Amino-Acid Composition of Heated Oyster Shells

(Pliocene/fossils)

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Contributed by Ralph W. G. Wyckoff, January 20, 1972

ABSTRACT More than 90% of the protein in the shells of the modern oyster, *Ostrea angelica*, is decomposed when shells are heated at 130° for 1 week. There remains, however, a stable residue that is little changed as heating is continued for 9 weeks. This residue resembles, in both amount and composition, the material previously recovered from fossil oyster shells deposited before the Pliocene era.

Recent analyses have shown that the insoluble proteinaceous residues from several fossil oyster shells (1) that date back to before the Pliocene era have essentially the same composition. This similarity of composition has also been found true for the proteins from a group of fossil scallop shells (2, 3), and is apparent in earlier analyses of *Mercenaria* shells (4). The amino-acid ratios in these fossil proteins are, however, so unlike those in their recent counterparts that it is important to establish the relation between the two. Very possibly, the fossil proteins are exceptionally stable products of the denaturation of one or more of the proteins of the fresh shells. If this is true, then heating of the shells should induce a similar denaturation. To test this hypothesis, we have analyzed samples of fresh shells heated for different lengths of time, comparing the amino-acid content of the end products with that of the fossil protein. The present note is a preliminary record of such a study with the modern oyster, *Ostrea angelica*.

MATERIALS AND METHODS

Oyster shells sufficient for the entire study were thoroughly washed, crushed, and mixed to provide a homogeneous sample. 1-g Portions were dried at 110°, sealed in glass ampules, and heated at 130° for periods up through 9 weeks. Three ampules were taken without heating, and three were removed from the oven every week for analysis. The contents of each ampule were dissolved in 2 N HCl to yield a sediment containing undecomposed protein, and a supernatant containing the soluble products of protein decomposition.

After they were washed, the proteinaceous sediments were hydrolyzed with 6 N HCl in a closed container under nitrogen for 20 hr at 110°. Hydrolysates were freed of HCl by evaporation under reduced pressure at 60°, and their amino acids were converted to volatile *n*-butyl *N*-trifluoroacetic derivatives by the procedures of Gehrke *et al.* (5). These derivatives were analyzed in a Hewlett Packard dual-column (model 700) gas chromatograph, equipped with a hydrogen flame detector.

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The supernatants were dried under reduced pressure to remove HCl and redissolved in water; their calcium was precipitated by the addition of appropriate amounts of HF. After removal of the precipitate, each supernatant was dried under reduced pressure, taken up in 6 N HCl, and hydrolyzed under the conditions stated above. This dried hydrolysate contained salts that were removed by passage through an Amberlite 120-8W resin column, its amino acids being eluted with 4 N NH₄OH. After the eluate was dried under reduced pressure, these acids were converted to their volatile derivatives and analyzed as were the proteinaceous sediments. The amino acids found in the soluble fraction thus were the sum of the free acids and those liberated by hydrolysis from soluble peptide fragments of the original protein molecules. Norleucine, added before hydrolysis, served as an internal standard. Duplicate runs were made on each sample; since there were triplicate samples, the values given in the tables are averages of six analyses.

RESULTS

The total amount of protein in the insoluble sediment after the various periods of heating, and the amino-acid composition

TABLE 1. Composition of insoluble unheated and heated oyster proteins (in mole %)

Amino acid	Unheated	Time of heating (weeks)					Average of heated
		1	2	3	6	8	
Hyp	0.1	0.6	1.0	1.5	2.9	2.4	1.4
Asp	33.5	15.4	11.3	14.3	10.4	11.1	12.0
Thr	0.4	3.4	3.7	2.7	3.7	2.4	3.1
Ser	0.9	5.1	8.9	6.0	10.0	7.6	7.7
Glu	0.5	6.1	7.8	8.9	5.6	8.5	8.6
Pro	0.6	5.0	5.9	4.5	4.1	5.3	4.7
Gly	61.5	28.1	18.5	20.7	18.2	15.8	18.8
Ala	0.6	11.2	18.2	14.0	19.7	15.4	16.3
Val	0.4	4.8	5.2	5.1	4.4	5.7	5.0
Ile	0.3	4.3	4.2	5.3	5.6	4.2	4.6
Leu	0.4	5.0	5.9	5.2	5.7	6.2	5.7
Tyr	0.4	3.3	3.1	4.2	4.1	3.3	3.6
Phe	0.2	3.4	3.7	3.9	3.5	4.0	3.7
Orn	0.1	1.4	1.0	1.8	1.6	3.0	2.0
Lys	0.2	2.7	1.5	1.9	1.5	4.9	2.6
Total							
(μg/g)	1554.0	44.3	40.9	36.5	28.6	20.8	

