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The origin of amino acids in lunar regolith samples

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Abstract

We analyzed the amino acid content of seven lunar regolith samples returned by the Apollo 16 and Apollo 17 missions and stored under NASA curation since collection using ultrahigh-performance liquid chromatography with fluorescence detection and time-of-flight mass spectrometry. Consistent with results from initial analyses shortly after collection in the 1970s, we observed amino acids at low concentrations in all of the curated samples, ranging from 0.2 parts-per-billion (ppb) to 42.7 ppb in hot-water extracts and 14.5–651.1 ppb in 6 M HCl acid-vapor-hydrolyzed, hot-water extracts. Amino acids identified in the Apollo soil extracts include glycine, D- and L-alanine, D- and L-aspartic acid, D- and L-glutamic acid, D- and L-serine, L-threonine, and L-valine, all of which had previously been detected in lunar samples, as well as several compounds not previously identified in lunar regoliths: α-aminoisobutyric acid (AIB), D- and L-β-amino-*n*-butyric acid (β-ABA), DL-α-amino-*n*-butyric acid, γ-amino-*n*-butyric acid, β-alanine, and ε-amino-*n*-caproic acid. We observed an excess of the L enantiomer in most of the detected proteinogenic amino acids, but racemic alanine and racemic β-ABA were present in some samples.

We also examined seven samples from Apollo 15, 16, and 17 that had been previously allocated to a non-curation laboratory, as well as two samples of terrestrial dunite from studies of lunar module engine exhaust that had been stored in the same laboratory. The amino acid content of these samples suggested that contamination had occurred during non-curatorial storage.

We measured the compound-specific carbon isotopic ratios of glycine, β-alanine, and L-alanine in Apollo regolith sample 70011 and found values of −21‰ to −33‰. These values are consistent with those seen in terrestrial biology and, together with the enantiomeric compositions of the proteinogenic amino acids, suggest that terrestrial biological contamination is a primary source of the amino acids in these samples. However, the presence of the non-proteinogenic amino acids such as AIB and β-ABA suggests the possibility of some contribution from exogenous sources.

We did not observe a correlation of amino acid content with proximity to the Apollo 17 lunar module, implying that lunar module exhaust was not a primary source of amino acid precursors. Solar-wind-implanted precursors such as HCN also appear to be at most a minor contributor, given a lack of correlation between amino acid content and soil maturity (as measured by I_s/FeO ratio) and the differences between the $\delta^{13}C$ values of the amino acids and the solar wind.

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1. INTRODUCTION

One of the most eagerly studied questions upon the collection of Apollo lunar samples was whether significant amounts of organic compounds were present. Throughout the 1970s, numerous studies probed the returned lunar soil in search of organic compounds. These studies detected small amounts of certain compounds, including hydrocarbons, urea, and amino acids (Hare et al., 1970; Harada et al., 1971; Nagy et al., 1970, 1971; Gehrke et al., 1972; Fox et al., 1973; Modzeleski et al., 1973). Interest in the nature of lunar organic matter has continued to the present day, with indigenous complex organic material recently identified for the first time in Apollo 17 samples (Thomas-Keprta et al., 2014).

Amino acids, which are the monomers of proteins and essential to all life on Earth, were the subject of intense interest and investigation in lunar samples; these compounds were especially intriguing because an abiotic synthetic route for them was known and extraterrestrial amino acids had recently been discovered in the Murchison meteorite (Kvenvolden et al., 1970). Only a few follow-up studies have been carried out on the lunar soils in the last 40 years (Brinton and Bada, 1996), despite significant advancements in analytical techniques and instrumentation used for organic analyses and the discoveries of amino acids in other extraterrestrial bodies such as meteorites and comets (e.g., Pizzarello et al., 2006; Elsila et al., 2009).

Early studies of Apollo lunar samples detected up to 70 parts-per-billion (ppb) of a range of amino acids. The most abundant was glycine, followed by alanine, glutamic acid, aspartic acid, serine, and threonine, with minor amounts of other proteinogenic and non-proteinogenic amino acids (Fox et al., 1973). Both hot-water extracts and acid-hydrolyzed hot-water extracts were analyzed. Amino acid yields in lunar samples increased after acid hydrolysis (Fox et al., 1975), in some cases at rates much higher than the ~100% increase in yield after acid hydrolysis typically observed for carbonaceous meteorites (Cronin, 1976; Glavin et al., 2006, 2010). This increase indicates that most amino acids in lunar soils are generated from a chemical precursor during sample processing (Fox et al., 1976; Brinton and Bada, 1996). Acid-hydrolyzable precursors to amino acids include HCN and other nitriles or polymers, which are more stable in the lunar environment than free amino acids; biological proteins also liberate free amino acids upon acid hydrolysis.

There was a lively contemporaneous discussion in the literature reports of these early studies about the source of the detected amino acids. Four primary potential sources of the amino acids were identified. Two of these sources involved biological and spacecraft-derived contamination of the lunar samples, while the other two considered indigenous sources.

The first contamination-based explanation is introduction of terrestrial biological amino acids during sample acquisition, handling, or analysis. Methodical efforts were made to reduce organic contamination of the lunar samples from potential sources including collection tools, sample return containers, curation facilities, and analytical laboratory equipment and chemicals (Flory and Simoneit, 1972). Surfaces coming into direct contact with lunar samples were cleaned to a level of $<10^{-9}$ g/cm^2 of total organics, with <0.1 ppm total organic carbon contamination measured during Apollo 12 sample processing (Flory and Simoneit, 1972). Controls and procedural blanks were performed during amino acid analysis, and the amino acid signature was also compared with that expected from handprints. However, interpretations of these tests varied, with some researchers finding that the detected amino acids correlated with handprint contamination (Nagy et al., 1971), while others found the opposite (Harada et al., 1971).

A second potential contamination source was exhaust from the lunar module engine. The relatively simple molecular distribution of amino acids was regarded as suggestive of chemical synthesis (Fox et al., 1976). Researchers attempted to address possible exhaust contamination by comparing amino acid distributions in Apollo 17 sample 70011, collected beneath the lunar module engine, and a sample (72501) collected ~6.5 km distant. Again, however, interpretations varied, with some reports concluding that the rocket exhaust could not account for the relatively high concentration of amino acids in the distant sample (Fox et al., 1976), while others argued that the pattern of amino acids detected and the variation with distance from the lunar module pointed towards the exhaust as the primary source (Gehrke et al., 1975). A similar study with an Apollo 15 sample from beneath the lunar module exhaust concluded that there was no evidence of exhaust contamination (Fox et al., 1973).

