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A search for amino acids and nucleobases in the Martian meteorite Roberts Massif 04262 using liquid chromatography-mass spectrometry

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Abstract–The investigation into whether Mars contains signatures of past or present life is of great interest to science and society. Amino acids and nucleobases are compounds that are essential for all known life on Earth and are excellent target molecules in the search for potential Martian biomarkers or prebiotic chemistry. Martian meteorites represent the only samples from Mars that can be studied directly in the laboratory on Earth. Here, we analyzed the amino acid and nucleobase content of the shergottite Roberts Massif (RBT) 04262 using liquid chromatography-mass spectrometry. We did not detect any nucleobases above our detection limit in formic acid extracts; however, we did measure a suite of protein and nonprotein amino acids in hot-water extracts with high relative abundances of β-alanine and γ-amino-*n*-butyric acid. The presence of only low (to absent) levels of several proteinogenic amino acids and a lack of nucleobases suggest that this meteorite fragment is fairly uncontaminated with respect to these common biological compounds. The distribution of straight-chained amine-terminal *n*-ω-amino acids in RBT 04262 resembled those previously measured in thermally altered carbonaceous meteorites (Burton et al. 2012; Chan et al. 2012). A carbon isotope ratio of −24‰ ± 6‰ for β-alanine in RBT 04262 is in the range of reduced organic carbon previously measured in Martian meteorites (Steele et al. 2012). The presence of *n*-ω-amino acids may be due to a high temperature Fischer-Tropsch-type synthesis during igneous processing on Mars or impact ejection of the meteorites from Mars, but more experimental data are needed to support these hypotheses.

INTRODUCTION

Martian meteorites were ejected from Mars by impact events and eventually landed on Earth. Laboratory analyses of Martian meteorites provide an excellent opportunity to study Mars' past geochemistry and could provide additional insight into the interpretation of data from Mars missions such as the Mars Science Laboratory (MSL) mission that includes organic detection and bulk carbon isotopic analysis capability with the Sample Analysis at Mars (SAM) instrument suite (Mahaffy et al. 2012). Organic compounds, including amino acids and polycyclic aromatic hydrocarbons (PAHs), have previously been detected in Martian meteorites, although their origin has been intensely debated (Wright et al. 1989; McDonald and Bada 1995; McKay et al. 1996; Becker et al. 1997; Bada et al. 1998; Glavin et al. 1999; Jull et al. 2000; Sephton et al. 2002).

McDonald and Bada (1995) analyzed water- and acid-soluble extracts of the Antarctic shergottite Elephant Moraine (EET) A79001 for amino acids and found almost exclusively L-enantiomers (the biologically favored form) and amino acids that are common to proteins. The absence of α-aminoisobutyric acid (α-AIB), a nonprotein amino acid abundant in CM2 carbonaceous chondrites (Kvenvolden et al. 1971), and an amino acid distribution similar to Antarctic ice meltwater led to the conclusion that the detected amino acids in EETA79001 were terrestrial contaminants. Bada et al. (1998) reached a similar conclusion regarding Allan Hills (ALH) 84001; the detected amino acids appeared to be terrestrial in origin derived from the Antarctic ice, although an extraterrestrial origin for traces of D-alanine could not be ruled out. Glavin et al. (1999) analyzed water- and acid-soluble extracts of Nakhla, a Martian meteorite that fell in the Nile Delta in Egypt in 1911, and found a much more diverse suite of amino acids than the Antarctic meteorites ALH 84001 and EETA79001 that included D- and L-aspartic acid, D- and L-glutamic acid, glycine, D- and L-alanine, β-alanine (β-ala), and γ-amino-*n*-butyric acid (γ-ABA), but no α-AIB. The same suite of amino acids was also measured in Nile Delta sediment with very similar D/L ratios for aspartic and glutamic acids and alanine and the most likely source of the D-amino acids can be attributed to peptidoglycan remnants from bacterial cell walls (McCarthy et al. 1998); therefore, Glavin et al. (1999) concluded that the D- and L-amino acids detected in the Nakhla meteorite were most likely derived from terrestrial biological contamination from the landing site.

All of the previous evidence points toward a significant contribution of terrestrial amino acids in the Martian meteorites analyzed to date for two reasons (1) ALH 84001 (and other Antarctic SNCs) and Nakhla have very different amino acid distributions that are similar to their landing site environments, and (2) the D/L ratios of protein amino acids in the SNCs match very well with terrestrial D/L ratios from the respective landing site environments. However, a recent publication by Steele et al. (2012) reported that reduced organic compounds associated with magmatic minerals were likely indigenous to Martian meteorites and that the organic compounds formed via igneous processes on Mars. Pyrolysis data from solvent-extracted Martian meteorites also had some similarities to organic matter in carbonaceous chondrites, which suggested a nonterrestrial origin (Sephton et al. 2002). Recently, Burton et al. (2012) measured a variety of extraterrestrial amino acids in thermally altered type 3 CV and CO carbonaceous chondrites and ureilites recovered from Antarctica. In contrast to aqueously altered carbonaceous chondrites, thermally altered meteorites are dominated by small, straight-chain terminal-amine amino acids (*n*-ω-amino acids) possibly produced by Fischer-Tropsch-type (FTT) reactions (Burton et al. 2012). Thus, it is possible that igneous processes on Martian meteorites or shock heating during impact ejection from Mars may have led to the formation of indigenous amino acids after cooling (below 300 °C for amino acids to have survived decomposition) (Li et al. 2006, 2007, 2008).

