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EXPERIMENTAL ALTERATION OF THE NAUTILUS SHELL BY FACTORS OF DIAGENESIS AND METAMORPHISM

Part II. — Amino acid patterns in the conchiolin matrix of the pyrolysed modern mother-of-pearl

BY

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In attempts at identification of the organic residues detected previously in fossil mother-of-pearl (GRÉGOIRE, 1958, 1959 a b, 1966 b; GRANDJEAN, GRÉGOIRE and LUTTS, 1964) nacreous layers of the shell wall of the modern *Nautilus* have been exposed to factors involved in fossilisation, such as heat and pressure, in presence and in absence of oxygen.

In former papers (GRÉGOIRE, 1964, 1966 a b, and in Part I, 1968), the structural aspects of a stepwise degradation of the conchiolin matrix have been examined by means of the electron microscope in samples heated at temperatures in the range of 150 °C to 900 °C for periods of time extending from 5 minutes to 21 days. The results showed that pyrolysis of modern mother-of-pearl can reproduce a number of structural changes observed in the electron microscope in fossil remnants of conchiolin matrix.

The present paper deals with the changes produced by pyrolysis in the amino acid patterns of the polypeptidic residues of the material. These patterns have been compared with those recorded previously in fossil nacreous conchiolin (nautiloids and ammonoids) from various geological periods (Oligocene to Devonian) (GRÉGOIRE, 1964; 1966 a b; GRÉGOIRE et VOSS-FOUCART, 1970; VOSS-FOUCART et GRÉGOIRE, 1971).

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MATERIAL AND METHODS

Fragments of the inner nacreous layers of the shell wall of *Nautilus pompilius* LINNÉ were selected in the living chamber, more adorally than the muscle scars, in order to avoid the myostracum (helle Schicht).

The procedures used for pyrolysis have been described in details in part I.

In a first group of experiments, fragments were placed in ceramic boats and heated dry in electric ovens and muffle furnaces to temperatures ranging from 150 °C to 900 °C for various periods of time (5 hours to 21 days).

In another group, quartz tubes (vitriosil) containing fragments of nacre, were sealed under vacuum, then heated dry to temperatures in the range of 150 °C to 900 °C for 5 hours.

In still another group, sea or distilled water was added to the samples. In this group, the inner volume of the tube was 5.77 cm^3. The total volume of the fragments was about 30 mm^3 and that of the fluid added about 40 mm^3. The pressure inside the tubes, appreciated by Dr. HESTERMANS (I. B. H. P.), reached values in the range of 1 bar (150 °C - 5 hours) and 38 bars (900 °C - 5 hours).

The samples selected for electron microscopy were decalcified in saturated aqueous solutions of E. D. T. A. (titriplex III Merck) at pH 4. and pH 8. The samples heated in open vessels in the range of 600 °C to 800 °C could not be demineralized in titriplex and were dissolved, in some cases incompletely, in 3 N solutions of hydrochloric acid.

For the biochemical analyses, fragments were demineralized in 0.5 N solutions of hydrochloric acid. The soluble and insoluble residues were dialyzed, hydrolyzed, and analysed by ion exchange chromatography, using a Beckman Spinco automatic analyser (see details in VOSS-FOUCART and GRÉGOIRE, 1971).

In samples heated dry or wet (sea water) to 150 °C., two fractions, soluble and insoluble in hydrochloric acid, were isolated and analysed separately.

RESULTS

Tables 1 and 2 give the amino acid composition of the hydrolyzed samples. This composition is expressed as the number of amino acid residues per cent total amino acid residues.

The results may be summarized as follows :

1. Polypeptides are present in all the pyrolysed samples.

2. The amino acid pattern of the insoluble fraction of the conchiolin from samples heated in open vessels for 5 hours at 150 °C does not differ, except for a slight increase in Alanine, from that of conchiolin

from unheated samples. Comparison of the 150 °C samples heated in open vessels with a sample heated with sea water in a sealed tube only reveals in the latter higher values in Glutamic acid (slight), and in Alanine (more distinct) and a slightly lower value in Serine and Glycine.

