

Amino acids in the sedimentary humic and fulvic acids

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Humic and fulvic acids isolated from a few sediment samples from Arabian Sea and Bay of Bengal were analysed for total hydrolysable amino acids concentration and their composition. The amino acids content of fulvic acids was higher than in the humic acids in the coastal Arabian Sea sediments; whereas amino acids content of fulvic acids was lower than that of humic acids in the coastal sediments of Bay of Bengal. Slope sedimentary humic acids were relatively enriched in amino acids as compared to the coastal sedimentary humic acids implying higher association of amino acids with the carbonaceous and fine grained sedimentary humic acids. Both the humic and fulvic acids are composed of neutral, acidic, basic, aromatic and sulphur containing amino acids. Low molecular weight, as compared to higher molecular weight, fractions of humic acids indicated higher abundance of amino acids. Lysine, aspartic acid and the straight chain-glycine and alanine were the major basic, acidic and neutral amino acids, respectively.

Large quantities of organic matter are produced in the world oceans and on terrestrial areas. Terrestrial organic matter finally reaches the sea by land drainage and river runoff¹⁻³. Atmospheric transport of land derived natural compounds to the sea sediments can also take place. The dissolved and particulate organic matter in seawater is largely composed of a variety of organic compounds. The main sources of organic matter in marine sediments are phytoplankton, zooplankton, the microbial population and higher plants. Carbohydrates and amino acids are probably the most predominant components of organic matter. A vast group of microorganisms attack proteins and organic compounds to produce amino acids and it is generally recognised that most carbohydrates and amino acids in water column and marine sediments exist in some sort of association, forming macromolecular material that is resistant to degradation⁴. Further transformation of this material results in the production of humic compounds.

Amino acids derived from proteinaceous as well as non proteinaceous sources participate in various geochemical processes that lead to the formation of humic molecules and are probably one of the major aliphatic structural units of the peripheral portion of these compounds⁵. Amino acids constitute apparently about 7.5 to 10% of humic substances in recent marine sediments and soils⁶, whereas Felbeck Jr.⁷ stated that 20 to 50% of the nitrogen in most humic compounds is in amino acid form and that amino acids make up approximately 10% by weight of the

humic components extracted from soil. Though amino acids are one of the major building blocks of humic compounds, there are only few reports on the amino acids content of humic compounds in marine sediments. The present study reports concentration and composition of amino acids in humic substances in shelf, and slope sediment samples from the Arabian Sea and a coastal sediment sample in the Bay of Bengal.

Materials and Methods

One surfacial sediment sample was collected at each station during cruises of *RV Gaveshani* {cruise 141 (October 1984), 151 (May 1985)} and *ORV Sagar Kanya* {cruise 37 (October 1987) and 44 (April 1988)}. The station locations and the characteristics of the sediment samples analysed for humic and fulvic acids are shown in Table 1. The extraction of humic and fulvic acids from the sediments was carried out by treating a known amount of the wet sediment with a mixture of equal volumes of 0.05M sodium hydroxide and 0.05M sodium pyrophosphate under inert atmosphere of nitrogen. The extract was separated by centrifugation and the residue was again treated several times with 0.05M sodium hydroxide under nitrogen until the extract was colourless. All the extracts were combined and pressure filtered twice through GF/C filter. The residue was then extracted as above with 0.5M sodium hydroxide and finally refluxed with 0.5M sodium hydroxide for 20 hours. The residue was then washed with distilled

Table 1—Location, depth, sediment texture, organic carbon, nitrogen and the total humic and fulvic acids content in the sediments at different stations

Area	Station	Location		Depth (m)	Sediment texture	C (%)	N (%)	C/N
		Lat. (N)	Long. (E)					
Arabian Sea	G-3464	17° 19'	72° 50.5'	50	Silt & clay	2.36	0.07	32.7
"	SK37F8	12° 35.24'	74° 04.89'	890	Clay	5.6	0.37	15.1
"	SK44/11	15° 52.5'	74° 24'	1121	Clay	4.8	0.27	17.7
Bay of Bengal	BB-05	21° 05.7'	88° 58.5'	25	Clay	0.33	0.02	16.5

water till neutral. All the 0.5M sodium hydroxide extracts along with the washings were combined and pressure filtered twice through GF/C filter.