Here, we report on measurements of the abundance, distribution, and carbon isotope ratios of amino acids in the Martian meteorite Roberts Massif (RBT) 04262. RBT 04262 is a relatively young lherzolitic shergottite with a Lu-Hf age of 225 Myr (Lapen et al. 2008; Usui et al. 2010). It was ejected from Mars 2.9 Myr ago, impacted Earth 0.7 Myr ago, and experienced moderate weathering while in Antarctica (Satterwhite and Righter 2007; Nishiizumi and Caffee 2010) before being recovered as a single stone. In addition, we applied our methods (Callahan et al. 2011) to target nucleobases and nucleobase analogs in formic acid extracts of RBT 04262 as well as ALH 84001, Allan Hills (ALH) A77005, EETA79001, and Miller Range (MIL) 03346. Nucleobases are nitrogen heterocyclic compounds that are essential components of the informational monomers of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The canonical nucleobases (adenine, uracil, guanine, cytosine, and thymine) as well as a few nucleobase analogs have been reported in a variety of carbonaceous chondrites (Van der Velden and Schwartz 1977; Stoks and Schwartz 1979, 1981; Martins et al. 2008; Callahan et al. 2011). Therefore, the possibility exists that nucleobases were delivered to the Martian surface by carbonaceous meteorites and eventually incorporated into Martian rocks. To our knowledge, this study represented the first search for nucleobases and nucleobase analogs in Martian meteorites, the first amino acid analysis for RBT 04262, and the first compound-specific carbon isotope ratio measurement of amino acids in a Martian meteorite.

EXPERIMENTAL

Chemicals and Reagents

All of the chemicals used in this study were purchased from Sigma-Aldrich, Fisher Scientific, and Acros Organics. Ultrapure water (18.2 MΩ, <3 parts-per-billion [ppb] total organic carbon provided by a Millipore Milli-Q Integral 10 system) was used exclusively for this study. To prepare amino acid standard solutions, individual compounds were dissolved in ultrapure water and then combined to enable their measurement in a single chromatographic separation. To prepare nucleobase standard solutions, individual compounds were dissolved in 0.1 M ammonium hydroxide and then combined to enable their measurement in a single chromatographic separation.

The *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) reagent used for amino acid derivatization was prepared by mixing 300 μL 0.1 M OPA (in methanol) with 15 μL 1 M NAC and 685 μL 0.1 M sodium borate buffer (pH 9) (Glavin et al. 2006). Solutions of sodium borate were prepared from solid sodium tetraborate decahydrate (Sigma Ultra 99.5–100% purity) that was heated in air at 500 °C for 3 h to remove any organic contaminants prior to dissolution in water. A hydrazine solution was prepared by vacuum triple-distillation of anhydrous hydrazine (98% purity) over slush chloroform followed by freezing into sufficient ultrapure water to prepare 0.1 M. The 6 M HCl solution for the acid hydrolysis procedure was prepared by dilution of concentrated HCl followed by double distillation. For the LC-MS analyses, ammonium formate buffer was prepared by NH_4OH titration of a 10 mM or 50 mM formic acid solution to the appropriate pH and then adding methanol. Methanol was Optima® grade from Fisher Scientific.

Sample Handling and Extraction Procedures

The Martian meteorites ALH 84001 (SNC orthopyroxenite, specific 425, parent 15), ALHA77005 (shergottite, specific 233, parent 100), EETA79001 (shergottite, specific 666, parent 510), MIL 03346 (nakhlite, specific 213, parent 79), and RBT 04262 (shergottite, specific 59, parent 0) were provided by the NASA Johnson Space Center Antarctic Meteorite Collection. Interior chips were requested and no fusion crust was observed on these meteorite samples.

Sample-handling tools, ceramics, and glassware were all rinsed with ultrapure water, wrapped in aluminum foil, and heated in air at 500 °C for 24 h to remove any organic residue. Individual meteorite fragments were powdered and homogenized using a ceramic mortar and pestle in a Class 100 laminar flow hood (Labconco) under HEPA-filtered positive pressure. A procedural control sample consisting of a crushed serpentine sample (hydrated magnesium silicate) that had been heated at 500 °C for 36 h in air was taken through the same extraction and analytical procedures as the meteorites.

For the nucleobase analyses, each powdered meteorite sample (approximately 200–250 mg) was extracted with 3 mL 95% formic acid at 100 °C for 24 h in flame-sealed glass ampoules. The ampoules were frozen in liquid nitrogen before opening, centrifuged, and the supernatant transferred to a test tube to dry under vacuum (Labconco Centrivap). After the residue was dry, it was dissolved in 200 μL 0.1 M ammonium hydroxide and transferred to an autosampler vial. Meteorite extracts were stored in a −86 °C freezer when not in use.