The hydrochloric acid used for decalcification of the pyrolysed and of the control samples contained non dialysable peptides. These peptides differ in quality and in amount in both groups : the soluble fraction is distinctly more important in the pyrolysed samples than in the controls. In addition, the percentage of Glycine is by far higher than that of the same amino acid in the soluble fraction of the control sample.

3. Heating in open vessels at 225 °C for 5 hours thoroughly alters the relative proportions of the amino acids of the polypeptidic residues : Alanine becomes predominant (42.5 %) followed by Glycine (26.2 %). In samples pyrolysed for 21 days under the same conditions, Glycine amounts to about 50 per cent of the total amino acids. The four other relatively important amino acids are, in decreasing order, Aspartic acid, Alanine, Serine and Glutamic acid.

4. The polypeptidic residues of the three samples heated in open vessels at 300 °C (Fig. 3) are characterized by the predominance of Glycine and by a relatively low content in Alanine, as in the 225 °C - 21 days sample.

However, the polypeptidic residues of these three 300 °C samples differ from each other, namely in the relative proportion of Serine, Glycine, Glutamic acid, Alanine and Arginine.

In samples heated in sealed tubes at the same temperature, either dry or with sea or distilled water (Fig. 4), the composition of the polypeptidic conchiolin residues differs from that of the samples heated in open vessels : the amounts in Glycine are much lower, those of Serine and Glutamic acid higher.

5. From the 500 °C stage up to 900 °C, the polypeptidic composition does not distinctly change. Glycine, Serine and Glutamic acid are the three predominant amino acids, followed by Alanine and Aspartic acid. This composition is similar in samples heated in open vessels and dry and wet in sealed tubes. The Serine content only is slightly higher in the samples dry heated in sealed tubes. The concentration in Glutamic acid is more important in sealed tubes in the presence of sea water.

DISCUSSION

1. Origin of the polypeptides recorded by biochemical analysis

Thermal energy has been used for polymerisation of free amino acids to polypeptides. The resulting products can contain the eighteen amino acids common to proteins (see disc. in Part 1 : Fox and Middlebrook,

TABLE 1. — *Nautilus pompilius* L. Protidic components of the pyrolyse

	Asp	Thr	Ser	Glu	Pro	G
1 Pyrolysis in open vessels						
150 °C. 5 hours (1010)	7.8	1.3	9.3	4.5	+	31
225 °C. 5 hours (721-225)	5.2	0.4	3.2	5.4	0.7	26
225 °C 21 days (721-225-21)	13.4	2.1	8.4	7.2	tr	46
300 °C. 5 hours (721-300)	7.8	1.4	7.7	6.4		49
300 °C. 5 hours (991)	6.3	3.8	14.7	10.4	2.1	36
300 °C. 5 hours (904)	7.7	3.1	13.2	10.3	tr	39
500 °C. 5 hours (905)	8.3	5.1	23.0	13.5	tr	21
700 °C. 5 hours (906)	7.6	4.3	21.7	14.7	tr	18
900 °C. 5 hours (907)	8.1	5.4	11.2	11.9	4.9	15
900 °C. 5 hours	8.0	4.4	19.5	15.2	+	18
2 Pyrolysis in tubes sealed under vacuum						
— dry						
300 °C. 5 hours	8.5	4.9	22.4	16.4	+	20
500 °C. 5 hours	10.6	5.7	25.6	9.4	+	19
700 °C. 5 hours	10.0	4.9	26.8	9.0	+	18
900 °C. 5 hours	8.5	4.7	20.9	14.0	+	21
— with sea water						
150 °C. 5 hours (997)	7.3	1.3	8.0	5.5	+	27
300 °C. 5 hours (992)	7.5	4.4	16.7	12.6	+	23
500 °C. 5 hours (994)	9.8	4.8	17.1	20.4	tr	18
700 °C. 5 hours (996)	10.7	4.8	18.8	20.2	+	18
— with distilled water						
300 °C. 5 hours (1006)	9.7	6.1	16.8	10.6	+	22
500 °C. 5 hours (1014)	10.4	4.5	17.1	14.1	tr	23