For the total humic and fulvic acids, the extracts were acidified to pH 2 by 6N hydrochloric acid to separate the humic and fulvic acids. The humic acid precipitate was separated by centrifugation, dialysed against distilled water till neutral and freeze dried. The supernatant solution of fulvic acid was concentrated by rotary evaporation, dialysed against distilled water till neutral and freeze dried. The molecular weight fractionation of humic substances was carried out at stations 37F8 and 44/11 by passing the extracts through Amicon ultrafiltration unit using different molecular weight cut-off diaflo ultrafilters. The fractions were then separated into humic and fulvic acids by acidification and purified as above. The fractions that were available in appreciable quantities were analysed for their amino acids content.

For the analysis of amino acids, the samples were digested with 6N HCl for 22 hours at 110°C and filtered through 0.4 µm Nucleopore filters. Filtered samples were then concentrated to dryness at 40°C using a rotary vacuum evaporator to remove HCl. Traces of acid were removed by adding 500 µl of water and again evaporating to dryness. After this the samples were dried over KOH under vacuum. About 1 ml of water was added to dried sample and a subsample was reacted with 50 µl of *o*-phthalaldehyde and analysed by high performance liquid chromatography (System Gold HPLC Beckman). The liquid chromatography equipment used for amino acid analyses consisted of two pumps, a reverse phase column (C₁₈), column heater, fluorescence detector, a system controller and a computer (PS -30) based data handling system (System Gold software). A binary solvent system was used to resolve amino acids in 28 minutes using a Beckman 15 cm length, 4.6 mm i.d., 5 µm particle size C₁₈ column maintained at 34°C. Solvent A consisted of 50 mM sodium acetate

solution and 3.0% of HPLC grade tetrahydrofuran, adjusted to pH 6.5 with acetic acid. Solvent B was glass distilled methanol. Both solvents were degased by filtering under vacuum. Peaks were integrated using System Gold software and peak areas from both calibration and sample runs were automatically transferred to speedsheets. Amino acid yields were calculated relative to amino butyric acid as internal standard. The precision of the analytical techniques based on replicate analyses of samples was better than +15%.

Results and Discussion

The contents of organic carbon, nitrogen and hydrogen of humic and fulvic acids and their ratios at different stations are shown in Table 2. The distribution of amino acids in humic and fulvic acids in the sediments is listed in Table 3. Station 37F8 sedimentary humic acids show higher concentration of total amino acids with relative enrichment in low molecular weight fraction. The coastal samples in the Arabian Sea show higher concentration of amino acids in fulvic acid fraction compared to that in the Bay of Bengal. The most striking difference in these coastal amino acids is in their higher content of acidic amino acids in the fulvic acids fraction than in the humic acids fraction of coastal Arabian Sea sediments. The acidic amino acids content of humic acids was higher than fulvic acids in the coastal sediments of Bay of Bengal. In the slope region the humic acids contained higher acidic amino acids than fulvic acids. Carter & Mitterer⁸ observed that only in the case of plant debris the acidic amino acids are more in fulvic acids than humic acids indicating different source material in the two regions. These regional differences in the acidic amino acids contents of humic and fulvic acids may also be the result of selective preservation or utilisation of certain amino acids over others in these different types of depositional environments⁹. However, besides these apparent reasons for the enrichment of some groups of amino acids, the individual amino acids may

Table 2—The contents of carbon, hydrogen, nitrogen and their ratios in humic and fulvic acids

Station	Humic material	C (%)	H (%)	N (%)	C/N	C/H
G-3464	Total HA (0.05M)	55.1	5.7	4.0	13.7	9.6
G-3464	Total FA (0.05M)	46.3	3.6	----	----	----
SK37F8	>300000HA(0.5M)	50.3	2.5	6.9	20.1	7.3
SK37F8	>300000FA(0.5M)	43.0	8.9	1.6	26.3	4.8
SK37F8	>10000HA(0.5M)	35.6	2.4	6.0	14.8	5.9
SK37F8	>10000FA(0.5M)	26.7	6.3	----	----	4.2
SK44/11	>300000HA(0.5M)	48.2	3.7	5.8	13.0	8.3
SK44/11	>10000HA(0.5M)	21.7	4.9	1.6	13.5	4.4
BB-05	Total HA(0.5M)	59.0	2.4	4.6	24.6	12.8
BB-05	TotalFA(0.5M)	43.4	4.2	2.4	10.3	18.0