For the amino acid analyses, each powdered meteorite sample (approximately 190–210 mg) was extracted with 1 mL ultrapure water at 100 °C for 24 h in flame-sealed glass ampoules. The meteorite extracts were centrifuged and the supernatant was split into two approximately equal fractions. One half of the water supernatant (500 μL) was transferred into a separate glass tube, dried under vacuum, and subjected to acid hydrolysis under 6 M HCl vapor at 150 °C for 3 h to liberate amino acids (Cronin and Moore 1971). The acid-hydrolyzed extract (representing the total amino acid content) was dried under vacuum and redissolved in 1 mL of water. Both nonhydrolyzed and acid-hydrolyzed water extracts were then desalted by prepacked cation exchange column (AG 50W-X8, 100–200 mesh, hydrogen form, BIO-RAD) using water followed by 2 M NH_4OH. The NH_4OH eluate was dried under vacuum and redissolved in 100 μL water. Meteorite extracts were stored in a −86 °C freezer until precolumn derivatization and LC-MS analysis.

Chemical Derivatization for Amino Acid Analysis

For OPA/NAC amino acid derivatization, 10 μL meteorite extract (or amino acid standard solution) was mixed with 20 μL 0.1 M sodium borate buffer (pH 9) in an HPLC vial and the solution was dried under vacuum. The dried residue was dissolved in 20 μL ultrapure water and derivatized with 5 μL OPA/NAC. The derivatization reaction was then quenched after 15 min at room temperature with 75 μL 0.1 M hydrazine, and 25 μL of the solution was immediately injected into the LC-MS. A 15 min derivatization time was used because it typically results in a much cleaner chromatogram (compared to a 1 min derivatization) and greater sensitivity for both α-AIB and isovaline, which are rare amino acids and used as an indicator of extraterrestrial origin. OPA/NAC amino acid derivatives were analyzed using a Waters ACQUITY ultraperformance liquid chromatograph and Waters ACQUITY fluorescence detector connected in series to a Waters LCT Premier time-of-flight mass spectrometer (LC-FD/ToF-MS).

LC-MS Parameters for Amino Acids

Our LC-FD/ToF-MS conditions used for amino acid analyses have been described elsewhere (Glavin et al. 2006, 2010). In brief, amino acid separation was achieved using two sequential UPLC columns; the first column was a Waters BEH C18 column (2.1 × 50 mm, 1.7 μm particle size) followed by a Waters BEH Phenyl-Hexyl column (2.1 × 150 mm, 1.7 μm particle size) maintained at 30 °C. Mobile phase (A) was composed of 50 mM ammonium formate buffer pH 8.0 with 8%

methanol, and mobile phase (B) was 100% methanol. Mobile phase (B) was changed over a linear gradient as follows: 0–35 min 0–55% B, 35–45 min 55–100% B, 45–50 min 100% B, and 50–60 min 0% B. A flow rate of 150 μL min^{-1} was used. We identified amino acids in the meteorite and control samples by correlating sample peaks with known standards using both detected masses and chromatographic retention times, as detected by fluorescence and mass spectrometry. A detection limit for amino acids was previously determined to be about 10^{-15} to 10^{-16} mol for both fluorescence and mass detection (Glavin et al. 2006).

LC-MS Parameters for Nucleobases

Nucleobase and nucleobase analog separation was achieved using two sequential HPLC columns, which were both Phenomenex Luna Phenyl-Hexyl columns (2.0 × 150 mm, 3 μm particle size) maintained at 50 °C. Mobile phase (A) was composed of 10 mM ammonium formate buffer pH 3.9 with 2% methanol, and mobile phase (B) was 100% methanol. Mobile phase (B) was changed over a linear gradient as follows: 0–20 min 5–25% B, 20–21 min 25–95% B, 21–35 min 95% B, 35–36 min 95–5% B, and 36–46 min 5% B. A flow rate of 100 μL min^{-1} was used.

The following lists the source parameters for positive ion electrospray (ESI). The ESI source voltage was 4.0 kV. The sheath and auxiliary gases for desolvation of the electrospray were both nitrogen and set to 35 and 10, respectively (both arbitrary units). The ion transfer capillary voltage and ion transfer capillary temperature were 21 V and 275 °C, respectively. The tube lens was set at 55 V. Full scan spectra were acquired over a mass range of m/z 50 to 500. To maintain a sufficient number of data points across chromatographic peaks, a mass resolution setting of 30,000 (at full-width-half-maximum for m/z 400) was used. Data-dependent MS/MS acquisition in both the linear ion trap (normalized CID energy 30% and isolation width of 2 Da) and the Orbitrap mass analyzer (HCD energy 60% and 100% and isolation width of 2 Da) was used. External calibration for positive ion mode in the range of m/z 120 to 2000 was performed using a mixture of caffeine, MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate hydrate) peptide, and Ultramark 1621 in an acetonitrile-methanol-water solution containing 1% acetic acid, which resulted in a typical mass accuracy of <3 ppm.

Compound-Specific Carbon Isotopic Analysis

Three acid-hydrolyzed hot-water extracts of 956.0 mg, 1014.0 mg, and 1840.6 mg of RBT 04262 (shergottite, specific 65, parent 63) were desalted by cation exchange chromatography as previously described and then combined to compose one solution. The total volume was dried, reacted with acidified isopropanol and trifluoroacetic anhydride to form volatile derivatives, and analyzed via gas chromatography coupled with mass spectrometry and isotope ratio mass spectrometry (GC-MS/IRMS) as described previously (Elsila et al. 2011).