The solar wind was suggested as an indigenous (non-contaminant) source of acid-hydrolyzable precursors to amino acids (Harada et al., 1971; Fox et al., 1976; Brinton and Bada, 1996). The solar wind is a primary contributor to the H, C, and N found in lunar surface materials (Haskin and Warren, 1991). The large increase in amino acids released upon hydrolysis suggests that HCN is a possible precursor, and HCN and DCN were detected in Apollo 14 and 15 samples at levels of 10–60 nmol/g (Holland et al., 1972). Analyses of samples from trenches and the lunar surface suggested a decreasing abundance of amino acid precursors with depth (Harada et al., 1971). This observation is consistent with solar wind implantation of amino acid precursors. In addition, it was argued that the most probable reason for the agreement in detected amino acids among twelve different lunar collections was a single source, such as solar wind implantation (Fox et al., 1973, 1976).

The fourth identified potential source of the amino acids was delivery to the lunar surface by exogenous material such as carbonaceous chondrites, cometary material, or interplanetary dust particles (IDPs). The cratering record of the Moon bears witness to the period of heavy bombardment early in the history of the solar system, during which meteorites, comets, and cosmic dust would have delivered organic material to the early Earth and the lunar surface (Chyba, 1990; Chyba et al., 1990; Chyba and Sagan, 1992). Meteoritic infall has also been argued to be the source of complex organic material recently analyzed in Apollo 17 samples (Thomas-Keprta et al., 2014), with the

carbonaceous chondritic component of lunar soils estimated at 1–4% (Haskin and Warren, 1991). Gibson and Moore (1973) suggested that the high volatile content in an Apollo 16 sample (61221) may be attributed to a volatile contribution from a nearby cometary impact. However, lunar entrapment and survivability of volatiles and organics after a cometary impact on the Moon, which has no atmosphere to slow an impactor, is unknown, although recent models suggest some amount of volatile retention is possible (Ong et al., 2010; Stewart et al., 2011).

These four sources of amino acids should exhibit differences in the molecular distribution of amino acids, their isotopic ratios, and their variation with soil maturity and sample locations. For example, one primary tool to discriminate between biological and abiotic sources of amino acids is measurement of their enantiomeric ratios and molecular distributions. Terrestrial biological processes almost exclusively use the L-enantiomer of 20 proteinogenic amino acids, while abiotic chemical processes form racemic mixtures with much larger diversity. Thus, a predominance of L enantiomers of proteinogenic amino acids may indicate significant terrestrial contamination. Early analyses of lunar amino acids were performed primarily with two techniques that could not determine chirality of analytes: ion exchange chromatography (IEC) (Hare et al., 1970; Harada et al., 1971; Nagy et al., 1971; Fox et al., 1973, 1976) and gas chromatography–mass spectrometry (GC–MS) (Gehrke et al., 1972; Modzeleski et al., 1973). The subsequent analysis by Brinton, however, utilized a more sensitive high performance liquid chromatography with fluorescence detection (Brinton and Bada, 1996) that was capable of determining the chirality for a few, but not all, of the observed amino acids.

Stable isotopic ratios have also been used to distinguish terrestrial contamination from indigenous extraterrestrial organic compounds in meteorites (e.g., Engel et al., 1990; Pizzarello et al., 2001; Huang et al., 2005) and to identify indigenous glycine in comet-exposed material from the Stardust spacecraft (Elsila et al., 2009). The four potential sources of amino acids described above should possess different stable isotopic signatures. Meteoritic amino acids are enriched in heavier isotopes compared to terrestrial amino acids because of their cold interstellar or nebular history. In contrast, the $\delta^{13}C$ and $\delta^{15}N$ values of solar wind measured in materials returned by the Genesis spacecraft or in lunar regolith (Hashizume et al., 2004; Marty et al., 2011) show depletions in the heavier isotopes compared to terrestrial values. Although measurements of bulk $\delta^{13}C$ values for Apollo 11 and 12 samples were made in the 1970s (e.g. Epstein and Taylor, 1970; Kaplan et al., 1970; Chang et al., 1971), compound-specific isotopic measurements were not possible until 1978 (Matthews and Hayes, 1978), and to our knowledge have never been applied to amino acids in lunar samples.

Here, we present analyses of the amino acid content in seven curated lunar regolith samples from the Apollo 16 and 17 missions and the compound-specific stable carbon isotopic ratio for three amino acids in one sample. We also discuss amino acid analyses of Apollo 15, 16, and 17 samples that had been stored in a non-curatorial laboratory environment for over 40 years and the potential for terrestrial contamination of those samples. We compare the amino acid data with expected observations from the four potential sources outlined above and draw conclusions about the likely sources of these compounds.

2. MATERIALS AND METHODS

2.1. Lunar samples

We analyzed seven Apollo 16 and 17 regolith samples allocated from the lunar collection at NASA Johnson Space Center (JSC); for some regoliths, multiple samples were received (Table 1). These samples were fresh allocations that had been stored since their original collection in the JSC lunar curation facility, where they are held under an atmosphere of gaseous N_2. All samples were selected to span a range of maturities as measured by I_s/FeO ratio (Morris, 1978) and included two samples from Apollo 17 that were collected to test exposure to the lunar module exhaust. Two larger samples (9–12 g, Apollo 70011 and 69961) were allocated for in-depth analyses.

We also analyzed seven additional samples (Table S1) that had previously been allocated to Dr. Everett Gibson upon the return of the Apollo 15, 16, and 17 missions, and which were stored in their original stainless-steel containers with Teflon caps under terrestrial atmosphere in a safe in the Gibson lab since allocation. The safe was briefly stolen from the laboratory in 2002, and the exact handling of the samples during this time is unknown. In addition, we analyzed two samples of dunite (Table S1) that had been used to test contamination from exposure to exhaust from model firings of the lunar modules (Flory and Manned Spacecraft Center, 1972) and which had also been stored in Dr. Gibson's safe since 1970.