Table 3—Distribution (nmols/mg) of amino acids in the humic and fulvic acids in the sediments at different stations (A-->300000HA, B-->300000FA, C-->10,000HA, D-->10,000FA)

Amino acids	G-3464 Total HA	G-3464 Total FA	37F8 A	37F8 B	37F8 C	37F8 D	44/11 A	44/11 C	BB-05 Total HA	BB-05 Total FA
Threoni.	2.74	20.35	traces	10.13	5.97	5.80	traces	5.76	traces	traces
Serine	6.75	9.04	2.49	9.40	1.80	7.40	3.70	4.09	4.86	4.25
Glycine	29.31	17.95	9.59	23.74	15.68	24.76	8.42	17.76	9.08	13.35
Alanine	12.17	13.29	14.98	16.93	11.78	16.31	12.79	11.06	15.33	14.11
Valine	5.46	0.48	traces	6.03	0.27	6.65	traces	8.54	2.42	5.05
Isoleucine	3.78	1.66	6.78	1.75	4.0	2.81	10.37	6.27	8.18	6.95
Leucine	8.44	2.11	7.54	2.26	5.50	3.62	8.72	9.02	9.64	7.20
Asp.acid	5.95	15.75	14.87	5.79	14.91	8.07	13.84	10.47	11.45	11.29
Glu.acid	6.98	8.97	9.14	5.36	10.0	7.15	7.13	9.29	10.45	10.65
Lysine	8.0	5.06	13.97	4.60	18.46	6.85	11.87	8.82	9.09	12.97
Histidine	0.7	traces	1.72	0.94	5.36	0.95	1.87	0.86	traces	traces
Arginine	3.42	traces	2.95	4.59	1.58	0.03	3.13	2.20	3.86	traces
Tyrosine	0.96	2.84	4.50	1.06	4.02	0.68	4.67	0.28	3.44	4.08
Phenylal ²	3.73	1.27	6.66	1.25	4.41	3.10	7.82	7.65	6.44	4.84
Methioni.	0.36	traces	4.77	----	traces	----	5.62	traces	5.69	5.33

constitute useful indicators of depositional environments.

The relative concentrations of individual amino acids in different humic and fulvic acid samples are presented in Table 4. Among the basic amino acids analysed, lysine is the major basic amino acid in all the samples and is relatively more abundant in the slope samples. Lysine has a net positive charge near pH 7 and would interact with the negatively charged exchange sites provided by clay minerals and humic substances in sediments¹⁰. These adsorption properties of lysine may be responsible for higher concentration of lysine in humic substances. Within the acidic fraction aspartic acid concentration is higher than the glutamic acid in all the samples and accounts for 5.95-15.75% of the total amino acids, the maximum being in the coastal fulvic acids. Aspartic

acid is reported to be abundant in terrestrial humic hydrolysates¹¹ and in sediments receiving large terrestrial organic inputs¹² whereas glutamic acid is more abundant in plankton. Moreover, it is the dominant amino acid in bacteria¹³. The calcareous material is characterised by a strong predominance of aspartic acid and a high aspartic acid : glycine ratio was observed in the sediment trap material¹⁴. In the present study the slope sedimentary humic acids show a high aspartic acid : glycine ratio (0.95-1.64) except the low molecular weight humic acid fraction. The coastal humic and fulvic acids fractions and the slope fulvic acid fractions show the characteristics of diatomaceous origin which is characterised by a predominance of glycine, low aspartic acid : glycine ratio (0.20-0.87) and a relatively higher content of hydroxy amino acids (serine and threonine). These

Table 4—Relative distribution (mol%) of amino acids in the humic and fulvic acids of the sediments at different stations (A-->300000HA, B-->300000FA, C-->10,000HA, D-->10,000FA)