RESULTS AND DISCUSSION

Search for Nucleobases and Nucleobase Analogs in Martian Meteorites

A variety of nucleobases (cytosine, thymine, uracil, adenine, and guanine) and nucleobase analogs (isocytosine, isoguanine, hypoxanthine, xanthine, purine, 2-aminopurine, 2,6-diaminopurine, 6,8-diaminopurine, and 5-aminoorotic acid) were searched for in formic acid extracts of the five Martian meteorites. For the majority of these target compounds (cytosine, adenine, guanine, isocytosine, hypoxanthine, purine, 2-aminopurine, 2, 6-diaminopurine, and 6,8-diaminopurine), we can detect amounts that would correspond to a 1 ppb concentration in meteorite extracts from a few hundred milligrams of sample (Fig. 1).

We did not detect these nucleobases or nucleobase analogs above our detection limit in any of the five Martian meteorites studied (Fig. 1). There may be trace amounts (approximately 0.1 ppb) of guanine in ALH 84001, but we designate this detection as only tentative based on a low number of scans acquired across the chromatographic peak. Nevertheless, trace amounts of guanine in ALH 84001 could be attributed to terrestrial contamination because trace amounts of guanine have been previously measured in Antarctic ice samples (Callahan et al. 2011). Compound-specific isotope measurements of guanine in ALH 84001 would be required to establish the origin of this nucleobase in the meteorite; unfortunately, based on current GC-IRMS detection limits and the abundance of guanine in ALH 84001, much more sample than available for this study would be required for this measurement (>1 kg).

The lack of nucleobases is not surprising because these compounds typically decompose at high temperatures and Martian meteorites have experienced varying degrees of igneous processing. Pyrolysis experiments have shown that the pyrimidines cytosine and uracil (under N_2 or CO_2 atmosphere) cannot survive temperatures higher than 700 °C. For purines, guanine decomposed at temperatures above 400 °C; however, adenine displayed very high thermal stability and survived a temperature of 1000 °C (Basiuk and Douda 1999). This is also consistent with our previous

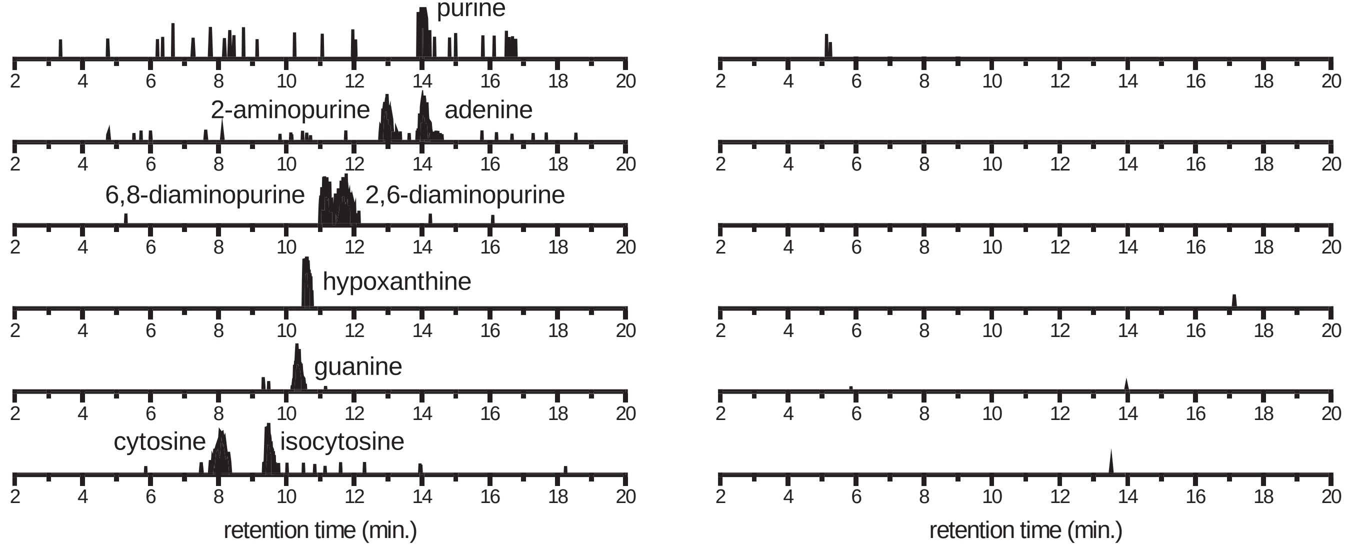

Fig. 1. Extracted ion chromatograms (using 5 ppm mass windows) of reference standards at 1 ppb concentration (left) and the formic acid extract for RBT 04262 (right). Nucleobase and nucleobase analogs that were targeted were not observed above detection limits in RBT 04262 or any of the Martian meteorites analyzed.

observation of only trace levels of adenine in the thermally altered ureilite Almahata Sitta (Callahan et al. 2011), suggesting that high temperature processing is incompatible with the formation or survival of significant amounts of nucleobases (assuming that adenine in Almahata Sitta is indigenous). On the other hand, the composition and conditions experienced on the ureilite parent body may be drastically different from Mars so a comparison between the two may not be completely relevant. Finally, these observations suggest that exogenous delivery and incorporation of nucleobases into Martian rocks (postigneous processing) may not be significant.