TABLE 2. Amino-acid content of the soluble fraction from unheated and heated oyster shells (in $\mu\text{g/g}$)

Amino acid	Total shell content	Time of heating (weeks)				
		1	2	3	6	8
Hyp	2.4	—	2.0	3.3	1.1	1.0
Asp	945.8	370.9	670.3	519.8	331.2	375.5
Thr	19.1	7.7	8.7	—	—	0.6
Ser	63.2	39.5	24.6	19.3	9.5	5.6
Glu	35.8	86.5	122.4	90.4	75.9	99.0
Pro	30.2	52.9	61.2	63.0	32.5	32.1
Gly	948.1	451.3	491.0	412.5	251.2	290.0
Ala	23.2	54.4	70.7	67.0	59.8	85.1
Val	24.6	32.6	38.8	30.1	23.5	30.5
Ile	18.5	20.7	25.8	10.6	10.7	9.4
Leu	18.6	21.5	25.1	24.9	15.4	17.6
Tyr	21.1	38.0	50.0	30.6	22.3	29.7
Phe	12.0	11.6	18.5	14.1	9.5	8.1
Orn	4.4	17.8	15.8	9.8	5.9	5.9
Lys	9.0	31.3	36.1	22.3	12.1	13.5
Totals:						
Soluble	623.0	1236.8	1661.1	1331.1	860.6	1003.3
Insoluble	1554.0	44.3	40.9	36.5	28.6	20.8
Shell	2176.6	1281.1	1702.0	1367.7	889.2	1024.1

of these residues, are given in Table 1. Less than 3% of the initial protein remained at the end of 1 week, but this residue was remarkably stable; only about half of it was lost over the succeeding 8 weeks. Its composition, very different from that of the original protein, showed only minor fluctuations after the first week of heating. During this first week, 99% of the original aspartic acid and glycine, and from 50 to 80% of the other amino acids were lost from the insoluble protein fraction.

The amino-acid content of the soluble fractions, together with the total content of the unheated shells, are stated in Table 2. Individual acids increase during the first 2 weeks due to the initial massive protein decomposition; thereafter, the quantities of most, but not all, remain sensibly constant or gradually decline. It is known (4, 6) that some amino acids transform into others when heated; further evidence for this is seen in the fact that the absolute amounts of certain

acids, e.g., alanine, are increased by heating. The quantities in the bottom row of Table 2 indicate that somewhat more than half the total amino-acid content of the shells is lost during the entire course of the heating.

Comparison with the analyses of fossil oysters permits several conclusions. The amount of stable proteinaceous residue in the heated samples (20–30 $\mu\text{g/gram}$) is nearly the same as in the older (1) fossils (10–20 $\mu\text{g/gram}$), and in neither is there a well-defined shift in composition once stability has been reached. Significant differences in composition are evident. Though glycine is the most prominent amino acid in both, the fossil residue is relatively poor in aspartic acid and especially in alanine. This may be due to the fact that the quick protein decomposition brought about by heat only roughly parallels that produced by slow aging. Invertebrate shells have been shown to contain more than one protein (7); for the oyster, these probably have different thermal stabilities. It is also possible that, at elevated temperatures, products of decomposition of the original proteins can recombine to form insoluble hydrolyzable complexes. Additional experiments at temperatures above and below 130° are necessary to ascertain if either of these hypotheses explains the differences seen between the fossil and the heated proteinaceous residues. There are a number of conceivable sources of the proteins found in fossils, but the results given here strongly support the idea that the proteins isolated from the analyzed oyster shells are denatured residues of those originally present.

This work has been supported by Grants 5 TO1 DE00126 and 5 RO1 DE01919 from the National Institutes of Health, and Grant GB-17516 from the National Science Foundation.

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