Amino acids	G-3464 Total HA	G-3464 Total FA	37F8 A	37F8 B	37F8 C	37F8 D	44/11 A	44/11 C	BB-05 Total HA	BB-05 Total FA
Threoni.	3.94	20.53	traces	16.26	2.18	10.4	traces	3.63	traces	traces
Serine	6.75	9.04	2.49	9.4	1.8	7.40	3.70	4.09	4.86	4.25
Glycine	29.31	17.95	9.59	23.74	15.68	24.76	8.42	17.76	9.08	13.35
Alanine	12.17	13.29	14.98	16.93	11.78	16.31	12.79	11.06	15.33	14.11
Valine	5.46	0.48	traces	6.03	0.27	6.65	traces	8.54	2.42	5.05
Isoleucine	3.78	1.66	6.78	1.75	4.0	2.81	10.37	6.27	8.18	6.95
Leucine	8.44	2.11	7.54	2.26	5.5	3.62	8.72	9.02	9.64	7.2
Asp. acid	5.95	15.75	14.87	5.79	14.91	8.07	13.84	10.47	11.45	11.29
Glu. acid	6.98	8.97	9.14	5.36	10.0	7.15	7.13	9.29	10.45	10.65
Lysine	8.0	5.06	13.97	4.60	18.46	6.85	11.87	8.82	9.09	12.97
Histidine	0.70	traces	1.72	0.94	5.36	0.95	1.87	0.86	traces	traces
Arginine	3.42	traces	2.95	4.59	1.58	0.03	3.13	2.20	3.86	traces
Tyrosine	0.96	2.84	4.50	1.06	4.02	0.98	4.67	0.28	3.44	4.08
Phenylal	3.73	1.27	6.66	1.25	4.41	3.10	7.82	7.65	6.44	4.84
Methioni.	0.36	traces	4.77	---	traces	---	5.62	traces	5.69	5.22

significantly different characteristics of individual amino acids indicate that amino acids in humic and fulvic acids are derived from different source materials¹⁴. As far as the neutral fraction is concerned, the relative abundance of straight chain (glycine and alanine) amino acids is characteristics of all samples, the total branched chain (valine, leucine and isoleucine) group forms the second largest group of neutral fraction. Within the total hydroxyacids fraction (threonine and serine) there is a general predominance of serine over threonine except the coastal Arabian Sea sample where the latter dominates.

Aromatic amino acids, especially phenylalanine are relatively abundant in humic acids of the offshore region. Although it is difficult to assess the environmental significance of this fact, Nissenbaum & Kaplan¹⁵ related this predominance to the oxidising conditions of the deposition in the Dead Sea, and Morris¹⁶ found phenyl alanine as a major amino acid in recent Atlantic marine sediments. However, sulphur containing amino acids probably related to the depositional characteristics of the sediments with respect to its redox conditions are present in the slope and offshore regions as well as in the Bay of Bengal coastal humic and fulvic acids, but it is present in traces in the low molecular weight fraction of humic acids of the slope region and in coastal fulvic acid fraction of the Arabian Sea. These amino acids are reported to have a tendency to disappear during

geochemical degradation of the sedimentary organic matter¹⁷.

Amino acids belonging to different groups are listed in Table 5. The order of abundance for hydrolysable amino acids is neutral, acidic, basic, aromatic and sulphur containing amino acids. The highest concentration of all the types of amino acids is found in the humic acids from the sediments of slope region (37F8) with the maximum concentration being observed in the low molecular weight fraction (>10,000) of the humic acids. This fraction contains almost equal amount of basic and acidic amino acids. Basic amino acid fraction is found to be relatively more abundant in the slope samples. These data are well in agreement and support the earlier observation of Gonzalez *et al.*¹⁷ where slope sediments showed higher abundance of basic amino acids than that exhibited by plankton. The sediments at stations 37F8 and 44/11 are clayey and relatively rich in organic matter content (organic carbon 5.6 and 4.8% respectively); whereas sediments at station G-3464 were of silt and clay. Sediments at station BB-05 are also clayey but were very low in organic matter content (organic carbon 0.33%). It is well documented that large grain sediment particles are deficient in organic matter specially in total amino acids. In contrast fine grain sediments exhibit organic matter enrichment. Evidently, clayey sediments contain absorbed humic substances which are bound to clay minerals by amino acids of proteins.

