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Stable isotope mass fragmentography: identification and hydrogen–deuterium exchange studies of eight Murchison meteorite amino acids

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Abstract—The concentration of eight protein amino acids found in extracts of the Murchison carbonaceous chondrite has been measured by quadrupole mass fragmentography. This result was obtained by using deuterated amino acids as internal standards. In addition, hydrogen–deuterium exchange in amino acids was studied by two methods. First, nondeuterated amino acids were added to the meteorite and the amount of deuterium incorporated after extraction with deuterium oxide was determined. Second, deuterated amino acids were added to the dry meteorite and the loss of deuterium after extraction with H_2O was measured. It was observed that the degree of hydrogen–deuterium exchange increased with increasing severity of extraction conditions. This exchange resulted in some racemization, presumably catalyzed by constituents of the meteorite. The degree of racemization for each amino acid was determined by gas chromatography of the corresponding N-trifluoroacetyl-O-(+)-2-butyl esters.

INTRODUCTION

THE PRESENCE of organic compounds in meteorites has had a long and controversial history which has been the subject of a thorough and critical review by HAYES (1967). His conclusions regarding the presence of amino acids in meteorites were that at best, the results were inconclusive and at worst, the amino acids identified were present strictly as a result of terrestrial contamination. Since that review, two major events have occurred to help resolve this problem.

The first event was the recent fall (September 1969) of the Murchison carbonaceous chondrite, which, because of its brief terrestrial history (LOVERING *et al.*, 1971), might be expected to have a minimum of contamination. The second has been the application of recently developed analytical techniques, particularly the gas chromatographic separation of D and L isomers of amino acids (POLLOCK and OYAMA, 1966; NAKAPARKSIN *et al.*, 1970) and the combination of the gas chromatograph with the mass spectrometer (GC/MS).

Numerous investigations have recently been carried out on amino acids in the Murchison meteorite (CRONIN and MOORE, 1971; KVENVOLDEN *et al.*, 1970, 1971; LAWLESS, 1973, in press ORO *et al.*, 1971). The suite of amino acids found in these studies is extremely complex and includes both protein and non-protein types. All amino acids with an asymmetric center and whose D and L isomers could be separated by gas chromatography showed nearly equal concentrations of each isomer.

It has been pointed out, however, that the state of the amino acids prior to extraction and the chemical reactions which they may undergo during extraction are not known (CRONIN and MOORE, 1971). Recently, deuterium oxide has been used for the extraction of the Murchison meteorite (LAWLESS, in press). All amino acids identified in that study showed the presence of carbon–hydrogen bonds, suggesting that these bonds existed before extraction. However, some amino acids showed varying amounts of deuterium incorporation. This result was believed to be due either to (a) a catalytic replacement of hydrogen by deuterium in existing amino acids or (b) the synthesis of amino acids during the extraction procedure.

The technique of mass fragmentography using stable isotopes (HAMMAR *et al.*, 1968; GORDON and FRIGERIO, 1972; KNAPP *et al.*, 1972; PEREIRA *et al.*, 1973) has become widely recognized as a reliable, quantitative, analytical method for the determination of submicrogram quantities of biologically significant compounds. In this method, the mass spectrometer is used to detect and identify only specific pre-selected ions in the mass spectrum of the compound to be analyzed and its deuterated analog (the internal standard). Thus, by spiking samples and extracts of the Murchison meteorite with deuterated amino acids, mass fragmentography has enabled us to (1) measure the amount of amino acids extracted from the meteorite and (2) to investigate the mechanism of carbon–hydrogen bond formation during the extraction procedure. This paper reports the results of this study.

EXPERIMENTAL

Chemicals

A deuterated amino acid mixture was obtained from Merck Laboratory Chemicals, New Jersey. It consisted of glycine (d_2) and the L isomer of each of the following amino acids: alanine (d_4), arginine (d_7), aspartic acid (d_3), glutamic acid (d_5), histidine (d_5), isoleucine (d_{10}), leucine (d_{10}), lysine (d_9), methionine (d_8), phenylalanine (d_8), proline (d_7), serine (d_3), threonine (d_5), tyrosine (d_7), and valine (d_8). Tabsorb column packing was purchased from Regis Chemical Co., Illinois. A standard solution of L-amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, tyrosine, and valine) was obtained from Pierce Chemical Co., Illinois.

