ACID-LABILE AMINO ACID PRECURSORS IN THE MURCHISON METEORITE

1: *Chromatographic Fractionation**

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Abstract. The amino acid content of a hot water extract of the Murchison meteorite can be increased by over 100 per cent by subjecting the extract to acid hydrolysis. The acid-labile compounds in the extract that account for this increase were fractionated by column chromatography on a cation exchange resin. Seventy mole per cent behaved as neutral or acidic compounds and were eluted from the column with an initial water wash. The remaining 30 mole per cent (basic precursors) were retained on the column and were eluted with the free amino acids by aqueous NH_4OH. The acid-labile amino acid precursors in the water eluate could be retained and further fractionated on an anion exchange column, indicating that they are acidic compounds.

The presence of amino acids in hot water extracts of samples of the Murchison meteorite, a C2 chondrite, has been demonstrated in several laboratories (Kvenvolden *et al.*, 1970; Cronin and Moore, 1971; Oro' *et al.*, 1971a). Qualitative (Kvenvolden *et al.*, 1971; Lawless, 1973), quantitative (Cronin and Moore, 1971; Pereira *et al.*, 1975), and stereochemical (Kvenvolden *et al.*, 1970; Oro' *et al.*, 1971b) analyses of the amino acids have been carried out. The results of these studies, taken with those of analyses of the Murchison meteorite for other organic species, provide evidence that these compounds are indigenous to the meteorite and the result of an abiotic synthetic process. Thus C2 chondrites such as Murchison offer the most direct evidence found in nature in support of the Oparin–Haldane hypothesis of chemical evolution (Kenyon and Steinman, 1969).

It was previously reported that water extracts of samples from both the Murchison and Murray (C2) chondrites contain, in addition to free amino acids, compounds that give rise to amino acids upon subjecting the water extracts to acid hydrolysis (Cronin and Moore, 1971). Similar observations have been made with the Nogoya (C2) carbonaceous chondrite (Cronin and Moore, 1976) and with the lunar fines returned by the Apollo 11 and 12 missions (Harada *et al.*, 1971). Characterization of these amino acid precursor or derivative compounds is desirable in terms of understanding the chemical evolution of amino acids. The work described in this paper was aimed at isolating these compounds for further study and partially characterizing them by ion exchange chromatography.

1. Methods

Meteorite samples were fragments of a larger stone that had been broken in the laboratory. Where present, ablation crust was chiselled away and the remaining fragments crushed in a steel mortar and pestle. The resulting powder was suspended in water (3 ml water per g meteorite) that had been glass distilled and then

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redistilled from a quartz sub-boiling distillation apparatus. The suspension was refluxed for 20 hour. After cooling, the insoluble meteorite residue was removed by vacuum filtration using a fine (4–5.5μ) frit glass funnel. The meteorite residue was washed on the filter with a small amount of water. The wash and original filtrate were combined and concentrated about ten fold with a rotary evaporator. The calcium sulfate that precipitated on concentrating the extract was removed by filtration as before and the filtrate and precipitate wash were combined. The resulting yellow solution was apportioned among test tubes, each portion representing the extract from about 1 g of meteorite, and taken to dryness in a vacuum desiccator over NaOH pellets. The dry extract fractions were stored in a refrigerator until used.

Cation exchange chromatography of the meteorite extract was carried out using a procedure similar to one described previously for desalting meteorite amino acids (Kvenvolden *et al.*, 1971). The dry extract from 1 g meteorite was redissolved in approximately 1 ml water and adjusted to pH 2. This solution was applied to a 1.1 × 5.3 cm column of AG50W-X4 resin (Bio Rad Laboratories, minus 400 mesh, H^+ form) and the column eluted with water (10 ml) followed by 3M NH_4OH (10 ml).

The water eluate from the cation exchange column was concentrated with a rotary evaporator, made alkaline, and applied to a 1.1 × 5.0 cm column of the anion exchange resin, AG1-X8 (Bio Rad Laboratories, 200–400 mesh, acetate form). The column was then eluted with (1) 0.1M pyridine (25 ml), (2) 0.1M pyridine adjusted to pH 5.5 with glacial acetic acid (50 ml), (3) 1.0M acetic acid (100 ml), and (4) 0.5M HCl (25 ml).

Amino acid analyses were performed in some cases using a commercial amino acid analyzer with ninhydrin detection and in others with a high sensitivity system employing o-phthalaldehyde and fluorometric detection (Benson and Hare, 1975). Hydrolysis of samples was carried out in constant boiling HCl.