Amino Acid Distribution in RBT 04262

We analyzed the shergottite RBT 04262 by LC-FD/ToF-MS, which is a technique that has been applied in our laboratory to the analyses of over 30 different meteorites (covering different groups and different degrees of alteration) with great success (Glavin et al. 2006, 2010; Glavin and Dworkin 2009; Herd et al. 2011; Burton et al. 2012). The mass chromatograms of the 6 M HCl-vapor-hydrolyzed, hot-water extracts from RBT 04262 and the procedural blank show several peaks that were identified by comparison of retention time and mass with amino acid standards (Fig. 2). Fluorescence retention time was used as additional confirmation. The total procedural-blank-corrected amino acid abundances (free + bound) of identified C2–C6 amino acids in the 6 M HCl-hydrolyzed, hot-water extracts of RBT 04262 was 906 ppb. The most abundant amino acid was ε-amino-*n*-caproic acid (ε-ACA, 683 ± 12 ppb), followed by γ-ABA (130 ± 11 ppb), β-ala (59 ± 11 ppb), glycine (23 ± 4 ppb), L-alanine (5.6 ± 1.7 ppb), δ-amino-*n*-valeric acid (δ-AVA, 4.3 ± 0.2 ppb), and D-alanine (0.6 ± 0.2 ppb) (Table 1). The mass and fluorescence chromatograms of the unhydrolyzed, hot-water extracts from RBT 04262 were similar in appearance to the acid-hydrolyzed, hot-water extracts, but with lower abundances of amino acids (this data is available upon request).

Glycine in RBT 04262 exists entirely in its free form. Free amino acids have been suggested as an indicator of extraterrestrial origin because protein-bound amino acids (that are terrestrial in origin) would probably require acid hydrolysis to release them. In addition, "free" glycine in Antarctic blue ice collected from the Graves Nunatak and La Paz regions were only 0.02 parts-per-trillion (pptr) and 9.6 pptr, respectively (Glavin et al. 2006; Burton et al. 2012). However, the transfer efficiency of amino acids found in Antarctic ice to meteorites is presently unknown, so a direct quantitative comparison should be taken with caution. Thus, at this time, we cannot rule out the possibility that glycine in RBT 04262 is terrestrial in origin. High levels of ε-ACA (approximately 91% bound) in RBT 04262 was attributed to terrestrial contamination, the most likely source being Nylon-6, which is present in the storage bags for meteorite samples and exists largely in the bound form (>99%) (Glavin et al. 2006). After accounting for nonbiological ε-ACA contamination, low levels of glycine and L-alanine along with the absence of

