WOODS HOLE OCEANOGRAPHIC INST.
NSR-22-014-001

SEPARATION OF PROTEINS IN MOLLUSC SHELLS BY GEL-FILTRATION

Proteins and glycoproteins are involved in biological mineralization processes. For example, collagens in bone structures of vertebrates promote the formation of apatite seeds[1], whereas glycoproteins in the exoskeleton of crabs provide a set of highly specific templates for calcite nucleation[2]. In molluscs, a wide,array of structurally different proteins are contained in the shell structures, attesting to the heterogenity of calcified tissues[3].

It has been suggested that independent of the kind of protein participating in crystal-seed formation, the most essential factor,in nucleating a mineral phase appears to be the availability of free carboxyl groups provided by certain acidic amino acids and free amino groups by certain basic amino acids and hexosamines[1-4]. It is thus inferred that throughout the animal kingdom, Nature adheres to the same principles when it comes to the formation of mineral nuclei.

To test this assumption, mollusc shell tissues of widely different biochemical composition and phylogeny have been studied by means of enzymatic and non-enzymatic degradation techniques and by gel-filtration. Tryptic digests did not result in the dissolution of shell matrix proteins but treatments with urea, hydroxylamine and formic acid degraded the tissues. According to preliminary studies, the hydroxylamine treatment resulted in a cleavage of the protein molecule to units of molecular weight of approximately 20,000 to 80,000[4].

The analytical scheme followed during the present investigation is outlined in Figure 1. The decalcified organic tissue was treated with a

hydroxylamine/6 M Urea solution for 24 hours at 45°C and subsequently with 95% formic acid for 4 hours at 45°C. Each treatment was followed by centrifugation and by gel-filtration of the supernatent, on G-25 Sephadex columns.

A variety of buffer systems were tested and the molality of the buffers (0.1 to 1) did not play a significant role in the efficiency of separation. Although some buffer systems proved to be slightly better than the routinely used borax buffer of pH 9 (hydroxylamine/urea fraction) and formic acid buffer of pH 3.0 (formic acid fraction) the two latter ones interfered least with the subsequent amino acid analysis. The high-molecular fractions (M.W.>5.000) were hydrolyzed and examined for their amino acid composition[4/]. To facilitate a comparison, the amino acid analysis of the original samples and the residues are included in Table I.

The analytical data indicate that throughout the molluscan phylum the urea/hydroxylamine fraction is consistently enriched in aspartic acid, lysine and amino sugars although this relationship is not necessarily displayed when these three amino compounds are highly concentrated in the original shell. It is tentatively suggested that this peptide fraction contains the active sites for the deposition of the mineral phase and that the remaining part of the proteinaceous matrix in the shells is not involved in the actual calcification. It is reasonable to assume that similar structures are contained in other mineralized tissues such as are found in bones or teeth. A full account on the phylogenetic and chemical aspects of these experiments will be presented elsewhere, together with several hundred amino acid analyses from calcified and uncalcified proteins[5/].

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6. The work was sponsored by the National Aeronautics and Space Administration and by a grant from the Petroleum Research Fund administered by the American Chemical Society. Contribution No. 1961 from the Woods Hole Oceanographic Institution.

Figure 1 Flow diagram of analytical procedures

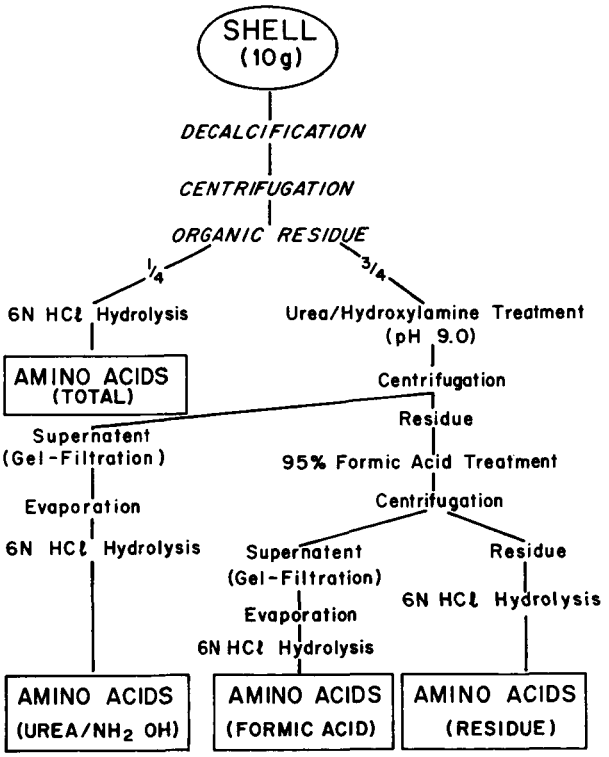

TABLE 1 A

AMINO ACID COMPOSITION OF MOLLUSC SHELL PROTEINS AND ISOLATED PEPTIDE/PROTEIN FRACTIONS - (IN RESIDUES PER 1000)

