.7	15.5
	60- 70	13.8	12.7	(b) 19.0	—
	120-130	12.5	30.1	(c) 31.8	20.5
	180-190	14.2	24.1	(d) 31.6	16.1
	240-250	14.7	~24.0	(e) 33.2	17.1
				(f) 16.4	13.3
	mean S.D.	13.9±0.85	23.8±6.7	24.8±8.2	16.5±2.6
(Asp+Glu)/Total (mole %)	0- 10	12.9	5.0	(a) 16.5	21.5
	60- 70	16.4	10.8	(b) 17.8	—
	120-130	12.0	9.9	(c) 14.6	19.6
	180-190	14.7	tr.	(d) 12.4	19.0
	240-250	23.4	12.8	(e) 15.4	20.6
				(f) 18.5	21.1
	mean S.D.	15.9±4.5	9.6±3.3	15.9±2.2	20.4±1.0

* after HECKEY *et al.* (1973); (a) *Navicula pelliaulosa*, (b) *Melosira nummuloides*, (c) *Melosira granulata*, (d) *Cyclotella stelligera*, (e) *Cyclotella cryptica*, (f) *Nitzschia brevirostris*.

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