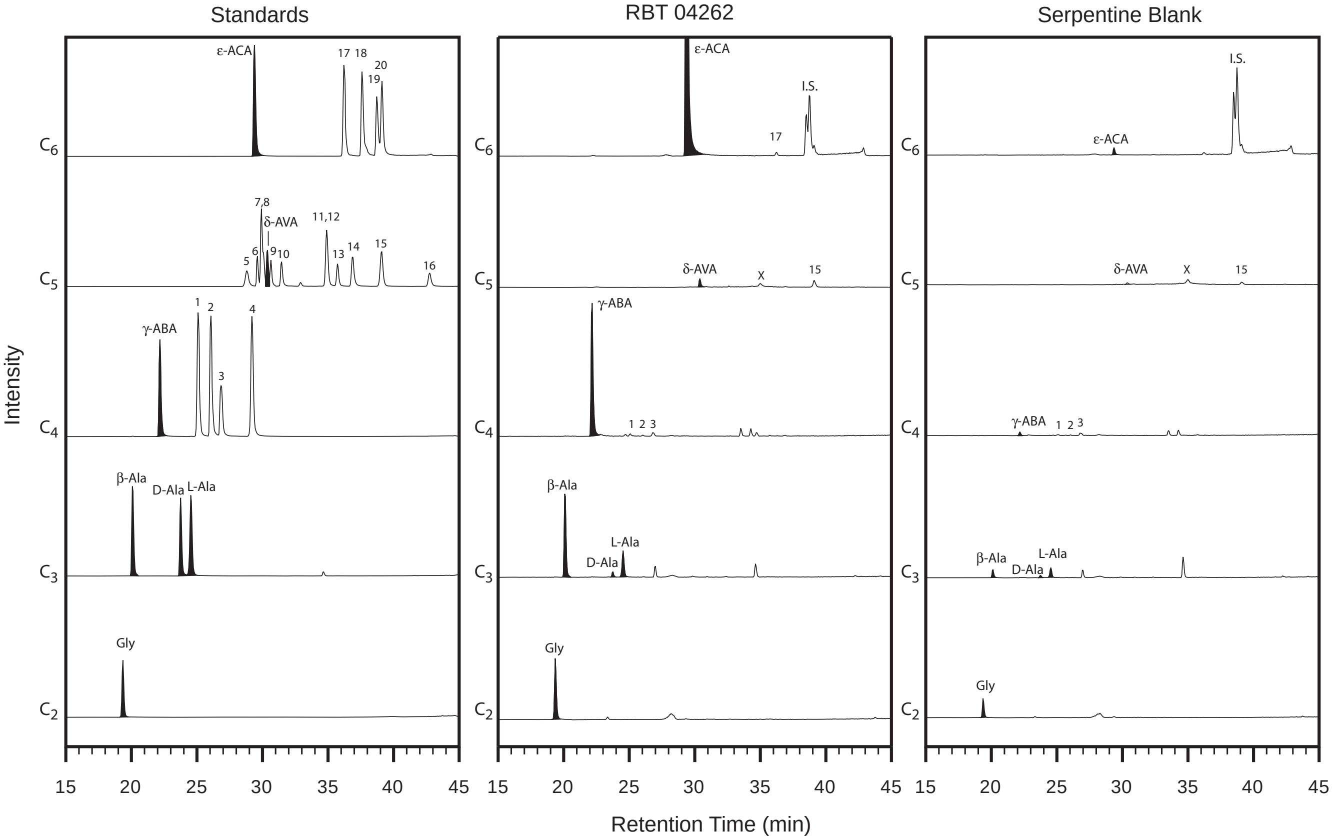

Fig. 2. LC-ToF-MS single ion chromatograms of acid-hydrolyzed RBT 04262, partial amino acid standards, and procedural blank analyzed in this study. Chromatograms shown are for the C2 through C6-carbon primary, aliphatic, acyclic amino acids. The masses plotted represent a 0.07 Da window (the peak width at half-maximum) centered around the corresponding theoretical monoisotopic masses (derivatized positive ionization mass-to-charge ratios of 351.10, 365.12, 379.13, and 393.15). The traces have been set to the same scale to each other to facilitate comparison. Peak numbers correspond to (1) D-β-amino-*n*-butyric acid, (2) L-β-amino-*n*-butyric acid, (3) α-aminoisobutyric acid, (4) D,L-α-amino-*n*-butyric acid, (5–10) 3-amino-2, 2-dimethylpropanoic acid; D,L-4-aminopentanoic acid; 4-amino-3-methylbutanoic acid; 3-amino-2-methylbutanoic acid; 3-amino-2-ethylpropanoic acid; 3-amino-3-methylbutanoic acid, (11) D-3-aminopentanoic acid, (12) D-isovaline, (13) L-isovaline, (14) L-3-aminopentanoic acid, (15) L-valine, and (16) D-valine. Not shown: D,L-norvaline; 4-amino-2-methylbutanoic acid; the "x" denotes a contaminant with a mass similar to the C5 aliphatic amino acids; I.S. is the internal standard.

common proteinogenic amino acids such as Asp, Glu, Ser, and Val suggested that RBT 04262 (or at least the fragment we analyzed) is relatively uncontaminated with respect to terrestrial biological contamination. Other *n*-ω amino acids, β-ala, γ-ABA, and δ-AVA, exist mostly in bound form ranging from 73% to 90% and are nonproteinogenic, although they do occur in the biosphere (Weinstock et al. 1939; Syrjanen et al. 1990; Owens and Kriegstein 2002).

The distribution of β-Ala, γ-ABA, and δ-AVA in RBT 04262 is similar to those previously measured in thermally altered meteorites (ureilite, CV3, and CO3), which were dominated (>75% of the amino acids) by these straight-chain *n*-ω-amino acids (Burton et al. 2012), and is shown in Fig. 3. Additionally, high relative abundances of β-Ala and γ-ABA compared with glycine were also measured in the CO3 chondrite Colony (Chan et al. 2012); however, terrestrial contamination (inferred by the D/L ratio of proteinogenic amino acids) in Colony makes it difficult to assess the extraterrestrial contribution of *n*-ω-amino acids compared with the total amino acid content. The relative distributions of *n*-ω-amino acids are very different in terrestrial sources (Chan et al. 2012) including Antarctic ice as well as the aqueously altered Murchison meteorite (in which amino acids are thought to be formed predominantly by Strecker-cyanohydrin synthesis). Burton et al. (2012) proposed that Fischer-Tropsch-type (FTT) reactions (high temperature surface-catalyzed reactions of H_2, CO, and NH_3) may have been responsible for the distinct amino acid distribution in these thermally altered meteorites (because catalytic surface processes would favor the formation of straight-chain molecules).

Table 1. Summary of the average procedural blank-corrected free (nonhydrolyzed) and total (6 M HCl acid-hydrolyzed) amino acid concentrations in the hot-water extract of RBT 04262.

	RBT 04262	
Amino acid	Free	Total
D-asp	<1	<1
L-asp	<6	<1
D-glu	<1	<1
L-glu	<2	<1
D-ser	<1	<2
L-ser	<19	<2
gly	23 ± 2	23 ± 4
β-ala	7.2 ± 0.4	59 ± 11
γ-ABA	15 ± 1	130 ± 11
D-ala	3.1 ± 0.3	0.6 ± 0.2
L-ala	7.3 ± 0.4	5.6 ± 1.7
D-β-ABA	<4	<4
L-β-ABA	<4	<4
α-AIB	<4	<5
DL-β-AIB*	<5	<5
DL-α-ABA*	<5	<5
D,L-iva*	<5	<8
3-a-2,2-dmpa	<1	<1
δ-AVA	1.6 ± 0.1	4.3 ± 0.2
ε-ACA	69 ± 2	683 ± 12
D-val	<1	<1
L-val	<2	<1
Total	126	906

All values are reported in parts-per-billion (ppb) on a bulk sample basis. Extracts were analyzed by OPA/NAC derivatization and HPLC separation with UV fluorescence detection and by LC-ToF-MS at NASA Goddard. The uncertainties are based on the standard deviation of the average value of two to six separate measurements; in some instances, co-elution in the fluorescence chromatogram prevented quantitation. Numbers containing "<" are upper limits.