Instrumentation

A Varian Model 1200 gas chromatograph was coupled by means of an all-glass membrane separator (HAWES *et al.*, 1969) to a Finnigan 1015 Quadrupole mass spectrometer which was interfaced to the ACME computer system of the Stanford University Medical School (REYNOLDS *et al.*, 1970). Gas–liquid chromatographic (GLC) separations of the N-trifluoroacetyl (N-TFA)-O-*n*-butyl derivatives were carried out with a 2 m × 4 mm (i.d.) coiled glass column packed with Tabsorb. The flow rate of the carrier gas (He) was 60 ml/min. The GLC separation of the diastereoisomeric amino acid N-TFA-O-(+)-2-butyl ester derivatives was achieved using a 46 m × 0·05 cm capillary column coated with UCON 75-H-90,000. A Perkin–Elmer Model 990 gas chromatograph programmed from 100 to 155°C at 1°C/min was used.

Sample preparation

An interior sample of the Murchison meteorite was reduced to a fine powder using a porcelain mortar and pestle, thus yielding a common sample for all the experiments described.

Procedure

Two 1 g samples of the Murchison meteorite were analyzed for the presence of eight amino acids (alanine, valine, glycine, isoleucine, leucine, proline, aspartic acid and glutamic acid) by

mass fragmentography. The first meteorite sample was refluxed with water (3 ml) for 20 hr, centrifuged, and the extract evaporated to dryness. To the dry extract was added 50 μl. of a solution of deuterated amino acids (1 mg of mixture/ml H_2O). Fifty μl of the deuterated amino acid solution were added to the second sample prior to hot water reflux. After being refluxed, the solution was centrifuged and the residue washed with 2 ml of water. The extract and wash were combined and evaporated to dryness. The dry extract from each sample was separately dissolved in 0·5 ml of a solution of 0·09 N HCl in methanol* and applied to a dry column (0·9 × 10 cm) of silica gel. The amino acids were eluted with 0·09 N HCl in methanol* and isolated in the first 1·5 ml of eluate. The eluate from each sample was centrifuged (to remove silica gel fines), the supernatant evaporated to dryness, and the N-TFA-O-*n*-butyl derivative prepared (Roach and Gehrke, 1969). An aliquot of each isolated sample (1–2 μl) was injected into the gas chromatograph (oven temperature, 80°C) and after a 10 min delay, the temperature was programmed at 2°C/min to 140°C, and then at 4°C/min to 210°C. Data acquisition commenced after 15 min. The mass spectrometer was operated at 70 eV and an ionizing current of 250 μA.

Calibration curves

To each of four tubes containing 2 ml of the deuterated amino acid standard solution (1 mg of mixture/ml) were added 150, 200, 250 and 300 μl, respectively, of a standard amino acid