TABLE 2. — *Nautilus pompilius*
Insoluble residue and fraction which passes into solution in hydrochlor

	Asp	Thr	Ser	Glu	Pro	G
Normal conchiolin						
Insoluble residue	7.1	1.2	9.6	4.4	0.7	31
Fraction soluble in 0.5 N HCl	14.7	5.6	12.5	8.8	7.0	24
Pyrolysed conchiolin						
150° (open vessel) :						
Insoluble residue	7.8	1.3	9.3	4.5	+	31
Fraction soluble in 0.5 N HCl	14.2	1.7	11.0	5.1	1.9	44
150° (with sea water in sealed tubes) :						
Insoluble residue	7.3	1.3	8.0	5.5	+	27
Fraction soluble in 0.5 N HCl	19.1	1.6	9.1	6.1	+	37

other-of-pearl. Amino acid residues per cent total amino acid residues

Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
25.8	tr	1.6	1.2	1.3	2.1	1.0	6.0	0.3	0.5	5.3
42.5	0.3	1.8	0.1	1.1	2.2	0.7	5.5	3.7	0.3	0.4
8.7	—	1.9	—	1.6	2.1	1.2	1.9	3.9	1.4	+
10.9		1.7			1.4			4.2	3.0	6.4
7.5	tr	2.2		1.0	1.9	1.3	1.7	5.0	3.1	2.9
10.5	tr	+	tr	1.4	2.8	tr	tr	6.8	4.1	+
8.8	0	+	tr	2.4	4.7	+	+	8.8	4.1	+
8.6	0	3.8	0	2.0	3.6	+	+	9.0	5.5	+
9.7	0	5.1	tr	3.1	5.5	2.1	3.3	6.0	3.2	4.8
8.2	0	3.8	0	2.0	2.4	+	+	7.4	4.6	5.4
8.7	0	4.5	+	2.2	3.7	+	+	4.0	4.2	+
9.0	0	4.0	tr	2.3	3.2	+	+	8.5	2.4	+
9.3	0	3.6	tr	4.5	5.3	+	+	7.7	+	+
7.0	0	5.4	tr	+	3.9	+	+	9.3	4.7	+
32.5	0	1.6	0.5	1.3	2.3	1.1	5.0	0.5	0.4	5.6
10.0	tr	3.3	tr	1.5	2.7	1.4	1.8	7.7	3.7	3.2
7.6	0	3.9	tr	2.7	4.4	2.2	2.2	6.4	+	tr
6.3	0	3.4	tr	4.0	4.2	+	+	6.2	2.9	+
13.5	0	4.8	tr	3.9	5.5	+	+	6.1	+	tr
8.4	0	4.1	tr	2.4	4.5	1.9	2.4	6.9	+	+

ormal and pyrolysed nacre conchiolins.
id. Amino acid residues per cent total amino acid residues

Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
26.6	tr	1.5	0.5	1.4	2.1	1.8	5.1	0.6	0.5	5.3
8.4	tr	3.3	+	2.1	4.8	1.8	3.9	2.1	+	+
25.8	tr	1.6	1.2	1.3	2.1	1.0	6.0	0.3	0.5	5.3
12.4	—	1.2	tr	1.4	1.2	0.5	2.2	0.6	0.6	1.4
32.5	—	1.6	0.5	1.3	2.3	1.1	5.0	0.5	0.4	5.6
11.6	—	1.9	—	1.5	1.9	1.8	5.0	1.0	0.6	1.6

1954; HARADA and FOX, 1964; FOX, 1964; HARADA, 1967; ROHLFING, 1967). However, the routine control with the electron microscope of the material analysed here permitted to discard immediately this possibility : all the residues consisted of interlamellar conchiolin matrices variously altered by pyrolysis (see Part 1 and the present figures).

2. Thermal changes in conchiolin (biochemical composition and ultrastructure)

Comparison of the control and the pyrolysed mother-of-pearl of the modern *Nautilus* shell shows the following changes :

A. — In the 150 °C samples heated in open vessels (group 1), the structural alterations in the conchiolin matrices were moderate or indistinct (Fig. 1). Similarly, the biochemical analysis does not reveal important transformations of the insoluble conchiolin matrix. However, in the 150 °C samples heated in sealed tubes with sea or distilled water, there are slight modifications, namely as regards Glycine and Alanine. Moreover, the amount of the insoluble fraction is lower in the samples heated wet.