2. Results and Discussion

In Table I the amounts, both before and after acid hydrolysis, of fifteen amino acids found in a hot water extract of the Murchison meteorite are given. In this experiment an extract derived from 0.98 g meteorite was divided into two equal portions, one half was dried and analyzed directly and the other half subjected to acid hydrolysis, dried, and analyzed in the same way. Qualitative identification of the amino acids was based almost entirely on coincidence of elution times with those of known standards. It should be noted that the amino acids listed in Table I, with the exceptions of threonine and serine have also been identified in the Murchison meteorite by combined gas chromatography-mass spectrometry (Lawless, 1973; Pereira *et al.*, 1975). The amounts obtained are in good agreement with earlier data (Cronin and Moore, 1971; Pereira *et al.*, 1975). These results show the meteorite aqueous extract to contain about equal amounts of free amino acids and acid-labile precursors, the overall increase on acid hydrolysis being 110 mole per cent for the entire suite of amino acids. Individual amino acids show substantial differences in the magnitude of increase: the dicarboxylic amino acids, aspartic acid

TABLE I

Amino acids in the Murchison water extract before and after acid hydrolysis

Amino acid	Water extract	Hydrolysed water extract	Increase	Percent increase
	Nanomoles per gram meteorite			
Aspartic acid	1.0	8.8	7.8	780
Threonine	1.2	4.7	3.5	292
Serine	1.7	5.1	3.4	200
Sarcosine	8.6	12.5	3.9	45
Proline	4.4	10.5	6.1	139
Glutamic acid	3.5	20.5	17.0	486
Glycine	31.0	63.9	32.9	106
Alanine	17.1	28.7	11.6	68
α-Aminoisobutyric acid	19.9	27.4	7.5	38
α-Amino-n-butyric acid	5.3	7.9	2.6	49
Valine/Isovaline	4.6	11.5	6.9	130
Isoleucine	0.9	4.9	4.0	444
Leucine	0.8	5.2	4.4	550
β-Alanine	6.9	11.8	4.9	71
β-Aminoisobutyric acid	1.7	4.7	3.0	176
Total	108.6	228.1	119.5	110

and glutamic acid, increase by 486 and 780 per cent, while α-amino-isobutyric acid and sarcosine increase by only 38 and 45 per cent, respectively.

In Table II are shown the results of cation exchange chromatography of the Murchison extract. A water extract from 0.98 g meteorite was applied, and the column eluted with water and 3M NH_4OH. Both the water and NH_4OH eluates were collected and analyzed for amino acids before and after acid hydrolysis. It can be seen that the free amino acids of the extract are quantitatively recovered and found almost exclusively in the NH_4OH eluate. The water eluate of the column is nearly devoid of free amino acids. However, after acid hydrolysis, this fraction yields 83.6 nanomoles amino acids per gram thus accounting for about 70 per cent of the amino acid precursors. Acid hydrolysis of the NH_4OH eluate also leads to an increase in the amino acid content, accounting for the remaining 30 per cent. The acid-labile amino acid precursors of the meteorite extract thus fall into two classes: basic compounds that elute with free amino acids and neutral or acidic species that are not retained by a cation exchanger.

The results of anion exchange column chromatography of the amino acid precursors found in the water eluate of the cation exchange column are given in Table III. A four step elution scheme was used in which the eluant pH was progressively decreased from a slightly alkaline to a strongly acidic value. The overall recovery of the amino acid precursors was about 80 per cent. The precursors require at least moderately acid conditions for their elution and are found in appreciable amounts in both the 1.0M acetic acid and 0.5M HCl eluates. Thus these compounds behave as acidic species. Their fractionation between the acetic acid and HCl eluates suggests that there are two distinct classes of acidic precursors, possibly differing in their acid strength or number of acidic functional groups. The acid-labile amino

TABLE II

Behavior of meteorite amino acids and acid-labile amino acid precursors on a cation exchanger.

	Water eluate		NH_4OH eluate		Total
	not hydrolysed	hydrolysed	not hydrolysed	hydrolysed	hydrolysed
Amino acid	Nanomoles per gram meteorite				
Aspartic acid	0.2	5.3	1.3	4.2	9.5
Threonine	0.1	1.6	1.4	3.1	4.7
Serine	0.5	1.9	2.3	4.2	6.1
Sarcosine	0	6.0	6.3	5.9	11.9
Proline	0	6.1	5.3	6.8	12.9
Glutamic acid	1.4	15.1	2.5	5.9	21.0
Glycine	0.6	19.4	31.8	45.2	64.6
Alanine	0.2	8.3	17.4	20.8	29.1
α-Aminoisobutyric acid	0	5.3	21.3	21.3	26.6
α-Amino-n-butyric acid	0	2.4	5.5	6.0	8.4
Valine/Isovaline	0	3.9	5.4	7.4	11.3
Isoleucine	0	1.8	1.2	2.2	4.0
Leucine	0	2.5	1.0	2.6	5.1
β-Alanine	0	3.5	6.8	8.5	12.0
β-Aminoisobutyric acid	0	0.5	1.8	3.0	3.5
Total	3.0	83.6	111.3	147.1	230.7