2.2. Extraction and processing

All glassware and sample handling tools were rinsed with Millipore Integral 10 UV (18.2 MΩ cm, <3 ppb total organic carbon) ultrapure water (hereafter "water"), wrapped in aluminum foil and heated in air at 500 °C overnight. Individual aliquots of regolith were extracted with water at 100 °C for 24 h in flame-sealed glass ampoules. For samples < 1 g in mass, 1 mL of water was used for extraction. The larger samples were split into 0.5–2 g aliquots and each aliquot was extracted with 2 mL water in a separate ampoule. After extraction, the tubes were cooled and centrifuged at 3500 rpm for 5 min. in a Hanil Fleta 5 centrifuge to separate solid particulate from water supernatant. For select samples (see Table 1), the supernatant was split in two, with half of the extract being analyzed directly in the non-hydrolyzed form (which reveals the free amino acid content), while the other half was hydrolyzed under 6 M HCl vapor to detect both free and bound amino acids. For other samples, the entire extract was acid hydrolyzed prior to analysis. Hydrolysis was performed by drying the water supernatants under vacuum and hydrolyzing with

Table 1
Curated lunar regoliths investigated in this study.

Sample #	Specific, parent	I_s/FeO ratio (maturity)[a]	Mass analyzed (g)
73131	5, 0	16, immature	0.33[d]
73241	8, 0	18, immature	0.27[d]
78501	247, 234	36, submature	0.46
70011[b]	211, 26	54, submature	0.27[d]
70011[b]	35, 0	54, submature	9.78
72501[c]	59, 0	81, mature	0.29[d]
78421	20, 5	92, mature	0.25[d]
69961	150, 44	92, mature	11.82

[a] From Morris (1978).
[b] Collected beneath lunar module as an exhaust-exposed sample.
[c] Collected 6.5 km from lunar module as lunar module exhaust control.
[d] Both hydrolyzed and non-hydrolyzed fractions analyzed.

6 M HCl acid vapor at 150 °C for 3 h (Glavin et al., 2006). Both hydrolyzed and non-hydrolyzed extracts were desalted using prepacked columns filled with cation-exchange resin (AG50W-X8, 100–200 mesh, hydrogen form, BIO-RAD) and the amino acids recovered by elution with 2 M NH_4OH (prepared from water and NH_3(g) (Airgas), in vacuo). During ion exchange column loading, a DL-norleucine internal standard was added to each sample to estimate the amino acid recoveries from desalting and OPA/NAC derivatization; DL-norleucine was not used in the extractions of the two large samples to avoid having excessive amounts of this standard upon recombination of the separate aliquots. Amino acid abundances were corrected to reflect the desalting recoveries (typically 60–70%) using the norleucine standard and to subtract the backgrounds observed in the procedural blanks. However, analysis of standards indicates that these desalting losses do not impact the relative ratios nor isotopic values. After desalting, the amino acids in the NH_4OH eluates were dried under vacuum to remove excess ammonia; the residues were then re-dissolved in water. The separate aliquots of the 11.82 g sample of Apollo 69961 were recombined at this point for analysis. For the 9.78 g sample of Apollo 70011, each aliquot was analyzed separately (11 aliquots in total) to examine intra-sample variability. Procedural blanks were carried through the extraction and hydrolysis procedure in parallel with each regolith extraction.

2.3. Determination of amino acid abundances

Each desalted extract was analyzed for total amino acid content using a Waters ACQUITY and LCT Premier for ultrahigh-performance liquid chromatography with fluorescence detection and time-of-flight mass spectrometry (LC-FD/ToF-MS) coupled with *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) derivatization. Specific details about the derivatization protocol and LC-FD/ToF-MS conditions used for amino acid analyses are given elsewhere (Glavin and Dworkin, 2009; Glavin et al., 2010). In brief, derivatized samples were separated using tandem Waters BEH C18 column (2.1 × 50 mm, 1.7 μm particle size)-Waters BEH phenyl column (2.1 × 150 mm, 1.7 μm particle size). Chromatographic conditions were: column temperature, 30 °C; flow rate, 150 μL/min; solvent A, 50 mM ammonium formate, 8% methanol, pH 8.0; solvent B, methanol; gradient, time in minutes (%B): 0 (0), 35 (55), 45 (100). As in related studies, amino acids in regolith samples were identified by comparison with known standards using the masses and fluorescence responses of the OPA/NAC amino acid derivatives at the expected chromatographic retention times.

2.4. Compound-specific stable carbon isotopic measurements

We combined six of the 11 separate desalted, acid-hydrolyzed extracts of the 9.28 g Apollo 70011 sample for compound-specific carbon isotopic measurements, eliminating those for which amino acid content was similar to that of controls (see Section 3.4 for details). The sample was dried under vacuum and esterified with isopropanol, and the isopropyl esters reacted with trifluoroacetic anhydride (TFAA) using established methods (e.g., Elsila et al., 2009). The TFAA-isopropanol derivative was then dissolved in 5 μL ethyl acetate (Fisher Chemical, Optima Grade), and 1–2 μL aliquots injected for analysis by gas chromatography coupled with mass spectrometry and isotope ratio mass spectrometry (GC–MS/IRMS). The GC–MS/IRMS system consists of a Thermo Trace GC outfitted with a 5-m base-deactivated fused silica guard column (Restek, 0.25 mm ID) and four serial 25-m Chirasil L-Val columns (Varian, 0.25 mm ID) connected using Press-Tight connectors (Restek); column output is split with approximately 10% directed into a Thermo DSQII electron ionization quadrupole mass spectrometer that provides mass and structural information for each eluting peak. The remaining ~90% passes through a Thermo GC-C III interface, where eluting compounds are oxidized and reduced to carbon dioxide, which then passes into a Thermo MAT 253 isotope ratio mass spectrometer (IRMS) for $\delta^{13}C$ measurement. GC conditions are described elsewhere (Elsila et al., 2011). Standard solutions of amino acids were used to verify compound identification and isotopic values were calibrated with working standards of L-alanine ($\delta^{13}C = -23.33‰$, Iso-Analytical) and CO_2 (calibrated against commercial reference gas, Oztech Corporation), and $\delta^{13}C$ values of underivatized amino acid standards were measured on a Costech ECS 4010 combustion elemental analyzer connected through a Thermo Conflo III interface to the IRMS to allow correction of the carbon added by the derivatization process (Elsila et al., 2012). The final $\delta^{13}C$ values of the amino acids in the samples and their precision were calculated as described elsewhere (Elsila et al., 2012).