	CRYPTOCHITON				NAUTILUS				HALIOTIS				ACHATINELLA			
	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE
	* (2)	(1)	(2)	(1)	(3)	(2)	(2)	(1)	(3)	(1)		(1)	(4)	(1)	(1)	(2)
ASPARTIC ACID	117	118	118	112	85	213	89	70	186	160		56	64	185	36	19
THREONINE	58	62	68	71	19	38	15	23	43	32		22	23	53	12	8
SERINE	75	49	68	77	113	55	111	109	101	144		159	35	79	53	8
GLUTAMIC ACID	103	103	110	111	52	56	37	59	50	32		72	42	131	27	11
PROLINE	105	136	144	120	10	53	22	5	36	37		9	61	52	63	50
GLYCINE	128	122	139	101	296	270	380	255	212	350		135	365	156	417	520
ALANINE	100	75	82	86	223	69	186	248	163	63		252	40	86	53	24
CYSTINE	19	12	18	25	9	21	8	7	3	-		12	3	1	18	11
VALINE	50	51	50	65	16	23	13	20	22	21		35	69	53	92	52
METHIONINE	16	15	12	25	5	1	4	5	4	2		1	5	3	1	1
ISOLEUCINE	31	33	32	30	15	16	13	18	12	12		13	58	32	63	39
LEUCINE	52	46	43	68	27	24	26	27	29	27		26	99	68	75	108
TYROSINE	23	19	16	16	13	38	5	9	20	6		12	13	10	14	17
PHENYLALANINE	39	38	32	29	59	58	55	61	31	29		69	64	36	59	89
OH-LYSINE	-	-	-	-	1	1	1	1	1	1		1	-	-	-	-
LYSINE	24	28	15	16	3	28	2	4	19	45		21	41	51	12	22
HISTIDINE	6	18	10	14	3	14	1	2	3	1		1	3	2	2	2
ARGININE	54	75	43	34	51	22	32	77	65	38		104	15	2	3	19
PROTEIN HEXOSAMINES	1.11	7.12	7.6	0.87	121	43	41	661	33	35		36	92	67	302	89
PER CENT PROTEIN IN TOTAL SHELL	2.87				2.24				1.12				0.68			

* Number in parentheses indicate the number of individual analyses.

TABLE 1 B

AMINO ACID COMPOSITION OF MOLLUSC SHELL PROTEINS AND ISOLATED PEPTIDE/PROTEIN FRACTIONS - (IN RESIDUES PER 1000)

	MYTILUS				AEQUIPECTEN				MERCENARIA				LAEVICARDIUM			
	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE	TOTAL	UREA/ NH_2OH	FORMIC ACID	RESIDUE
	* (5)	(2)	(1)	(2)	(4)	(1)		(1)	(4)	(3)	(1)	(2)	(4)		(1)	(1)
ASPARTIC ACID	95	159	118	72	296	340		245	148	196	187	115	125		141	101
THREONINE	19	56	17	18	36	15		30	47	54	47	46	57		28	73
SERINE	93	86	98	94	186	218		143	75	102	52	62	58		127	69
GLUTAMIC ACID	36	84	41	32	59	57		63	71	84	95	57	90		53	106
PROLINE	20	82	15	19	23	21		36	128	91	126	116	87		66	67
GLYCINE	357	226	293	367	214	211		228	146	103	103	248	195		227	138
ALANINE	183	84	216	205	62	44		55	70	57	77	72	68		81	45
CYSTINE	13	13	13	10	12	9		20	21	24	12	24	90		97	99
VALINE	27	36	29	34	18	15		37	32	35	35	34	56		16	78
METHIONINE	7	9	6	6	6	1		1	14	12	3	14	25		1	8
ISOLEUCINE	15	24	18	18	12	8		21	24	22	22	23	31		6	44
LEUCINE	49	52	48	50	25	19		31	35	35	46	35	44		54	74
TYROSINE	34	13	24	35	5	4		12	42	41	34	53	17		15	10
PHENYLALANINE	15	16	17	15	8	6		13	33	29	35	33	20		29	11
OH - LYSINE					1	1		1								
LYSINE	14	46	18	10	26	23		43	68	69	75	36	24		31	41
HISTIDINE	4	2	5	1	3	5		9	5	22	9	3	5		16	10
ARGININE	19	12	24	14	8	3		12	41	24	42	30	10		12	28
PROTEIN HEXOSAMINES	394	37	856	752	118	61		n.d.	102	45	85	128	179		48	250
PERCENT PROTEIN IN TOTAL SHELL	0.91				0.25				0.28				0.54			

* Number in parentheses indicate the number of individual analyses.