TABLE III

Fractionation of Murchison cation exchange water eluate by anion exchange chromatography

Amino acid	0.1M pyridine eluate	0.1M pyridine pH 5.5, eluate	1.0M AcOH eluate	0.5M HCl eluate	Total
	Nanomoles per gram (not hydrolyzed/hydrolyzed)				
Aspartic acid	0/0.3	0.1/0.3	0.2/1.1	0.3/2.3	0.6/4.0
Threonine	0/0.4		0.1/0.5	0.3/0.9	0.4/1.8
Serine	0/0.8	0.1/0.4	0.6/1.2	0.5/1.4	1.2/3.8
Glutamic acid	0/0.6	0.3/0.6	0/9.1	0.1/2.7	0.4/13.0
Glycine	1.4/4.1	0.9/1.1	0.6/8.9	0.9/8.8	3.8/22.9
Alanine	0.4/1.6	0.1/0.3	0.2/3.5	0.4/3.5	1.1/8.9
α-Aminoisobutyric acid			0/2.0		0/2.0
α-Amino-n-butyric acid			0/1.4	0/0.9	0/2.3
Valine/Isovaline	0/0.7		0/1.5	0/1.3	0/3.5
Norvaline			0/0.5		0/0.5
Isoleucine	0/0.3		0/0.5	0/0.4	0/1.2
Leucine	0/0.5		0/0.5	0/0.5	0/1.5
β-Aminobutyric acid			0/0.8	0/0.7	0/1.5
β-Alanine			0/2.1	0/2.0	0/4.1
β-Aminoisobutyric acid			0/0.9		0/0.9
Total	1.8/9.3	1.5/2.7	1.7/34.5	2.5/25.4	7.5/71.9

acid precursors of the Murchison chordrite are thus heterogeneous as judged by ion exchange chromatography: 30 per cent are basic compounds and 70 per cent are acidic, the latter being separable into two fractions.

Since inorganic salts, particularly $CaSO_4$, are extracted from the meteorite by hot water along with the various organic compounds, the possibility that stable inorganic complexes of the amino acids account for either the acidic or basic precursor fraction has been considered. This possibility does not appear likely because of the instability of known complexes in acidic solution and/or in the presence of competing ligands. For example, copper glycinate, which has one of the smaller dissociation constants of the known amino acid-metal chelate compounds (Greenstein and Winitz, 1961), readily dissociates in the sodium citrate buffer, pH 2.2, used for sample dissolution and application to the amino acid analyzer. Furthermore, the meteorite extract can be heated at 100° for one hour in 0.1M sodium ethylenediaminetetraacetate, pH 7.1, with no accompanying increase in the free amino acid content.

It is possible that the large increase in amino acid content achieved by acid hydrolysis of the meteorite extract is a result of the hydrolytic breakdown of relatively large polypeptides or "proteinoid" species (Fox and Harada, 1958). Since acid hydrolysis of the meteorite extract yields substantial amounts of the acidic amino acids, aspartic and glutamic acid, and relatively little, if any, of the basic amino acids, such polymers would be rather acidic (low isoionic point) and might not bind to the cation exchange resin. A preliminary experiment was carried out in which the meteorite extract was chromatographed on a molecular exclusion column having an exclusion limit for peptides of 1800 daltons. The acid-labile precursors were not separated from the free amino acids of the extract by this procedure indicating that they do not differ appreciably in molecular size from amino acids.

A third possibility is that amino acids exist in the meteorite as, or form during the extraction process, derivatives in which a carbonyl group is bonded to the amino group nitrogen atom. Some derivatives of this type (e.g., N-carbamyl and N-acyl amino acids) are acidic, and would elute from a cation exchanger and be retained on an anion exchanger as are 70 per cent of the amino acid precursors of the meteorite. Other derivatives of this kind (e.g., peptides) are basic and would be retained on a cation exchanger as are 30 per cent of the meteorite amino acid precursors. Work aimed at demonstrating the latter compounds, if present in the meteorite extract, is described in the following paper.

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