3. RESULTS

3.1. Amino acid abundances

Amino acids were detected in all regolith samples at abundances above background levels, with total procedural blank-subtracted concentrations ranging from 0.2 ppb to

Table 2
Summary of the average procedural blank-corrected free (non-hydrolyzed) and total (6 M HCl acid-hydrolyzed) amino acid concentrations in the hot-water extracts of the curated Apollo 16 and 17 soils.[a]

Sample	Apollo 70011		Apollo 72501		Apollo 73131		Apollo 73241		Apollo 78421		Apollo 69961	Apollo 78501
	Free (ppb)	Total (ppb)	Free (ppb)	Total (ppb)	Free (ppb)	Total (ppb)	Free (ppb)	Total (ppb)	Free (ppb)	Total (ppb)	Total (ppb)	Total (ppb)
D-Aspartic acid	0.6 ± 0.2	<0.5	0.2 ± 0.1	<0.9	<0.5	11 ± 3	<0.6	11 ± 1	1.7 ± 0.3	5.1 ± 0.8	0.8 ± 0.1	1.0 ± 0.5
L-Aspartic acid	1.1 ± 0.3	<4	3.5 ± 0.3	<4	1.0 ± 0.3	59 ± 6	<0.3	37 ± 3	3.3 ± 0.6	18 ± 3	1.9 ± 0.2	<1
D-Glutamic acid	<0.2	<0.1	0.3 ± 0.2	<0.5	0.3 ± 0.1[b]	111 ± 7[b]	<0.8[b]	55 ± 2[b]	<1[b]	25 ± 3[b]	1.3 ± 0.1	<1
L-Glutamic acid	<0.4	<7	0.4 ± 0.1	<4							5.9 ± 0.3	<1
D-Serine	<0.2	<0.1	0.5 ± 0.1	<1	<0.1	2.6 ± 0.3	<0.8	2.8 ± 0.3	1.7 ± 0.7	2.7 ± 0.4	0.9 ± 0.1	<1
L-Serine	<0.3	<0.8	6.9 ± 0.3	<4	1.1 ± 0.5	62 ± 9	<3	13 ± 3	4.0 ± 0.7	11 ± 2	1.7 ± 0.2	<1
D-Threonine	<0.1	<1	<0.2	<2	<0.8	<0.6	<1	<0.8	<0.5	<0.8	<1	<1
L-Threonine	<0.5	<2	0.8 ± 0.3	<2	<0.4	60 ± 11	<0.5	11 ± 1	0.9 ± 0.3	1.4 ± 0.7	3.0 ± 0.3	<1
Glycine	13 ± 1	15 ± 1	9.2 ± 0.4	13 ± 1	1.5 ± 1.1	41 ± 12	<3	45 ± 11	<1	24 ± 3	18 ± 1	1.8 ± 0.6
β-Alanine	8.5 ± 1.1	18 ± 2	4.3 ± 0.4	45 ± 4	1.2 ± 0.1	4.1 ± 1.2	<2	17 ± 1	<2	19 ± 1	2.4 ± 0.2	1.9 ± 0.7
γ-Amino-*n*-butyric acid	<0.6	24 ± 9	<0.9	16 ± 7	<0.7	<1	<1	22 ± 4	<2	<2	6 ± 2	5.4 ± 0.8
D-Alanine	1.7 ± 0.6	3.4 ± 1.1	0.8 ± 0.2	2.5 ± 0.2	<0.5	4.6 ± 0.8	<0.2	3.4 ± 0.5	<0.4	0.9 ± 0.1	0.5 ± 0.1	<1
L-Alanine	1.9 ± 0.1	3.0 ± 0.3	2.3 ± 1.0	2.5 ± 1.1	<0.4	48 ± 8	<1	15 ± 3	<0.2	5.0 ± 2.4	2.0 ± 0.1	<1
D-β-Amino-*n*-butyric acid	<0.6	0.7 ± 0.1	2.7 ± 0.2	3.3 ± 0.6	<0.1	<0.1	<0.3	0.2 ± 0.1	<0.2	<0.5	<1	<1
L-β-Amino-*n*-butyric acid	<0.5	0.6 ± 0.1	2.4 ± 0.4	3.9 ± 0.2	<0.1	<0.1	<0.3	0.1 ± 0.1	<0.3	0.4 ± 0.2	<1	<1
α-Aminoisobutyric acid	<1	1.3 ± 0.2	0.9 ± 0.2	0.8 ± 0.4	<0.2	0.7 ± 0.2	0.2 ± 0.1	1.6 ± 0.4	0.4 ± 0.1	0.8 ± 0.2	<1	<1
DL-α-Amino-*n*-butyric acid[b]	0.6 ± 0.4	6.4 ± 4.8	5.1 ± 2.9	7.3 ± 4.0	<3	<1	<0.8	<3	<1	<1	<1	<1
DL-Isovaline	<1	<2	<0.3	<2	<1	<2	<1	<2	<1	<2	<1	<1
D-Valine	<0.5	<1	<0.1	<2	<0.2	<1	<0.2	<1	<0.1	<1	<1	<1
L-Valine	<0.3	<5	1.0 ± 0.7	<2	<0.5	35 ± 22	<2	27 ± 6	<0.4	16 ± 6	2.0 ± 0.1	<1
ε-Amino-*n*-caproic acid[c]	0.9 ± 0.4	25 ± 8	1.4 ± 1.2	11 ± 2	<1	55 ± 6	<1	390 ± 77	<1	28 ± 5	28 ± 4	4.4 ± 2.6
Total	28.3	97.4	42.7	105.3	5.1	494.0	0.2	651.1	12.0	157.3	74.4	14.5

[a] All values are reported in parts-per-billion (ppb) on a bulk sample basis. Extracts were analyzed by OPA/NAC derivatization and UPLC separation with UV fluorescence detection and time-of-flight mass spectrometry at NASA Goddard Space Flight Center. The uncertainties are based on the standard deviation of the average value of two separate measurements.
[b] Enantiomers could not be separated under the chromatographic conditions.
[c] Known to be a primary amino acid contaminant from Nylon-6 storage bags used at JSC.

42.7 ppb in the non-hydrolyzed extracts and 14.5 ppb to 651.1 ppb in the hydrolyzed extracts. Table 2 presents the amino acid abundances in the extracts from the curated lunar regolith extracts with the exception of the multiple aliquots from the large sample of Apollo 70011, discussed more below. Fig. 1 shows a representative liquid chromatography-fluorescence detection chromatogram. The non-curated samples are discussed separately below (Section 3.2).