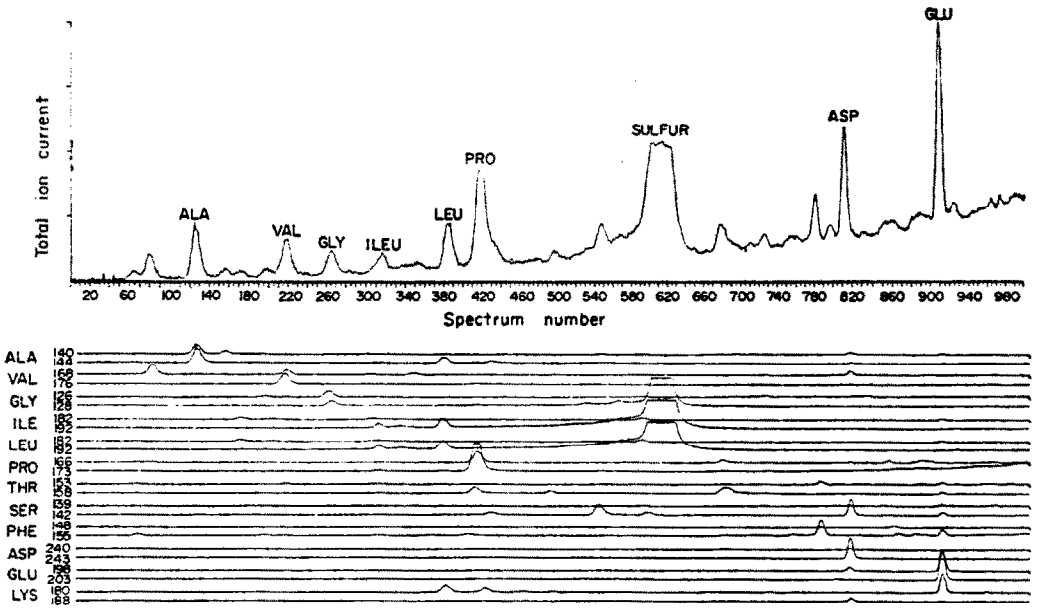

Fig. 1a. This mass fragmentogram results from the summation of the ion chromatograms (Fig. 1b) obtained from the indigenous meteorite and spiked (deuterated) amino acids.

Fig. 1b. The ion chromatograms were produced by selectively monitoring the indicated 22 ions throughout the GC–MS run. The ions selected are those characteristically produced by the N-TFA-O-*n*-butyl ester derivatives of the amino acids. Slight variations in retention time between non-deuterated and deuterated amino acid derivatives have been observed (Pereira *et al.*, 1973).

* The 0·09 N HCl in methanol was prepared by adding 0·36 ml of 12 N HCl to 48·0 ml of absolute methanol.

solution (2·5 μmole of each amino acid/ml). Each solution was evaporated to dryness and the residue isolated as the N-TFA-O-*n*-butyl derivative. Each such sample was subjected to mass fragmentographic analysis.

Mass fragmentography

In this technique, up to 25 integer mass values situated anywhere in the mass range of the Finnigan Quadrupole mass spectrometer (0–750 amu) may be monitored sequentially by computer control. The data acquisition software has been described (SUMMONS *et al.*, 1974). This procedure allows for small drifts in instrument calibration to be corrected on each mass on every scan. The precision intensity data for the 22 masses is collected and filed on disc at 2 sec intervals; typically, 750 such passes are made in the direction of each sample run. A sum of the precision intensities of the 22 preselected masses were used to construct the mass fragmentogram shown in Fig. 1a. Experimental values are reproducible to ±10 per cent using this technique.

Data analysis program

The program has, as its input, the series of ion chromatogram pairs shown in Fig. 1b. Each ion chromatogram is represented as a series of 12-bit digital samples measuring the ion current at that mass as a function of time. The program locates candidate peaks in the various ion chromatograms, selects those peak pairs corresponding to the deuterated and undeuterated analogs of each treated amino acid, and measures the area ratio for each pair for purposes of identification. Details of the data analysis program such as peak location, establishment of background levels, and peak area ratio calculations using the standard calibration curves are described elsewhere (SUMMONS *et al.*, 1974).

Racemization experiment

A 0·5 g sample of the meteorite was mixed with water (5 ml) containing the standard L-amino acid mixture (0·25 μmole of each amino acid). The reaction mixture was refluxed for 20 hr, cooled and centrifuged. The supernatant was reduced to about 1 ml, adjusted to pH 2 and passed through an ion exchange column (10 × 1·5 cm, AG 50W X-12, 50–100 mesh, H^+ form). Water (30 ml) was passed through the column and the amino acids were eluted with 3 N NH_4OH solution (40 ml). The NH_4OH eluate was evaporated to dryness and the N-TFA-O-(+)-2-butyl ester (POLLOCK and OYAMA, 1966) derivatives prepared. A control experiment was performed using the same analytical procedure but in the absence of meteorite. With the exception of aspartic acid, which showed an increase in the D isomer concentration equivalent to that obtained in the presence of meteorite, no racemization of the protein amino acids were observed.

A 250 μl standard L-amino acid solution (2·5 μmoles of each amino acid/ml) was evaporated to dryness, dissolved in 2 ml of D_2O, and added to a 1 g sample of the meteorite. Additional D_2O (8 ml) was added to the sample and the tube then sealed and heated at 150°C for 90 hr. The amino acids were recovered and the N-TFA-O-*n*-butyl esters prepared as described above.

RESULTS AND CONCLUSIONS

Identification

Previous studies of Murchison meteorite extracts have revealed the presence of six protein amino acids (valine, alanine, glycine, proline, aspartic acid and glutamic acid). In this study, leucine and isoleucine have also been identified. A typical mass fragmentogram of the eight N-TFA-O-*n*-butyl ester derivatives of the indigenous protein amino acids present in the meteorite, together with the spiked, deuterated amino acids, is shown in Fig. 1a. The resulting ion chromatograms are presented in Fig. 1b. Phenylalanine, tyrosine, lysine, serine and threonine were not detected; they were either absent or isolated in amounts below the

detectable limits of the analytical system used. Arginine and histidine would not have been eluted under the gas chromatographic conditions employed. The identification of the eight protein amino acids (Table 1) found in this study is in close agreement with the results of other investigators (CRONIN and MOORE, 1971; CRONIN, 1973).