These biochemical results are in agreement with those of HARE and MITTERER (1967-1968) obtained on shell fragments of *Mercenaria* heated at 160 °C : little or no reaction appeared in samples of *Mercenaria* heated dry, in contrast with the samples in which water was present.

As pointed out above, the polypeptidic fraction which is soluble in the hydrochloric acid used for decalcification is more important in the pyrolysed samples than in the controls, and differs from that of the latter with regard to the relative concentration of the amino acids. These results suggest a trend toward formation of soluble breakdown products, in agreement with the data of VALLENTYNE (1969) on pyrolysis of amino acids in the Pleistocene *Mercenaria* shells. According to this author, over 90 per cent of the total N in the shell powder pass into solution during the early phases of pyrolysis.

In our material, the insoluble residue left by pyrolysis of a sample of nacre at 150 °C for 5 hours in presence of distilled water represents about 11 per cent of the conchiolin amount present in an identical weight of normal unheated nacre.

B. — In the 225 °C sample (open air) the relative concentrations of the amino acids are modified. These concentrations differ with the duration of pyrolysis. The structural changes observed in the electron microscope are in agreement with these data : in the 225 °C - 5 hours stage the changes do not distinctly differ from those recorded in the 150 °C - 5 hours stage (see Part 1, p. 5 and Figs 2 to 8). On the other hand, the 225 °C - 21 days stage shows more distinct alterations (beginning fragmentation of the trabeculae : see Part 1, p. 6 and Fig. 10).

C. — The biochemical alterations in the 225 °C. - 21 days (open air) and in the 300 °C (open air) samples do not greatly differ.

The conchiolin alterations, observed in the electron microscope, in the range of 200-300 °C, have been described in details in previous studies (GRÉGOIRE, 1968, 1972). Persistance of the nautiloid pattern (Figs. 2 and 3), variously altered (e.g. widening and coalescence of the trabeculae) (Fig. 2), in scattered conchiolin fragments, swelling, flattening and progressive dislocation of the trabeculae of the matrices into rods and pebble-shaped corpuscles (Figs. 3 and 4) characterized in other fragments the modifications of the samples.

D. — From the 500 °C stage up to 900 °C (in open vessels and in sealed tubes), the composition of the polypeptidic residues of conchiolin seems to be stabilized : there are no distinct subsequent alterations in the amino acid patterns with increase in temperature. This distribution of the relative amounts of amino acids is similar in the samples heated in open vessels and in sealed tubes.

Examination of this material in the electron microscope reveals similar changes in the samples heated dry and wet in sealed tubes (Figs 5, 6 & 7). The samples heated in open vessels (not shown) are distinctly more brittle than those heated dry or wet in sealed tubes. In the former group disintegration of the original trabeculae into corpuscles, in the latter flattening and coalescence of the original trabeculae into fenestrate membranes are predominant. However, in each group (open vessels, sealed tubes , with further increase in temperature, the characteristic alterations observed at 500 °C remain similar except for subsidiary modifications, such as moulding of the conchiolin residues on the crystal faces of large calcite crystals and formation of organic geometrical structures (see Part 1, Figs. 80-85 and Part III, 1972, Figs. 124-125).

3. Thermal stability of amino acids from conchiolin and in solution

ABELSON (1956, 1957, 1959), JONES and VALLENTYNE (1960), VALLENTYNE (1964), investigated the thermal stability below 270 °C of chemically pure amino acids in aqueous solutions. These authors have shown evidence of a selective thermal destruction of these substances. Alanine, Glutamic acid and Glycine were found to be most stable and Serine was easily destroyed. HARE and ABELSON (1967), HARE and MITTERER (1968) furnished evidence of racemization of certain amino acids heated in aqueous solutions.