Amino acids detected in the hydrolyzed extracts of all seven curated samples were glycine, β-alanine, and ε-amino-*n* -caproic acid (ε-ACA). In addition, several other amino acids were detected in one or more samples, including D- and L-alanine, α-aminoisobutyric acid (AIB), D- and L-β-amino-*n*-butyric acid (β-ABA), DL-α-amino-*n*-butyric acid (α-ABA, enantiomers not separated), γ-amino-*n*-butyric acid (γ-ABA), D- and L-aspartic acid, D- and L -glutamic acid (enantiomers not separated in certain samples), D- and L-serine, L-threonine, and L-valine. Table 3 shows the D/L ratios for the enantiomeric pairs we detected. The data show a clear predominance of the L enantiomers in most of the proteinogenic amino acids detected (aspartic acid, glutamic acid, serine, threonine, and valine), implying a terrestrial biological contribution. The D/L ratio of the non-proteinogenic amino acid β-ABA, however, is approximately racemic in the samples in which it was detected, as expected from abiotic formation processes. The data for the amino acid alanine was mixed, with an L-enantiomeric excess evident in certain samples but a racemic composition in others, suggesting a mix of potential sources for this compound. This is the first measurement of enantiomeric composition in lunar regoliths for many of these compounds, as it was not possible to make these measurements with the techniques available for the early analyses. Brinton and Bada (1996) showed an excess of L-alanine over D-alanine and of L-aspartic acid over D-aspartic acid, the only amino acids for which they measured enantiomeric ratios in a sample of Apollo 78421.

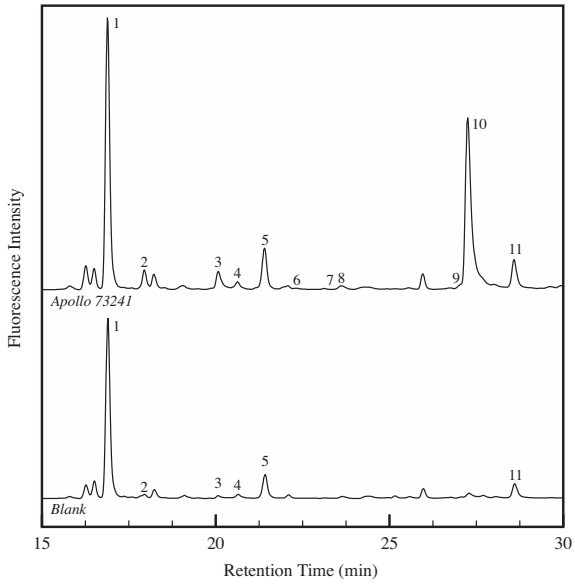

Fig. 1. Representative liquid chromatography-fluorescence detection chromatogram for the acid-hydrolyzed Apollo 73241 sample and a procedural blank. Chromatographic conditions are given in the Methods section (§2.3). Peak numbering is as follows: (1) glycine; (2) β-alanine; (3) γ-aminobutyric acid; (4) D-alanine; (5) L-alanine; (6) D-β-aminobutyric acid; (7) L-β-aminobutyric acid; (8) α-aminoisobutyric acid; (9) DL-α-aminobutyric acid; (10) ε-amino-*n*-caproic acid; (11) L-valine.

3.2. Heterogeneity of amino acid abundances

The abundances of amino acids vary across the seven curated lunar regolith samples analyzed here, showing a heterogeneity in distribution of these compounds. The correlation of amino acid abundances with sample characteristics is discussed further below. In addition to these inter-sample observations, we explored heterogeneity within a single regolith sample. We were allocated 9.78 g of sample 70011, which was collected as a 440.7 g unsieved fines sample located beneath the Apollo 17 lunar module. As described previously (Section 2.2), we split this sample into 11 aliquots for extraction, hydrolysis, and analysis. Table 4 shows the abundance data for selected amino acids in these multiple aliquots, as well as data from a separately allocated smaller sample of Apollo 70011. These analyses show high variability in the amino acid abundances, with the glycine concentration ranging from <1 ppb to 48 ppb. Such variability between these 0.5–1 g aliquots of a single larger sample implies that the amino acids are distributed heterogeneously throughout the soil. This could indicate the presence of small carbonaceous particles mixed inhomogeneously through the regolith or could reflect different exposures of portions of the soil sample to sources of amino acids. It is also possible that such heterogeneity may explain some of the differences observed in analyses of the same samples by multiple laboratories in the 1970s; for example, one study reported glycine abundances of 5–11 ppb in a hydrolyzed extract of Apollo 70011 (Gehrke et al., 1975) while another reported 115 ppb (Hamilton and Nagy, 1975).

3.3. Amino acids in non-curated samples

In addition to the curated samples presented in Table 1, we analyzed seven lunar regolith samples that had been allocated to Dr. Everett Gibson in 1971–1974 for gas-release analyses (Table S1). The samples we analyzed were portions remaining from the original allocations that had not been subjected to the gas-release analyses; their storage during curation is discussed in the Methods section above. We also analyzed two samples of terrestrial dunite that had been used in experiments testing contamination resulting from exposure to exhaust from lunar module-type engines; these samples had also been previously allocated to Dr. Gibson and stored in his laboratory since 1970. The amino acid abundances of these samples are shown in Tables S2 and S3 and show some common characteristics that are dissimilar to the curated samples. Overall amino acid abundances are higher in these lunar samples than in the curated ones. The D/L ratios (Tables S4 and S5) show

Table 3
Amino acid enantiomeric ratios (D/L) measured in the free (non-hydrolyzed) and total (6 M HCl acid-hydrolyzed) hot-water extracts of curated Apollo 16 and 17 soils.

	Apollo 70011		Apollo 72501		Apollo 73131		Apollo 73241		Apollo 78421		Apollo 69961	Apollo 78501
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Total	Total
D/L Aspartic acid	0.43 ± 0.29	n.d.	0.05 ± 0.04	n.d.	<0.5	0.19 ± 0.05	n.d.	0.29 ± 0.03	0.50 ± 0.12	0.28 ± 0.06	0.43 ± 0.13	n.d.
D/L Glutamic acid	n.d.	n.d.	0.75 ± 0.49	n.d.							<0.6	n.d.
D/L Serine	n.d.	n.d.	0.07 ± 0.02	n.d.	<0.09	0.04 ± 0.01	n.d.	0.21 ± 0.05	0.43 ± 0.19	0.24 ± 0.05	0.55 ± 0.18	n.d.
D/L Threonine	n.d.	n.d.	<0.3	n.d.	n.d.	<0.01	n.d.	<0.07	<0.6	<0.6	n.d.	n.d.
D/L Alanine	≈1	≈1	0.36 ± 0.18	≈1	n.d.	0.10 ± 0.02	n.d.	0.23 ± 0.07	n.d.	0.18 ± 0.09	0.26 ± 0.05	n.d.
D/L Valine	n.d.	n.d.	<0.1	n.d.	n.d.	<0.03	n.d.	<0.04	n.d.	<0.06	n.d.	n.d.
D/L β-Amino-*n*-butyric acid	n.d.	≈1	≈1	≈1	n.d.	n.d.	n.d.	≈1	n.d.	≈1	n.d.	n.d.

