Amino Acids in Samples of Surface Sea Water[1]

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ABSTRACT

Both paper and ion exchange chromatographic techniques have been used to identify and make a partial quantitative estimate of amino acids in surface water samples collected in four subtropical regions. The most abundant of the 18 amino acids identified were aspartic acid, threonine, serine, glutamic acid, alanine, valine, isoleucine, and leucine. In general, basic amino acids, possibly except for lysine, seem to be in low concentration. The amino acid composition of hydrolysates of proteinaceous material in sea water varies in the samples. These differences may be important in the ecology of marine organisms.

Interest in the dissolved organic compounds in sea water has increased recently because of their importance in the nutrition of phytoplankton, the ecology of marine organisms, the geochemistry of petroleum and marine sediments, and in other environmental phenomena such as surface adsorption, the formation of slicks, and trace element chelation. Saunders (1957) and Vallentyne (1957) have written review articles on the soluble organic compounds which have been identified in natural environments, including sea water.

Biochemical substances in sea water have been separated from sea salt by solvent extraction, dialysis, or adsorption: the isolation of vitamin B_{12} by Cowey (1956), the identification of organic acids of low molecular weight by Koyama and Thompson (1959), and the quantitative estimation of fatty acids in vertical profiles of the ocean by Slowey *et al.* (1959) and Williams (1961). Belser (1959) has used biochemically deficient mutant strains of bacteria for direct evaluation of the organic micronutrient content of sea water.

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The importance of proteins, peptides, and amino acids in sea water has long been recognized. Neuberg *et al.* (1957) have indicated the importance of proteinaceous material in the formation of carbamino carboxylic acids, a fact which may be significant in the marine geochemistry of calcium carbonate. Smith *et al.* (1960) have shown that the carbamino carboxylic acids are a preferred source of carbon dioxide for many marine phytoplankton. Largely because of the difficulties in separating dissolved proteinaceous material from sea salt, identification of these compounds has been extremely limited (Belser, 1959). Several amino acids have been identified in marine sediments by Erdman *et al.* (1956) and in older sediments by Abelson (1955). Fogg (1952) and Watanabe (1951) identified the amino acids in the filtrate of algal cultures. Evidence for proteins in sea water has been reported by Anderson *et al.* (1956).

Jeffrey and Hood (1958) have indicated that coprecipitation of organic matter with ferric hydroxide is an effective means of isolating a large portion of the organic materials from sea water. The purpose of the investigation here reported was to identity amino acids derived from acid hydrolysates of sea water concentrates which were isolated by this technique.

TABLE I. Sampling Locations; All Samples Taken at the Surface.

Region	Location	Depth (m)	Volume of Sample (L)
Gulf of Mexico	25°20′N, 90°24′W	1950	75
Yucatán Strait	25°20′N, 86°07′W	1200	75
Reef near British Honduras	17°05′N, 88°09′W	9.7	37.5
Caribbean Sea	21°33′N, 85°57′W	1050	75

Preparation of Samples. Four samples of sea water collected from the surface (see Table I) were filtered through a 0.45 μ Millipore filter within eight hours of collection. To each liter of water, 0.015 g of mercuric chloride was added and to each 18 liters, 20 ml of 2 M ferric chloride. The soluble organic fraction was coprecipitated with ferric hydroxide by adding 40 ml of 2 N sodium hydroxide. Upon our return to the laboratory, the iron precipitate was filtered through a 5 cm glass wool filter mat and dissolved in excess 6 N hydrochloric acid. This solution was hydrolyzed for 24 hours by refluxing at atmospheric pressure, then concentrated *in vacuo* at less than 70°C to reduce the volume and remove part of the hydrochloric acid. The concentrated solution, after being adjusted to pH 1 with NH_4OH, was passed through 2.5 L (7.5×55 cm column) of Dowex 50-X8 cation exchange resin in the hydrogen form. The loaded column was washed with 3 L of 0.1 N HCl and eluted with 8 L of 2 N NH_4OH. The eluate was concentrated to 500 ml *in vacuo* at less than 60°C and loaded on 100 ml of IRA-400 anion exchange resin in the chloride form (2×30 cm column). After the column had been washed with 200 ml of distilled water, the amino acids were eluted with 500 ml

of 1 N HCl. The material was then placed in the refrigerator for future use. An aliquot of 100 ml of the eluate was frozen and dried, then dissolved in 2 ml of 10% isopropyl alcohol, and used for paper chromatography. Another aliquot, reduced in volume, was used for ion exchange chromatographic separation of the amino acids.

Separation by Paper Chromatography

A. One-dimensional separation. Aspartic acid, glutamic acid, cystine, and threonine were identified by development on Whatman No. 3 filter paper. The solvent used was phenol: water in an 80: 20 mixture, with a small quantity of 8 hydroxy-quinoline having been added to the phenol prior to addition of water; beakers containing 10 ml of 1% NaCN and 25 ml of 0.3% ammonium hydroxide were placed in the chromatogram chamber. A 0.2% ninhydrin solution in acetone was used as the detecting agent.

Lysine, histidine, arginine, alanine, isoleucine, and leucine were identified with normal butanol: acetic acid: water in a 240: 60: 250 mixture. The upper layer was separated and used as a moving phase; the lower layer was placed in the chromatogram chamber.

Proline was identified with butanol: acetic acid: water in a 450: 50: 125 mixture. The color reagent used was 0.2% isatine in acetone. The color was developed after addition of the reagent to air-dried chromatographic strips by heating in an oven at 70°C for 10 minutes.