Table 1. Identification of eight protein amino acids ($\mu g/g$) in the Murchison meteorite

Amino acid	Nonhydrolyzed Murchison meteorite extract analyzed by mass fragmentography	Nonhydrolyzed Murchison meteorite extract (CRONIN, 1973)	Hydrolyzed Murchison meteorite (CRONIN and MOORE, 1971)
Ala	2·1	2·1	3·5
Val	1·2	0·8	1·6
Gly	3·1	3·4	6·1
Ileu	0·3	—	—
Leu	0·2	—	—
Pro	0·7	0·4	1·3
Asp	0·3	0·4	1·7
Glu	3·3	0·6	3·1

It was observed that when α-amino acids (unlabeled or labeled with deuterium) were added to the meteorite and heated with deuterium oxide or water, a significant loss of certain of these amino acids occurred. The most noticeable losses occurred with aspartic acid and phenylalanine, while tyrosine was not recovered at all. The degree of loss of each amino acid and the determination of whether this loss is due to adsorption to the meteorite matrix and/or destruction is currently under investigation.

Hydrogen–deuterium exchange

Previous work (LAWLESS, in press) has shown that when a sample of the Murchison meteorite is refluxed with D_2O, deuterium is found in some of the amino acids extracted. In an effort to understand the mechanism of deuterium incorporation (synthesis of carbon–deuterium bonds from precursors or hydrogen–deuterium exchange in existing amino acids), mass fragmentography was used to measure the loss of deuterium from the deuterated L-amino acids when they were added to the meteorite prior to the 20 hr reflux with water. The deuterium content of the deuterated amino acids was first determined. Their respective N-TFA-O-*n*-butyl ester derivatives were prepared and subjected to the following analysis. Each of the characteristic ions listed in Fig. 1b plus the corresponding isotopic species containing one, two or three less deuterium atoms was quantitated in a single analysis. A computer program was written to subtract the theoretical ^{13}C contribution from ions one mass lower in each amino acid ion series and then to calculate the isotopic composition of each of the deuterated isolated amino acids. In a completely analogous manner, the isotopic composition of the deuterated amino acid standards was determined. The quantitative results from both series of measurements are presented in Table 2. It is evident that there is a considerable

Table 2. Isotopic distribution analysis of deuterated amino acids by mass fragmentography

	Isotopic composition of deuterated amino acid standards		Deuterated amino acids added before H_2O extraction of meteorite
	m/e	%	%
Ala	141	0·2	24·6*
	142	1·3	3·1
	143	11·8	17·3
	144	86·7	55·0
Val	173	4·4	3·3
	174	3·3	3·5
	175	19·3	20·9
	176	73·0	72·2
Gly	127	16·0	81·9*
	128	84·0	18·1
Leu	190	3·8	5·7
	191	21·1	33·8
	192	75·1	60·5
Ileu	190	5·4	5·5
	191	25·6	32·4
	192	68·9	62·1
Pro	171	2·0	2·0
	172	15·7	16·3
	173	82·3	81·7
Asp	241	1·9	23·7*
	242	14·7	32·9
	243	83·4	43·4
Glu	201	1·9	7·5
	202	17·6	32·3
	203	80·5	60·2

* The intensity of these peaks registers abnormally high due to contributions from the indigenous amino acids.

loss of the isotopic label when the deuterated amino acid mixture is added to the meteorite prior to the hot water extraction, except in the cases of valine and proline. In these cases, virtually no loss of deuterium was observed. This result might have been due to selective catalytic activity resulting from steric or inductive effects (BADA, 1972; NAKAPARKSIN *et al.*, 1970). Further evidence for exchange was found when a mixture of pure L-amino acids was heated in the presence of the meteorite and deuterium oxide at 150°C. In this case substantial amounts of deuterium were incorporated into the eight protein amino acids studied (Table 3). It was assumed that if noncatalyzed hydrogen–deuterium exchange occurred, it would involve the α-hydrogen, but the data in Table 3 do not support this concept. While the major change in mass spectral peak intensities results from the replacement of one hydrogen atom by a deuterium atom, the increase in intensity of many of the peaks over the range of *m/e*'s investigated is indicative of an additional catalytic effect, resulting in multiple hydrogen–deuterium exchanges. The relatively low level of incorporation of deuterium in aspartic acid and glutamic acid