n.d. = not detected.

a predominance of the L-enantiomeric form of several proteinogenic amino acids in the lunar samples, although serine is nearly racemic in most of the hydrolyzed extracts. The laboratory-stored samples also contained higher concentrations of γ-ABA and ε-ACA compared to the curated samples. For the dunite samples, the control sample, intended to represent clean material, contains higher abundances of amino acids than the sample exposed to the exhaust of a hydrazine thruster. The detected amino acids likely reflect contamination of the laboratory-stored samples during the past >40 years, as the storage conditions were not intended to control exposure of the samples to organic compounds. The source of the contamination remains unknown, although ε-ACA is a monomer released upon hydrolysis of Nylon-6, commonly found in the storage bags used for curation (Glavin et al., 2006; Elsila et al., 2009). Plastic contamination has previously been observed in lunar samples (Steele et al., 2001). Our observations of contamination are also in agreement with previous observation of carbon isotopic values of lunar samples held in non-curation facilities (Swart et al., 1983). In the remainder of this manuscript, we primarily discuss the data from the curated samples.

3.4. Compound-specific carbon isotopic measurements

The ability to measure compound-specific stable isotopic ratios was not available during earlier analyses of lunar soils, although their potential utility in constraining the source of detected amino acids was acknowledged (Brinton and Bada, 1996). Even today, these measurements typically require >1 nmol of a compound for carbon compound-specific isotopic analysis (C-CSIA). The low abundances of amino acids in the lunar regoliths mean that large sample masses (~10 g) must be extracted to obtain sufficient abundance. Our experience is that large extractions are somewhat less efficient than extractions of smaller masses, so large samples must be split into smaller aliquots for extraction and processing before being recombined, which raises more possibilities of increasing background contaminant levels and interferences from other sample components.

In this work, we focused on C-CSIA of glycine, β-alanine, and L-alanine, three of the most abundant amino acids in the Apollo 70011 sample that did not exhibit interfering coelutions. Eleven aliquots of the 9.78 g were extracted, processed, and analyzed. Four aliquots (#2, 3, 4, and 5; Table 4) showed no detectable glycine above levels seen in the procedural blanks. Another aliquot (#11) was analyzed by GC–MS (data not shown) prior to isotopic analysis of the remaining aliquots to verify the absence of coeluting compounds or interference not detectable by LC-FD/ToF-MS analysis. The remaining six aliquots were combined and analyzed by GC–MS/IRMS; results are shown in Table 4 and in Fig. 2. A corresponding set of six procedural blanks were also combined and analyzed; amino acid concentrations in this combined blank were below GC–MS/IRMS detection limits. The $\delta^{13}C$ values for the three measured amino acids in Apollo 70011 ranged

Table 4
Summary of the average procedural blank-corrected total (6 M HCl acid-hydrolyzed) concentrations and carbon stable isotopic ratios for selected amino acids in the hot-water extracts of separate analyses of aliquots of Apollo soil 70011.[a]

	Aliquot 1	Aliquot 2	Aliquot 3	Aliquot 4	Aliquot 5	Aliquot 6	Aliquot 7	Aliquot 8	Aliquot 9	Aliquot 10	Aliquot 11	2013[b]	$\delta^{13}C$ value[c] (‰ VPDB)
Glycine	18 ± 7	<1	<1	<1	<1	37 ± 8	9.4 ± 1.5	21 ± 4	5.3 ± 3.4	48 ± 6	5.5 ± 0.6	13 ± 1	−33 ± 10
β-Alanine	15 ± 1	<3	2.7 ± 2.0	4.5 ± 1.3	4.5 ± 1.2	8.0 ± 0.7	5.1 ± 0.6	5.8 ± 1.3	4.9 ± 1.5	7.4 ± 2.3	7.2 ± 0.6	8.5 ± 1.1	−26 ± 9
γ-Amino-*n*-butyric acid	4.8 ± 1.4	2.7 ± 1.1	2.9 ± 1.3	3.3 ± 2.0	2.4 ± 1.2	4.2 ± 0.8	2.9 ± 0.9	3.2 ± 2.1	2.5 ± 1.3	3.3 ± 1.0	1.6 ± 0.4	24 ± 9	n.a.
D-Alanine	1.1 ± 0.1	<1	<1	<1	<1	2.4 ± 0.4	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.2	2.5 ± 0.1	0.3 ± 0.2	3.4 ± 1.1	n.a.
L-Alanine	0.9 ± 0.1	<1	<1	<1	<1	6.3 ± 0.7	<1	2.4 ± 0.4	1.6 ± 0.8	6.5 ± 0.6	0.5 ± 0.1	3.0 ± 0.3	−21 ± 2
α-Aminoisobutyric acid	1.30 ± 0.6	<1	<1	<1	<1	1.0 ± 0 .7	0.9 ± 0.2	1.1 ± 0.1	<1	1.1 ± 0.2	0.4 ± 0.3	1.3 ± 0.2	n.a.
ε-Amino-*n*-caproic acid	2.7 ± 0.5	1.5 ± 0.4	1.1 ± 0.2	1.0 ± 0.2	26 ± 14	3.6 ± 0.3	3.9 ± 1.1	2.1 ± 0.3	1.9 ± 0.1	4.0 ± 0.2	0.4 ± 0.1	25 ± 8	n.a.

n.a. = measurements not made due to insufficient abundance or interfering coelutions.
[a] All values are reported in parts-per-billion (ppb) on a bulk sample basis. Extracts were analyzed by OPA/NAC derivatization and UPLC separation with UV fluorescence detection and time-of-flight mass spectrometry at NASA Goddard Space Flight Center. The uncertainties are based on the standard deviation of the average value of two separate measurements. Aliquots 1–11 are portions of the 9.78 g sample allocated in 2014, while the 2013 sample was a 0.27 g sample allocated separately.
[b] Has been corrected for losses during desalting and derivatization using internal standard (65% recovery).
[c] Measurement of combined aliquots 1, 6, 7, 8, 9, 10.

from −21‰ to −3‰. The significance of these values is discussed in Section 4.3.