B. Two-dimensional separation. Here the solvent system was methanol: water: pyridine in the ratio 80: 20: 4, followed by *t*-butanol: methyl ethyl-ketone: water: diethylamine in the ratio 40: 40: 20: 4. Boric acid buffer was added to maintain a pH of 9.2; and a 0.2% ninhydrin solution in acetone was used to identify the amino acid spots.

Separation by Ion Exchange Chromatography. Amberlite CG 120 cationic exchange resin was graded to the correct particle sizes by the hydraulic flow method of Hamilton (1958) and was used to fill two columns, one 0.9 × 150 cm and the other 0.9 × 50 cm. The procedure used for amino acid analysis was identical to that of Moore *et al.* (1958), which is briefly described as follows: A 1–2 ml sample containing an amino acid mixture or protein hydrolysate was pipetted on the 150 cm column, and elution was begun with 0.2 N citrate buffer at pH 3.25 and a temperature of 30°C. Upon emergence of alanine from the column (approximately 300 ml effluent volume), the eluting buffer pH was raised to 4.25 and the temperature was increased to 50°C. This procedure served to separate the neutral and acidic amino acids.

In order to determine the basic amino acids, an identical sample was put on the 0.9 × 50 cm column and elution was begun with 0.38 N sodium citrate buffer at pH 4.26 and 30°C. After 400 ml of effluent had been collected,

the temperature was raised to 50°C, with a simultaneous change to another 0.38 N citrate buffer whose pH was 6.25. The type of separation provided by this procedure in our hands is shown in Fig. 1, which represents a recovery experiment with a known mixture of amino acids.

Recovery of Proteinaceous Material from Sea Water. The isolation of free amino acids in sea water by coprecipitation with ferric hydroxide was investigated with phenylalanine-3-C^{14}, and 53% of this amino acid was recovered. Jeffrey and Hood (1958) have found that nearly 100% of the total organic material in aged algal cultures is removed by the process of ferric hydroxide coprecipitation. However, it is believed that the free amino acids in sea water are present in very small quantities compared to the combined amino acids such as the proteins and peptides.

The demineralization of sea water by means of Dowex resin and IRA-400 using C^{14}-labeled methionine indicates that 96% of this amino acid is recovered from these resins by the technique used. Very small amounts of the basic amino acids were found in these hydrolysates; it is possible, therefore, that some of these were lost by incomplete adsorption on the IRA-400 resin.

TABLE II. Summary of the Results of Paper Chromatographic Studies on Amino Acid Composition of Acid Hydrolysates of Sea Water.

Region	Isoleucine	Leucine	Glutamic Acid	Alanine	Aspartic Acid	Valine	Cystine	Arginine	Histidine	Lysine	Serine	Proline	Threonine
Gulf of Mexico....	+++	+++	+++	++	++	+	+	±	±	±	±	±	–
Yucatán Strait.....	+	+	+	–	–	–	+	–	–	–	–	–	–
Reef near British Honduras	+++	+++	++	++	±	+	+	±	–	–	–	–	–
Caribbean Sea	+++	+++	++	++	+	+	+	–	±	–	–	–	–

± Indicates that a spot occurred at the proper R_f value for the respective amino acid but that positive identification was not made.

Results. The results presented in Table II were obtained with a combination of solvents and one- and two-dimensional techniques. The data show positive identification of isoleucine, glutamic acid, and cystine, in all the samples studied. Since the sample from the Yucatán Strait shows a much lower concentration of amino acids than do the others, it is likely that the quantities present in it were too small to identify.

Ion exchange analysis of hydrolysates provides a resolution of the amino acid fraction, and if the concentration level is adequate, it also permits a quantitative estimate of the amino acids present. Our analysis of water samples from

TABLE III. Ion Exchange Analysis of Amino Acid Composition of Hydrolysates of Surface Ocean Water.

Amino Acids	Gulf of Mexico* (mg/M3)	Reef near British* Honduras (mg/M3)
Aspartic Acid	+	++
Threonine	++	+
Serine	+	++
Glutamic Acid (and/or Citrulline)	+++	++++
Glycine**	+	++
Alanine**	+	++
Valine†	+	++
Methionine	+	–
Isoleucine	+	++
Leucine	+	+
Tyrosine	+	+
Lysine	+	++
Histidine	+	+
Tryptophan	+	+
Arginine	+	–
Ornithine	+	+
Cysteic acid or Phosphoethanolamine	+	+
Glucosamine	+	–

* Trace-3 mg/M3: +; 3–8: ++; 8–13: +++; 13– : ++++; –: not detected.
** Not well resolved.
† May be more than one component.

the Gulf of Mexico and the reef near British Honduras (Table III) shows the amino acid composition of the hydrolysates to be quite variable. It may be inferred that the peptides and proteins, from which most of the amino acids are thought to be derived, vary considerably in these two samples. Data were also obtained by ion exchange techniques from the Yucatán and Caribbean samples, but the concentration of amino acids was too low for reliable results. However, enough information was gained to indicate that these samples differ in amino acid content from the other pair of samples.

Summary. It seems reasonable to infer, at least for surface water, that there is variation in the organic nitrogen components. In general, basic amino acids, with the possible exception of lysine, seem to be in low concentration. Of 18 amino acids identified in four samples, the most abundant were aspartic acid, threonine, serine, glutamic acid, alanine, valine, isoleucine, and leucine. Differences in amino acid content may have importance in the ecology of organisms depending on this source of nutrients or stimulants for growth. Much more work is needed to determine the differences in amino acid composition of both surface waters and sub-surface waters collected in vertical profiles.

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