*Enantiomers could not be separated under the chromatographic conditions.

Conversely, recent laboratory reactions simulating FTT reactions using CO, H_2, and NH_3 (1:1:1 ratio) in the presence of meteorite powder or magnetite powder catalysts at 370 °C for 24 h did not produce the entire *n*-ω-amino acid suite (Pizzarello 2012). Instead, varying amounts of glycine, alanine (the form was not specified although it is inferred to be a racemic mixture of D- and L-alanine), α-ABA, and α-AIB were produced depending on the choice of catalyst. Trace amounts of γ-ABA were also detected. However, these experiments were conducted using a limited range of experimental conditions (i.e., a single compound ratio, temperature, and reaction time) so *n*-ω-amino acids by FTT synthesis as a result of Martian or impact chemistry cannot yet be excluded. It is not known whether Mars contained the necessary precursor gases to undergo FTT reactions to produce amino acids. Nevertheless, a high-temperature synthesis for *n*-ω-amino acids is still a tantalizing possibility; the full suite of *n*-ω-amino acids (β-Ala, γ-ABA, δ-AVA, and ε-ACA) along with glycine and alanine that were measured in RBT 04262 have also been synthesized in hydrothermal abiotic reactions (up to 400 °C and 25 MPa) in the laboratory (Islam et al. 2001, 2003).

Even though β-Ala, γ-ABA, and δ-AVA are nonproteinogenic, these amino acids are products of biology on Earth. For example, the bacterially mediated decarboxylation of aspartic and glutamic acid result in β-Ala and γ-ABA, respectively. While analyses of the Martian meteorite Nakhla as well as nearby Nile Delta sediment revealed significant concentrations of β-Ala and γ-ABA in both (100 ppb or greater), the Martian meteorites analyzed in the current study were all collected from Antarctica, with significantly less bioburden than Nile Delta sediment. In fact, β-Ala, γ-ABA, δ-AVA, or ε-ACA were not detected above the 0.01 parts-per-trillion (pptr) level in a 7.4 kg piece of Antarctic blue ice (Burton et al. 2012); it should be noted, however, that this ice sample was collected in a different year (2006) and region (Graves Nunatak) from the Martian meteorites studied here. This argues that most of the *n*-ω-amino acids detected in some Antarctic Martian meteorites are unlikely to be contaminants from Antarctic ice meltwater.

Compound-Specific Carbon Isotope Measurements for RBT 04262

We obtained a separate, larger fragment (approximately 4 g) of RBT 04262 for isotope measurements. This fragment was extracted in three portions (approximately 1, 1, and 2 g each) using our procedures described in the Sample Handling and Extraction Procedures section. Before the desalting step, the two 1 g extractions were combined. We analyzed the two desalted and acid-hydrolyzed extracts by LC-FD/ToF-MS to determine the amino acid distribution and abundance before combining them into single extract for isotope measurements. The relative abundances (glycine = 1.0) of β-ala, γ-ABA, and δ-AVA were 2.1, 3.9, and 0.2 for one extract and 1.6, 2.9, and 0.1 for the second extract. These results compared well with our analysis of the smaller fragment of RBT 04262 where the relative abundances (glycine = 1.0) of β-ala, γ-ABA, and δ-AVA were 2.2, 4.1, and 0.1 (and shown in Fig. 3). Additionally, the common proteinogenic amino acids were absent (below our detection limits). The observation that multiple fragments consistently had a similar amino acid distribution indicated that these amino acids were not specific to one fragment only. It is also worth mentioning that amino acid

