Amino Acids in the Proteins from Aragonite and Calcite in the Shells of Mytilus californianus

Abstract. *Hydroxylysine and hydroxyproline are absent in the calcified proteins of* Mytilus. *The organic matrices from the calcite layers have a consistently higher ratio of acidic to basic amino acids than the aragonitic shell units. The uncalcified shell units periostracum and outer ligament, have very few acidic residues, which may in part account for the lack of mineralization.*

The shells of *Mytilus californianus*, a common West Coast mussel, consist primarily of three calcified layers: an outer prismatic layer of calcite, a nacreous aragonite layer, and an inner prismatic calcite layer. In addition, two other calcified units (both aragonite) are present: the ligamental ridge and the inner part of the ligament, which is attached to the ligamental ridge. The outer part of the ligament and the periostracum are not mineralized but are included in the present study for comparative purposes.

Dodd (*1*) has shown that the development of the inner prismatic calcite layer in unworn shells of *M. californianus* is environmentally controlled. The shells for the present study, with well-developed inner prismatic layers, were taken from a moderately cool-water environment (mean annual water temperature 11° to 12°C) approximately 60 miles north of San Francisco, California.

The different structural units of the shells were mechanically separated and decalcified (if necessary) in 3*M* acetic acid or ethylenediaminetetraacetic acid (disodium salt) (*2*). The insoluble residues remaining after the decalcification of the various units were rinsed free of acid, dried, and hydrolyzed in 6*N* HCl (sealed tubes under nitrogen for 22 hours at 110°C). The amino acid concentrations were determined with a commercial automatic ion-exchange analyzer of the type described by Spackman *et al.* (*3*).

The data for the various structural units of the shells are shown in Table 1. In addition, tryptophan was detected in small amounts in NaOH hydrolyzates. Trace amounts of amino sugars were also present in most samples. The high number of glycine residues in the prismatic calcite and nacreous aragonite layers is suggestive of collagen, but the absence of hydroxyproline and hydroxylysine as well as the low proline content would seem to isolate these proteins from the collagen class (*4*). Furthermore, electron micrographs on similar organic matrix material from mollusk shells by Grégoire (*5*) do not resemble typical electron micrographs of collagen.

Systematic variations occur in the amino acid composition of the outer prismatic layer. The values in Table 1 for the amino acid composition of this layer are the averages of eight specimens ranging from 48 to 155 mm in length. The samples were taken from the growing edge opposite the hinge on each specimen and included insofar as possible the same relative proportion of each shell. The standard deviations for most of the amino acids are less than 1 or 2 residues per 1000. Samples taken from other positions around the growing edge of the shell show somewhat greater variations within single shells. For example, the outer prismatic layer near the beak area contains nearly 3 percent organic matrix and contains relatively fewer residues of glycine and alanine (275 and 245 residues per 1000, respectively) and slightly increased proportions of most of the other amino acids. The ratios of acidic (glutamic + aspartic − amide N) to basic (lysine + histidine + arginine) amino acids vary with the percentage of organic matrix but in no case approach those from the aragonite shell structures.

The values for the amino acid compositions for the nacreous and inner prismatic layers listed in Table 1 are averages of five samples each from various positions within the shell. No systematic variations were observed. The standard deviations for most of the amino acid values were less than 2 percent of the average value for that particular amino acid. For cystine, histidine, glutamic acid, and valine the standard deviations were between 5 and 10 percent.

The development of the inner prismatic calcite layer is temperature sensitive with increased development occurring at lower temperatures (*1*). Thus the same mantle cells in this species sometimes secrete aragonite and other times calcite. Seasonal variations at a single locality are often reflected in the intertonguing of the nacreous and inner prismatic layers (*1*). Significantly, the composition of the inner prismatic organic matrix is between that of the nacreous layer and the outer prismatic layer.

The ligamental ridge makes up a

Table 1. Distribution of mineral phases and amino acid composition of the structural units of some *Mytilus* shells.

Amino acid	Unit of shell						
	Outer prismatic layer	Inner prismatic layer	Nacreous layer	Ligamental ridge	Inner ligament	Outer ligament	Periostracum
	*Mineral phase**						
	Cal	Cal	Arag	Arag	Arag	None	None
	Approximate percentage organic						
	1	1	1	1	30	100	100
	Amino acid residues per 1000 total residues						
Aspartic acid	98.6	100	102	126	38.6	43.4	26.0
Threonine	13.4	15.4	19.2	60.0	23.8	20.0	8.7
Serine	102	100	101	126.5	26.8	157	69.0
Glutamic acid	24.4	27.0	28.6	89.0	71.0	21.0	25.0
Proline	11.4	12.6	14.7	38.0	139	9.0	23.7
Glycine	298	289	269	142	197	391	508
Alanine	274	266	246	57.0	16.0	66.4	8.0
Half-cystine†	11.4	12.7	16.5	13.6	25.3	1.0	14.4
Valine	24.6	24.7	29.4	60.0	7.4	34.5	32.0
Methionine	5.0	6.0	7.6	22.5	328†	9.2	4.0
Isoleucine	13.5	15.1	17.1	51.7	10.0	60.0	13.0
Leucine	47.0	47.4	49.5	52.5	13.7	92.5	9.0
Tyrosine	16.3	16.9	16.9	22.5	0.0	23.0	155
Phenylalanine	15.7	16.7	18.7	29.2	4.8	34.0	10.5
Lysine	16.7	22.7	26.7	54.7	91.6	6.0	21.7
Histidine	2.5	2.6	5.5	12.7	1.8	3.2	16.7
Arginine	26.2	26.6	30.9	43.5	7.8	27.7	55.0
Amide N	(58)	(61)	(68)	(123)	(35)	(63)	(41)
Acidic residues (Asp+Glu−NH_3)	65	66	63	92	75	1.4	10
Basic residues (Lys+His+Arg)	45	52	63	111	101	37	93
	Ratio of acidic to basic residues						
	1.4	1.3	1.0	0.83	0.74	0.038	0.11