Comparing the decomposition rates of amino acids in aqueous solutions and in powdered shells of the Pleistocene *Mercenaria* pyrolysed in sealed tubes below 270 °C, JONES and VALLENTYNE (1960) observed that the amino acids more stable to dry pyrolysis in *Mercenaria* were among the least stable during natural fossilisation or in pyrolysis in aqueous solutions (ABELSON, 1954, 1956). They suggested that either the laboratory experiments did no mimic the effects of low geologic temperatures over long periods of time, or that the relative geologic stabilities of amino acids in

Mercenaria shell were influenced by some factors other than temperature, such as pH, concentration of water, reaction between amino acids and other compounds.

Our results, in agreement with those of ABELSON (1961); PETITJOHN (1964); GRÉGOIRE (1964, 1968), HARE and MITTERER (1968); VALLENTYNE (1969); show that laboratory experiments at high temperatures for short periods of time simulate the effects of low temperatures over long geologic periods of time : ultrastructure (see Table 2, Part 1, 1968) and composition of conchiolin residues after pyrolysis at temperatures above 300 °C are similar to those of the fossil conchiolins.

However, our results indicate (see VOSS-FOUCART, 1970) that the data obtained in experiments using solutions of pure amino acids cannot always be compared with those recorded on protidic matrices sheltered between mineral layers. Recently, AKIYAMA (1971) arrived to the same conclusion.

The pyrolytic degradation of the conchiolin matrix is an extremely complex process : interactions occur, not only between mineral and organic phases, but also between the different components of the organic matrix. Certain transformations resulting in a partial solubilisation, seem to represent the first steps of the process. Accordingly, in future investigations, it will be necessary to separate at every stage the different fractions which compose conchiolin.

The divergences between our results and certain data of HARE and MITTERER (1968) and of VALLENTYNE (1969) proceed from the fact that these authors, in their studies of the effects of pyrolysis on the organic components of modern (HARE and MITTERER, 1968) and Pleistocene (VALLENTYNE, 1969) shells, have analyzed the entire content of the tubes, including the free amino acid fraction, the peptidic soluble fraction and the polypeptidic insoluble fraction. Our results concern exclusively the residual polypeptidic, non dialyzable fraction of conchiolin, which must be identified in order to establish a comparison with the composition of the remnants of fossil conchiolin.

CONCLUSIONS

Ultrastructural and biochemical differences between the conchiolin matrices of the modern *Nautilus* and those of fossil cephalopods might indicate, as already pointed out in our previous papers, patterns of structure now extinct or might be the result of diagenesis in originally identical or slightly different structures.

Detection of the nautiloid pattern in specimens from different geological ages (e.g. in Ordovician and in Pennsylvanian nautiloids, see GRÉGOIRE, 1966, disc.) already supported the second interpretation. The remarkable resistance of the conchiolin matrices of nacre to thermal stress and the possibility of simulating experimentally the different kinds of morphological alterations in this material, recorded in Paleozoic and in

Mesozoic cephalopods (GRÉGOIRE, 1964, 1966a, 1968, 1972) were also in agreement with this interpretation.

The present biochemical results bring additional evidence in favor of this interpretation :

A. — Polypeptides subsist in all the samples of modern *Nautilus* nacre pyrolysed in the range of 150 °C to 900 °C, in open vessels, and dry or wet in sealed tubes, in agreement with consistent findings of biuret-positive remains of interlamellar matrices.

B. — In the evolution of the experimental alterations induced by increasing temperature, a certain degree of stabilization, from the 300 °C stage up, in sealed tubes, was observed in the relative concentrations of the amino acids of these polypeptides and in the structural modifications of the nautiloid pattern previously reported (see Part 1, p. 9).

This stabilization of the conchiolin changes has been also recorded in the fossil shells : the composition of the non-dialysable residue of the organic matrices subsisting in the Miocene nautiloids does not greatly differ from that of the Devonian nautiloid studied, whereas it distinctly diverges from that of the modern *Nautilus*. Likewise, except for subsidiary variations, the structural changes in the fossil conchiolin from species of all the ages investigated show a certain degree of uniformity.

