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FOSSIL PROTEINS OF GRAPTOLITES

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Florkin et al.(1) have demonstrated, in the case of conchiolins of fossil Molluscan shells, that animal proteins may be preserved in fossils for several geological ages. This paper deals with the study of proteic remnants in external skeletons of Graptolites, extinct marine organisms of the paleozoic age.

Analyses have been carried out on three different samples of fossils belonging to the order of Graptoloidea. The first sample was composed of fragments of Pristiograptus gotlandicus and Pristiograptus dubius extracted from a calcareous concretion of Bardo (Silurian). The second sample was composed of Monograptidae (gn. sp.) contaminated with a few Retiolitidae, extracted from a marly calcareous erratic boulder (Silurian). These specimens have been identified by Professor Kozlowski. A third sample consisted of fragments of Climacograptus typicalis (provisional identification) isolated from a limestone originating in Ohio (Ordovician).

The calcareous rocks have been dissolved with cold HCl (0.5N). The Graptolites have been separated from the decalcified tissues with micropipettes and washed repeatedly with cold HCl and distilled water in order to remove the soluble components such as free amino acids. The insoluble fossil remnants were then dried, weighed and hydrolyzed by HCl 6 N for 24 hours under reflux. After removing HCl by evaporation, the different amino acids contained in the hydrolysates were identified by column chromatography on a Beckman Spinco automatic apparatus. The results obtained are shown in Table I.

It can be seen that in addition to high amounts of ammonia, amino acids are present in the hydrolysates of the three samples studied (Pristiograptus : 2.202 µg/g - Monograptidae : 5.604 µg/g - Climacograptus : 568 µg/g.)

TABLE I

Amino acids in hydrolysates of Graptolites, after decalcification and washings*

	Pristiograptus gotlandicus and P. dubius (Silurian)		Monograptidae gn. sp. (Silurian)		Climacograptus typicalis (Ordovician)	
	µg/g	mole-fraction p.100	µg/g	mole-fraction p.100	µg/g	mole-fraction p.100
Aspartic acid	218	9	560	8.6	68	10
Threonine	108	4.9	290	4.9	26	4.3
Serine	214	11	550	10.6	122	22.8
Glutamic acid	380	13.9	1100	15.3	96	12.8
Proline	(83)	(3.9)	(340)	(6)	tr	
Glycine	280	20.1	760	20.8	89	23.4
Alanine	103	6.3	410	9.5	40	8.9
Valine	116	5.3	(240)	(4.1)	tr	
Isoleucine	97	4	230	3.6	(15)	2.3
Leucine	190	7.8,	390	6.1	(13)	(2)
Tyrosine	(42)	(1.2)	(90)	(1.1)	-	
Phenylalanine	(87)	(2.9)	(110)	(1.3)	-	
Lysine	96	3.5	(310)	(4.4)	70	9.4
Histidine	60	2.2	(84)	(1.1)	29	3.7
Arginine	128	4	140)	(2.2)	tr	
Ammonia	223					

* The amounts indicated in brackets could only be calculated approximately on account of their low values.

As the soluble material of the Graptolites have been removed by repeated treatments, we can consider that the amino acids contained in the hydrolysates are of proteic origin. An identification of peptidic linkages by means of the biuret reaction, as used by Florkin et al.(1) in the case of conchiolin remnants, was not possible on account of the dark colour of the Graptolite fragments.

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Glycine
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Valine
Isoleucine
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.9	26	4.3
.6	122	22.8
.3	96	12.8
)	tr	
.8	89	23.4
.5	40	8.9
.1)	tr	
.6	(15)	2.3
.1	(13)	(2)
.1)	-	
.3)	-	
.4)	70	9.4
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TABLE II

Comparison between the amounts of amino acids of proteic origin in Graptolites extracted from a rock with those found in the rock itself.

	µg/g		mole. fraction	
	Graptolites*	Rock**	Graptolites	Rock
Aspartic acid	68	12.8	10	7.4
Threonine	26	8	4.3	(5.1)
Serine	122	45.4	22.8	33.4
Glutamic acid	96	9.6	12.8	5.1
Proline	tr	-	tr	-
Glycine	89	30	23.4	30.7
Alanine	40	12	8.9	10.4
Valine	tr	-	tr	-
Isoleucine	(15)	5.6	2.3	(3.3)
Leucine	(13)	7.4	(2)	(4.3)
Tyrosine	-	-	-	-
Phenylalanine	-	-	-	-
Lysine	70	-	9.4	-
Histidine	29	-	3.7	-
Arginine	tr	-	tr	-
Total	568	130.8	99.6	99.7

* Climacograptus typicalis

** Ordovician limestone containing Climacograptus typicalis (from Ohio) after decalcification and removal of the Graptolite fragments.

The question can be raised as to whether these proteins were contained in the Graptolites themselves or originated in the sediment from which the Graptolites were extracted. The analysis of one graptolitic rock compared to that of the Graptolites extracted from this rock allowed us to eliminate the latter hypothesis. The result given in Table II show that the amino acid concentrations are much lower than those of the Graptolites. On the other hand, some amino

acids present in the Graptolites have not been detected in the sediments (Table II). The organic composition of the Graptolite remnants are thus quantitatively and qualitatively distinct from the kerogen of the sedimentary rock.

It has also been possible to rule out the eventuality of a contamination of the Graptolites themselves. Indeed, observation of the fragments with the electron microscope did not reveal appreciable proportions of typical contamination such as bacteria, algal filaments, conchiolin remnants and so on. On the other hand, parallelism between the results obtained for the three samples of different origin and geological age could hardly be consistent with the possibility of a contamination by exogenous organic material.

The amino acids observed in the hydrolysates of washed and decalcified fragments of Graptolites can certainly be considered as originating in remnant fossil proteins belonging to the test of these animals. In all the three different species of Graptolites so far examined, these proteins show a similar amino acid pattern, characterized by high amounts of serine (mole-fraction : 10.6 to 22.8) alanine (6.3 to 9.5), glycine (20.1 to 23.4), aspartic acid (8.6 to 10) and glutamic acid (12.8 to 15.3). Such a composition with a high total amount of glycine, serine and alanine suggests that these graptolitic proteins are of scleroproteic nature. The study of this proteic material with the electron microscope is being continued by Dr.Ch. Grégoire in our laboratory.

Finally in contrast to what is generally believed (3, 2), the three samples examined did not contain any trace of chitin: this polysaccharide has not been detected by the specific enzymatic method of Jeuniaux (4) and the chromatograms of the hydrolysates did not reveal the presence of glucosamine. Furthermore, the test of the Graptolites has not revealed any trace of cellulose.

We are indebted to Prof. R. Kozlowski (Warsaw) for sending the samples of Graptolites, to Prof. G.Ubaghs forproviding us with Climacograptus limestone and for helpful discussion of the results, and to Dr. Ch. Grégoire for examination of our material with the electron microscope.

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