* Arag, aragonite; Cal, calcite. † Includes oxidation product(s).

small fraction of the shell. In thin section the structure of this aragonitic unit is seen to be prismatic, with a feathery arrangement of the prism axes similar to that described by Bøggild (*6*) for other shell groups. The amino acid composition of the organic matrix from this unit is distinctly different from that of the nacreous and the two calcite layers. It has relatively fewer glycine and alanine residues and more of most of the other amino acids. It has a relatively higher number of both acidic and basic residues with an excess of basic residues.

The inner ligament superficially appears to be entirely organic material but is actually more than two-thirds aragonite and less than one-third organic matrix. The amino acid composition of this unit is most unusual, with nearly one-third the total residues made up of methionine. The high proline content is also distinctive. Perhaps the most unusual feature of the composition of this unit is the complete absence of tyrosine. In three separate samples ranging up to 3 mg of protein no tyrosine was detected in any of the records.

The unmineralized outer ligament differs markedly in amino acid composition from the matrix of the inner ligament. Proline and methionine are both much lower, while glycine makes up nearly 40 percent of the total. The ratio of acidic to basic residues is significantly lower than for the calcified units.

The periostracum, also unmineralized, shows greater variations in amino acid composition than any of the other structural units. The values for the periostracum given in Table 1 are from a single sample (taken from the growing edge opposite the hinge). Samples from the growing edge around the periphery of a single specimen may vary from 10 to 15 percent in the number of residues of many of the amino acids. The physical appearance of the periostracum also varies considerably around the periphery of the shell. Profound changes occur in the periostracum composition during the growth of very small shells (15 mm). After a shell length of 70 to 80 mm is attained, relatively few further changes occur. In shells of 20 mm in length, for example, leucine is higher by nearly a factor of 10, while tyrosine is lower, by a factor of two, than the corresponding values for a larger specimen, as listed in Table 1. During these changes the ratio of acidic to basic amino acids remains low, apparently a characteristic of the noncalcified components.

In comparing the amino acid composition of the nonmineralized components with the matrix from the mineralized fractions, the most obvious difference seems to be in the relative number of acidic and basic amino acid residues. The inner ligament, which has a high organic matrix content (30 percent), has the lowest ratio of acidic to basic residues of any of the calcified units. The nonmineralized units have still lower ratios.

The role of the organic matrix in mineralization is probably to provide a set of highly specific templates which act as the sites for the nucleation of the mineral phase (*7*). Certain side groups in the protein matrix may concentrate Ca^{++} and CO_3^{--} in specific positions and thus provide an appropriate initial concentration of these ions to nucleate the mineral phase. Aspartic and glutamic acid side chains could provide negatively charged sites, which would attract calcium ions. Similarly, the basic side chains could provide sites for the concentration of carbonate (or bicarbonate) ions. The fact that the nonmineralized units have very few acidic residues may indicate a possible mechanism for preventing mineralization.

A comparison of the compositions of the aragonite and calcite matrices shows the calcite matrices to be consistently higher in the ratio of acidic to basic residues. This may indicate the presence of a mixture of protein components, one of which may be rich in the basic amino acids. That the organic matrix of some shells is indeed a mixture of different proteins has been shown by Grégoire *et al.* (*8*) and more recently by Tanaka *et al.* (*9*).

The specificity of the organic matrix for the formation of aragonite or calcite has been considered by numerous workers. Differences in composition of the respective organic matrices have been detected by Roche *et al.* (*10*) and Tanaka *et al.* (*11*). On the other hand, Grégoire (*5*) has demonstrated the presence of organic fragments of identical microstructure in calcite and aragonite from *Mytilus edulis*, and Beedham (*12*) could not detect any significant differences in the amino acid composition of the aragonite and calcite matrices of *M. edulis*. Recent studies (*13*) show that the differences in the aragonite and calcite matrices of *M. edulis* are of the same kind and degree as those of *M. californianus* listed in Table 1.

The present data indicate a consistent difference in composition between the organic matrices of aragonite and calcite as well as between the different structural units of aragonite. Further work should indicate the significance of these differences in the formation of the various shell structures and mineral phases (*14*).

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References and Notes

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Resistance to DDT in the Mosquito Fish, Gambusia affinis

Abstract. *Mosquito fish from waters near cotton fields that have had a long history of treatment with chlorinated hydrocarbon pesticides exhibited a marked resistance to DDT compared with fish from areas which had had no past exposure to insecticides.*

Among vertebrates the fishes are notable for their susceptibility to chlorinated hydrocarbon pesticides. Odum and Sumerford (*1*) obtained an LD_{50} for mosquito fish (*Gambusia affinis*) at 0.01 part of DDT per million (ppm DDT). On the basis of published toxicity data, at 0.1 ppm DDT all fish will die within 12 hours, at 0.01 ppm only a few fish will be able to survive, and at 0.005 ppm many but not most test fish will be killed (*2*).

Resistance to DDT, although quite