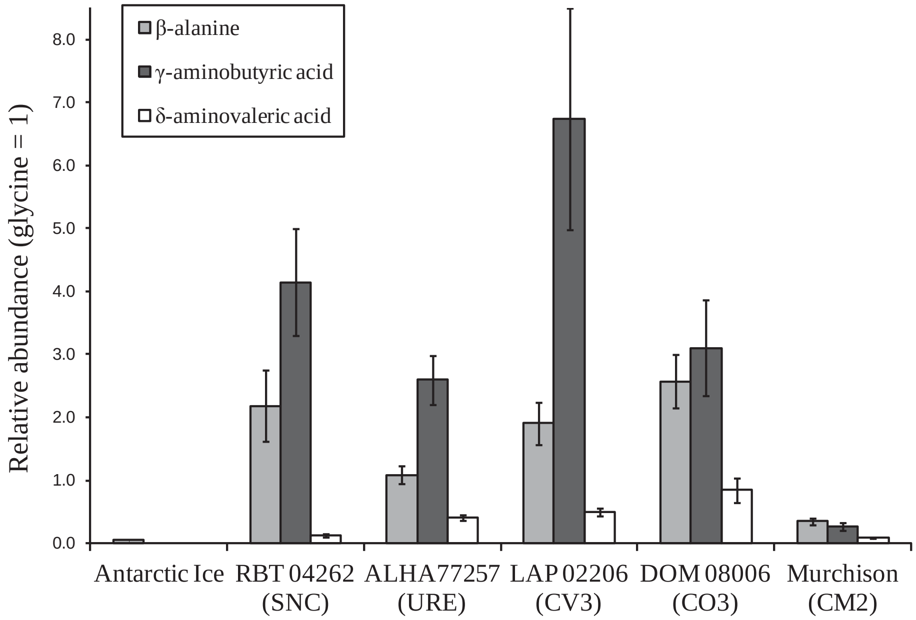

Fig. 3. A comparison of the relative molar abundances (glycine = 1.0) of β-ala, γ-ABA, and δ-AVA in the 6 M HCl-hydrolyzed, hot-water extracts of RBT 04262, thermally altered carbonaceous meteorites and Antarctic ice from Burton et al. (2012), and the aqueously altered Murchison meteorite from Glavin et al. (2010). The relative abundances were calculated from the data in Table 1 and the references listed above after correcting for the molecular weights of each amino acid. The uncertainties were calculated by standard error propagation of the absolute errors.

abundances in the meteorite fragment extracted for isotope analysis were significantly lower compared with the fragment we initially analyzed, which suggests that these compounds were heterogeneously dispersed.

We measured carbon isotope ratios ($\delta^{13}C$) of −24‰ ± 6‰ for β-Ala and −27‰ ± 5‰ for ε-ACA in RBT 04262, which, to our knowledge, is the first report of $\delta^{13}C$ for amino acids in a Martian meteorite. We were unable to determine the isotopic composition for the other *n*-ω-amino acids due to a low abundance (δ-AVA) or the presence of chromatographic interference (in the case of γ-ABA). The $\delta^{13}C$ value for ε-ACA in RBT 04262 was identical to the bulk carbon isotope measurements ($\delta^{13}C$ = −26.8‰ ± 0.2‰) of the Nylon-6 shipping and curation bags (Elsila et al. 2009), which supported that ε-ACA in RBT 04262 was due to terrestrial contamination. The $\delta^{13}C$ of β-ala in RBT 04262 was distinctly different from those previously measured in CI, CM, and CR carbonaceous chondrites, which had a range from −1 to +21‰ (Ehrenfreund et al. 2001; Pizzarello et al. 2004; Elsila et al. 2012). Rather, this value was more in the range of $\delta^{13}C$ for reduced organic carbon previously measured in Martian meteorites (including the recently recovered Tissint Martian meteorite), which on average was a $\delta^{13}C$ of –19.8 ± 4.3‰ (Steele et al. 2012). Similar depleted $\delta^{13}C$ of two *n*-ω-amino acids were measured in the thermally altered CV3 carbonaceous chondrite LAP 02206: −27‰ ± 8‰ for γ-ABA and −23‰ ± 7‰ for ε-ACA (Burton et al. 2012). Furthermore, hydrocarbons produced by FTT reactions were substantially depleted in ^{13}C (McCollom and Seewald 2006); thus, the $\delta^{13}C$ depleted values in thermally altered meteorites (including RBT 04262) may be a reflection of a similar mechanism for amino acid synthesis. On the other hand, a $\delta^{13}C$ of −24‰ ± 6‰ for β-Ala in RBT 04262 is within the range of proteinogenic amino acids ($\delta^{13}C$ of −73 to +11) from metabolically diverse microorganisms on Earth (Scott et al. 2006). Therefore, $\delta^{13}C$ measurements may not be particularly useful for differentiating between terrestrial and Martian sources. A terrestrial origin for the unusual amino acids in RBT 04262 cannot be completely ruled out.

CONCLUSION

While previous results strongly indicated that all amino acids in Martian meteorites were the result of terrestrial contamination, RBT 04642 provides the first reasonable suggestion of endogenous amino acids in a Martian meteorite. Nevertheless, more research will be necessary to conclusively support an extraterrestrial origin for amino acids in Martian meteorites (and to constrain their formation mechanism). The link between amino acids produced by FTT reactions and amino acids in Martian meteorites (as well as thermally altered carbonaceous chondrites) has yet to be firmly established and remains largely speculative at this time.

Thus, more laboratory experiments of FTT syntheses of amino acids should be conducted in the future to understand amino acid abundance, distribution, and potential depletion in ^{13}C.

It is also important to remember that these five different igneous stones only sampled small regions of Mars and are not representative of the entire Martian surface (McCoy et al. 2011). Regardless of their origin, our laboratory analyses of Martian meteorites help inform what amino acids may be present on Mars. Amino acids will be one class of organic compounds targeted by the SAM wet chemistry gas chromatograph mass spectrometer experiment and, from this study, *n*-ω amino acids may be of particular interest. SAM and MSL's discoveries may significantly improve our understanding of organic compounds on Mars and hopefully resolve ambiguities regarding the indigeneity of amino acids and other organic compounds that have been identified in Martian meteorites (assuming Martian sedimentary samples contain higher abundances of amino acids compared to Martian igneous samples). Finally, these results will also inform the scientific community if a Mars sample return mission may ultimately be needed to answer these questions.

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