Table 3. Deuterium incorporation into L-amino acids by heating to 150°C for 90 hr in a sealed tube in the presence of D_2O

	m/e	Standard undeuterated amino acids	L-Amino acids added prior to D_2O extraction of meteorite
Ala	140	73·2	33·7
	141	24·1	40·8
	142	2·6	11·6
	143	—	2·4
	144	—	11·4
Val	168	82·5	29·4
	169	13·4	48·5
	170	4·1	22·1
Gly	126	54·5	20·0
	127	43·3	19·4
	128	2·2	34·7
	129	—	25·9
Ileu	182	79·3	60·6
	183	19·0	30·5
	184	1·7	8·9
Leu	182	78·7	48·0
	183	19·0	38·0
	184	2·2	14·0
Pro	166	84·7	20·8
	167	14·1	61·1
	168	0·5	8·1
	169	0·0	7·0
	170	0·7	3·0
Asp	240	67·6	72·3
	241	27·1	22·4
	242	4·9	3·3
	243	0·3	2·0
Glu	198	86·9	83·8
	199	11·6	11·1
	200	1·5	5·1

is at present unexplained. These results preclude the determination of the degree of racemization which may occur with hydrogen–deuterium exchange.

In order to ascertain if racemization accompanied the hydrogen–deuterium exchange, a sample of the meteorite, fortified with a standard mixture of L-amino acids, was refluxed with water for 20 hr. The resulting degree of racemization is shown in Table 4. The results obtained by running a similar experiment but under more drastic conditions than those commonly employed, heating in a sealed tube with water at 150°C for 90 hr, show a large degree of racemization (Table 4). It has been previously observed (GREENSTEIN and WINITZ, 1961; GARNETT *et al.*, 1972) that the hydrogen–deuterium exchange with concurrent racemization of amino acids can occur in the presence of metal ions, with the rate of exchange of the α-proton being dependent on the structure of the side chain. JUNK and SVEC (1963) found that when amino acids were heated with deuterium oxide in a sealed tube, only those protons attached to the carboxyl

and amino groups were replaced by deuterium. Furthermore, JUNK and SVEC (1964) found multiple hydrogen–deuterium exchange in a pyridoxal–metal–amino acid system. Our results therefore suggest that the meteorite contains material having catalytic activity which is responsible for the incorporation of deuterium from deuterium oxide into the amino acids. This process also produces some racemization.

Table 4. Degree of racemization of L-amino acids added to Murchison meteorite and heated with water

Amino acid	% D-amino acid after heating	
	Refluxed 20 hr at 100°C	Sealed tube 90 hr at 150°C
Val	2	21
Ala	10	27
Ile	2	5
Leu	4	11
Pro	4	33
Asp	4	45
Glu	14	3

SUMMARY

1. It is concluded that within experimental errors, all the carbon–deuterium bond formation observed after extraction of the meteorite with deuterium oxide results from hydrogen–deuterium exchange in existing carbon–hydrogen bonds, rather than from the synthesis of carbon–deuterium bonds during the extraction procedure.

2. The results presented show the utility of quadruple mass fragmentography for isotopic distribution studies and for the quantitation of amino acids in meteorite extracts. Similar investigations of other extraterrestrial soils, should they become available, would be advantageous.

3. Since the presence of optical activity strongly indicates the presence of biological activity on a planet (ULBRICHT, 1962; LEDERBERG, 1965), the importance of using a method of extraction and isolation of optically active compounds free from significant racemization cannot be overemphasized. Thus, while the extraction techniques currently employed in meteorite investigations produce only a small degree of racemization, an extraction procedure which produces no racemization should be employed.

4. Since it was not possible for us to extract amino acids quantitatively from the meteorite, the amount and isomeric distribution of amino acids indigenous to meteorites has yet to be accurately obtained.

5. The use of quadrupole mass fragmentography for the detection of amino acids on other planets is suggested to be a viable approach to the question of whether life can, or may have, existed outside Earth.

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