4. DISCUSSION

4.1. AIB and survivability of chondritic organics

The detection of the amino acid AIB at concentrations of 0.7–1.6 ppb in five of the seven hydrolyzed regolith extracts is notable because it is commonly found in carbon-rich meteorites, but is rare in the terrestrial biosphere; thus, its presence is often used to argue for the indigenous nature of amino acids in carbonaceous chondrites (Zhao and Bada, 1989). Another amino acid that is rare in the terrestrial biosphere but common in carbonaceous chondrites is isovaline; we did not detect isovaline in any of the lunar samples. AIB, but not isovaline, has also been observed at trace levels in some Antarctic micrometeorites (Matrajt et al., 2004). A tentative identification of AIB was previously made in Apollo sample 78421 with a concentration of ⩽0.3 ppb (Brinton and Bada, 1996), but the current report is the first confirmed detection of AIB above background in a lunar sample.

Although we also detected higher levels of AIB in the non-curated lunar samples as well as low levels in the non-curated dunite samples (§3.3 and Supplemental Information), the other indications of contamination present in those samples (§3.3) combined with storage conditions that did not aim to minimize exposure to terrestrial organics suggest that a terrestrial origin of that AIB, potentially from fungal peptides (Bruckner et al., 2009; Elsila et al., 2011) or pollutants (Mita and Shimoyama, 1998), is most likely. It is therefore possible that the AIB in the curated samples also derives from an unknown terrestrial source; however, it may also be the result of the infall of carbonaceous material to the lunar surface. Isotopic measurements that may confirm the origin of the AIB were not possible given the small quantities. In the discussion that follows, we assume a fully extraterrestrial origin of the AIB in order to put some constraints on potential survivability of infalling carbonaceous material to the lunar surface.

The survival of organic compounds delivered by carbonaceous chondrites to the lunar surface has been of great interest. Some evidence for survival is seen in meteorites detected in Apollo regolith samples (Joy et al., 2012 and references therein), and observations of graphite phases within an Apollo 17 impact breccia also suggest that carbonaceous material from impacts at the time of the Late Heavy Bombardment may survive on the Moon (Steele et al., 2010). Space weathering and irradiation causes an amorphous rim in the top ~100 nm of a lunar grain (Keller and McKay, 1997); this weathering could destroy organics, and there is ongoing research into understanding the potential survival of such compounds (e.g., Matthewman et al., 2015). Subsequent impacts may also alter past infall products, meaning that infall can be both a source and a cause of loss for organic accumulation.

Brinton and Bada (1996) calculated that the AIB content of lunar soil from contribution from exogenous sources such as delivery by meteorites and IDPs should be 40 ppb,

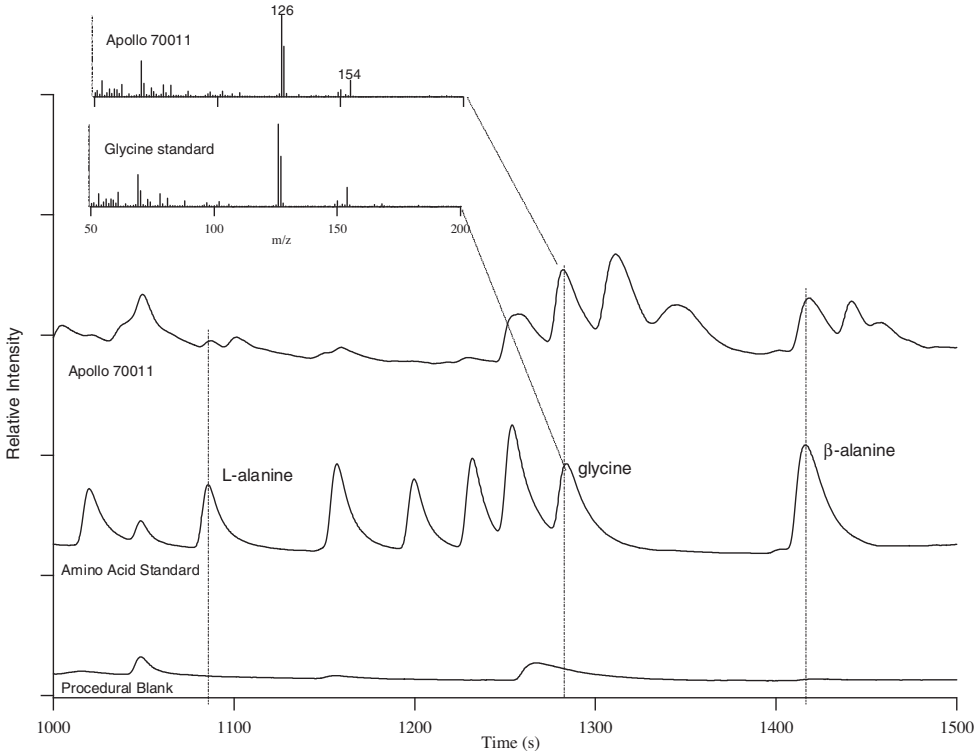

Fig. 2. GC–MS/IRMS analysis of the derivatized extracts from Apollo 70011 and of an amino acid standard and procedural blank. The traces show the m/z 44 ($^{12}CO_2$) peaks produced during GC-IRMS analysis. The inset shows the simultaneously collected mass spectral fragmentation pattern for the peak assigned to glycine in the Apollo sample and the standard, which indicate minimal contributions from non-glycine coelutions.

assuming 100% survivability and based on a carbonaceous chondritic component of the lunar soil of 1–4% (Haskin and Warren, 1991) and an AIB content of these carbonaceous chondrites of 1×10^{-5} g/g derived from measurements of the Murchison CM2 chondrite (Cronin and Pizzarello, 1983). Their results of $\leqslant$0.3 ppb AIB in the two lunar regoliths they analyzed implied an impact survivability of $\leqslant$0.8%. We observed AIB at concentrations up to 1.6 ppb in Apollo 73241, which would suggest a slightly higher survivability rate of up to 4% using the same assumptions. However, these assumptions may need to be updated based on newer results since the original calculations were made. For example, the AIB content of the Murchison meteorite is not representative of all carbonaceous chondrites; more recent measurements from CI, CM, CR, CO, CV, CH, CB, and CK chondrites show that total AIB content can range from $<1 \times 10^{-9}$ g/g to 1.4×10^{-5} g/g (Glavin et al., 2010; Burton et al., 2012, 2013, 2014, 2015), and that the value from Murchison used in the previous calculations is near the upper end of this range. Thus, the actual AIB content delivered to the lunar surface may have been lower than estimated, which in turn could increase the actual survivability rate above our calculated 4%. With such variability in AIB content of chondritic material, there is high uncertainty in any calculation of survival rate based on measurements of the lunar regoliths; a full knowledge of the impact history at the site and around the site, as well as the type of chondritic impactor, would be required.