Table 5—Amino acid content (nmols/mg) in different groups in sedimentary humic and fulvic acids at different stations (A-->300000HA, B-->300000FA, C-->10,000HA, D-->10,000FA)

Amino acids	G-3464 Total HA	G-3464 Total FA	37F8 A	37F8 B	37F8 C	37F8 D	44/11 A	44/11 C	BB-05 Total HA	BB-05 Total FA
Neutral	48.51	65.50	75.41	47.59	112.62	40.14	58.45	95.70	50.57	2.09
Acidic	8.98	24.52	43.76	6.95	68.05	8.49	27.85	31.32	22.36	5.21
Basic	8.43	5.02	33.98	6.32	69.38	6.03	22.41	18.84	13.23	3.08
Aromatic	3.26	4.08	20.34	1.44	23.05	2.11	16.59	12.58	10.12	2.12
Sulphur	0.25	traces	8.70	----	traces	----	7.46	----	5.81	1.24
Total	69.43	99.12	182.19	62.30	273.1	55.77	132.76	158.44	102.19	23.74

Table 6—Relative distribution (mol%) of different groups of amino acids in humic and fulvic acids of the sediments at different stations. (A-->300000HA, B-->300000FA, C-->10,000HA, D-->10,000FA)

Amino acids	G-3464 Total HA	G-3464 Total FA	37F8 A	37F8 B	37F8 C	37F8 D	44/11 A	44/11 C	BB-05 Total HA	BB-05 Total FA
Neutral	69.85	65.50	39.8	76.37	41.0	71.95	44.0	60.37	49.5	50.9
Acidic	12.93	24.52	23.1	11.15	24.9	15.22	20.9	19.76	21.9	21.9
Basic	12.12	5.02	17.9	10.13	25.4	7.83	16.9	11.88	12.95	12.9
Aromatic	4.69	4.08	10.7	2.31	8.4	3.78	12.5	7.93	9.88	8.9
Sulphur	0.36	traces	4.6	----	traces	----	5.6	----	5.7	5.2

Deamination of these complexes releases the humic materials from the clays¹⁸.

Table 6 shows the percentage of different groups of amino acids relative to the total amino acids in different humic and fulvic acid fractions. The humic acids show 40-70% neutral, about 13-25% acidic, 8-25% basic and 5-13% aromatic amino acids; whereas the fulvic acids show 51-76% neutral, 11-25% acidic, 5-13% basic and 2-9% aromatic amino acids. Humic as well as fulvic acids show almost equal proportion (5-6%) of sulphur containing amino acids except the low molecular weight humic acid fraction and the coastal (G-3464) fulvic acid fraction which contain traces of sulphur containing amino acids. Rashid⁶ reported 41-45% neutral, 25-26% acidic, 15-19% basic and 9-12% aromatic and about 4% sulphur containing amino acids in marine humic acids. However, he found 38% neutral, 23% acidic, about 10% basic and 12% aromatic amino acids in fulvic acids.

Between the coastal and slope sedimentary humic acids, the coastal humic acids show the minimum concentration of amino acids. In case of slope sedimentary humic acids the relative proportions of neutral and acidic amino acids are almost the same in high and low molecular weight humic acid fractions, whereas the basic amino acids are relatively higher in the low molecular weight fraction. The relative proportion of aromatic amino acids was marginally

high in the high molecular weight fraction. The amino acids in the humic acid fractions of the slope and offshore region sediments show little difference in their relative concentration.

A comparison of the amino acids of the coastal fulvic acids (sts. BB-05 and G-3464) sediments indicate higher proportion of neutral and acidic amino acids in fulvic acids of coastal Arabian Sea sediment whereas a higher proportion of basic and aromatic amino acids were observed in the coastal fulvic acids samples of the Bay of Bengal, indicating a greater proportion of terrigenous source material in the Bay of Bengal sediments.

Amino acids are useful indicators of the source material and are found to be derived from terrigenous as well as planktonic source material. The individual amino acids and their ratios are also significant indicators of depositional environments and suggest that amino acids in humic and fulvic acids are derived from different source materials. Amino acids are enriched in low molecular weight fraction of slope sedimentary humic acids.

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