C. — The amino acid patterns and the structural alterations in the stabilized pyrolysed samples of modern mother-of-pearl do not distinctly differ from those recorded in the organic remnants of thirty Paleozoic, Mesozoic or Cenozoic cephalopods (compare table 1 with tables 1 & 2 in VOSS-FOUCART and GRÉGOIRE, 1971, and, in GRÉGOIRE and VOSS-FOUCART, 1970, p. 197, plates 2, 5, 11, 13, 15 with plates 3, 6, 12, 14, 16).

These results furnish an additional support to our previous conclusions (GRÉGOIRE, 1966, 1968; VOSS-FOUCART and GRÉGOIRE, 1971) that the structural and biochemical differences between the nacreous conchiolin matrices of the modern *Nautilus* and of fossil cephalopods from various ages (Miocene to Devonian) are chiefly diagenetic in nature and do not bring evidence that in the extinct groups the structure and biochemical composition were originally different.

* * *

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EXPLANATION OF FIGURES

Fig. 1.

Nautilus pompilius LINNÉ.

Mural mother-of-pearl heated for 5 hours at 150 °C in open vessels (1010).
Interlamellar conchiolin matrices freed by decalcification of the sample.
No distinct alteration of the conchiolin in parts of the sample (upper right hand corner). In other parts of the field, slight diffuse inflation and coalescence of the trabeculae with protrusion of hemispheral elevations.

Shadowed with platinum. Negative print (black shadows).
× 48.000.

Fig. 2.

Nautilus pompilius LINNÉ.

Mural mother-of-pearl heated for 21 days at 225 °C in open vessels (721-225-21).
Double-stage carbon-palladium replica of the sample, polished in tangential orientation, parallel to the tabular 001 facets of the aragonite crystals, and etched with EDTA (60 seconds).
The interlamellar nacreous conchiolin matrices appear in the form of pseudoreplicas lying on the positive replicas. The structural changes in this material consist of flattening, widening and coalescence of the trabeculae into networks of membraneous structures. Fragmentation of the trabeculae into pebble-shaped corpuscles is visible in the right hand upper area of the figure (see another micrograph of this preparation in GRÉGOIRE, 1966b, Fig. 9).
× 42.000.

Fig. 3.

Nautilus pompilius LINNÉ.

Mural mother-of-pearl heated for 5 hours at 300 °C in open vessels (904).
In this field, two types of alteration of the remnants of decalcification of the sample are associated : on the left hand side, a fragment in which the nautiloid pattern in still recognizable, and in which trabeculae appear locally flattened or swollen, widened and fused into membraneous textures on to which rounded protuberances are scattered; on the right hand side, disintegration of the trabeculae into clusters of spheroidal, pebble-shaped corpuscles.
Shadowed with platinum. Direct print (white shadows).
× 48.000.

Fig. 4.

Nautilus pompilius LINNÉ.

Mural mother-of-pearl heated with distilled water for 5 hours at 300 °C in sealed tubes (1087).
Remnants of decalcification of the samples. The trabeculae are fragmented into twisted segments, or disintegrated into smaller corpuscles.
Shadowed with platinum. Direct print (white shadows).
× 48.000.

Fig. 5.

Nautilus pompilius LINNÉ.

Mother-of-pearl heated with distilled water for 5 hours at 500 °C in sealed tubes (1014).
Remnants of decalcification of the samples.
The original network of trabeculae composing the interlamellar conchiolin matrices has been transformed into membranes perforated by oval openings surrounded by thickenings in the form of ring-shaped pads.
Shadowed with platinum. Positive print (white shadows).
× 48.000.

Fig. 6.

Nautilus pompilius LINNÉ.

Mother-of-pearl heated with sea water for 5 hours at 500 °C in sealed tubes (994).
Remnants of decalcification of the samples.
Same alterations in the interlamellar conchiolin matrices as in Fig. 5.
Shadowed with platinum. Positive print (white shadows).
× 48.000.

Fig. 7.

Nautilus pompilius LINNÉ.

Mother-of-pearl heated for 5 hours at 700 °C in sealed tubes (778).
Same alterations in the conchiolin remnants as in Fig. 5 (see another micrograph of the samples in Part. I, Fig. 77).
Shadowed with platinum. Negative print (black shadows).
× 48.000.