4.2. Hydrolyzed vs. non-hydrolyzed samples and potential sources of amino acid precursors

We observe a marked increase in amino acid abundance upon acid hydrolysis. Table 5 summarizes the free and total amino acid contents in each sample. The percentage of free amino acids detected in the non-hydrolyzed extract ranges from 0.03% to 43% of the total amino acid content detected in the hydrolyzed extract. These results roughly agree with previous analyses of non-hydrolyzed and hydrolyzed lunar extracts, which showed free amino acids present ranging from trace levels up to ~50% of the total amino acid concentrations (Fox et al., 1975, 1976; Brinton and Bada, 1996). The increase upon acid hydrolysis suggests creation or liberation of amino acids from a precursor. This precursor could be a protein from terrestrial biological contamination, and the predominance of L- over D-enantiomers in several proteinogenic amino acids suggests that this is a

Table 5
Comparison of free and total amino acid abundances in lunar regoliths of various maturities.

Sample	I_s/FeO	Free (ppb)	Total (ppb)	Free (%)
73131	16	5.1	494.0	1.0
73241	18	0.2	651.1	0.03
70011	54	28.3	97.4	29
72501	81	42.7	105.3	43
78421	92	12.0	157.3	7.6

likely possibility. However, such a range in the percentage of free amino acids among samples that have been curated under the same conditions and protocols in the same facility suggests that other precursors may also be present. In carbonaceous chondrites, the percentage of free amino acids is typically in the 30% to 60% range (e.g., Glavin et al., 2010); the values seen for samples 70011 and 72501 fall within this range and could be indicative of an amino acid contribution from meteoritic infall. Previous analyses of these two samples also fell in the 20–50% range (Fox et al., 1975). These two samples also contain detectable amounts of AIB, another potential marker of meteoritic contributions.

Another possible precursor is hydrogen cyanide (HCN) polymer, which is hydrolyzable to amino acids under the conditions used for the workup of lunar sample extracts (Matthews and Moser, 1967; Fox et al., 1976; Yuasa et al., 1984). HCN was one of the primary gases observed in exhaust from the lunar module engine, which burned a 1:1 mix of nitrogen tetroxide and Aerozine 50 (a 50/50 mix of unsymmetrical dimethylhydrazine and hydrazine) (Simoneit et al., 1969). If lunar module exhaust were the primary contributor of the amino acid precursor, however, we would expect to see significantly higher amino acid concentrations in sample 70011, collected under the lunar module, than in sample 72501, which was collected 6.5 km away. Instead, we see similar values both for total concentration and for the free:total ratios in these samples. We thus conclude that lunar module exhaust was not the primary source of the observed amino acids in these samples. Our results agree with analysis of these samples by Fox et al. (1976) and contrast with the results of Gehrke et al. (1975).

We also considered the possibility that HCN derived from solar-wind-implanted H, C, and N was the precursor of the amino acids observed in these samples. Lunar soil maturity, as measured by the ratio of ferromagnetic resonance intensity to the total iron content (I_s/FeO), correlates with the amount of surface exposure (Morris, 1978). Solar-wind implantation only occurs in the top few hundred nanometers of a lunar grain, although gardening of the regolith means that most of the grains in the top few to tens of centimeters have been exposed to solar wind; even soils from the bottom of 2–3-m deep drill cores have non-zero I_s/FeO ratios (Morris et al., 1979). The concentration of solar-wind-originated amino acid precursors such as C and N correlates with soil maturity (Haskin and Warren, 1991; McKay et al., 1991). Thus, a more mature soil would be expected to have a higher amino acid content, if solar-wind-implanted HCN is the main source of these amino acids. In contrast to this prediction, our results show that the least mature samples have the highest total amino acid content (Table 5), suggesting that these least mature samples contain some acid-hydrolyzable amino acid precursor in greater abundance than the more mature samples. The prediction is complicated, however, by the fact that more mature soils would have experienced more exposure to irradiation and weathering, which could degrade volatiles and organic compounds and therefore reduce the concentrations of amino acid precursors, although weathering can also create defects that could adsorb migrating volatiles. Immature samples also typically contain coarse-grained ejecta from relatively recent craters, leading to the possibility that volatiles from micrometeorite impacts could be retained on broken mineral crystals in these immature soils. Nevertheless, our results, supported by the carbon isotopic data discussed below, indicate that the solar wind was not the main source of amino acids in the lunar regolith samples studied here.

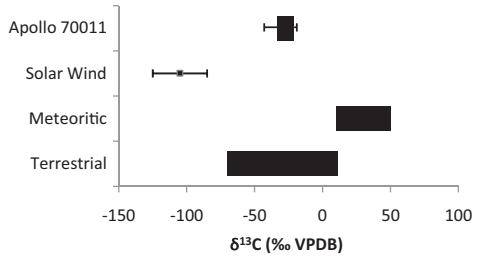

Fig. 3. Range of carbon isotopic ratios measured for Apollo 70011 amino acids compared with solar wind, carbonaceous chondrites, and terrestrial biological sources.

4.3. Carbon isotopic ratios and potential amino acid sources

As described in §3.4, we measured the compound-specific carbon isotopic values of glycine, β-alanine, and L-alanine in Apollo 70011. The $\delta^{13}C$ values for these three amino acids ranged from −21‰ to −33‰ (Table 4). Fig. 3 compares these values with those from the proposed sources for these amino acids. Our values are consistent with those of amino acids measured from terrestrial biological sources, which are typically in the −70‰ to +11‰ range (Scott et al., 2006). The carbon isotopic composition of glycine, β-alanine, and L-alanine in carbonaceous chondrites, however, is typically in the +10 to +50‰ range (Elsila et al., 2012), while the solar wind is depleted in ^{13}C, with a $\delta^{13}C$ value of −105‰ ±20‰ (Hashizume et al., 2004). Thus, these first carbon isotopic measurements of individual amino acids in lunar regolith samples strongly suggest terrestrial biological contamination as a primary source of glycine, β-alanine, and L-alanine in the Apollo 70011 sample we analyzed.

5. CONCLUSIONS

Distinguishing between the multiple potential sources of amino acids found in low concentrations in lunar regolith samples was difficult given the technology available during early analyses of these samples in the 1970s. Our re-examination of seven lunar regoliths with modern technology has added some additional constraints to the debate. The identities and abundances of amino acids measured in the current work are in good agreement with previous measurements. Our analysis of the enantiomeric composition of these amino acids shows a strong excess of the L-enantiomer of several proteinogenic amino acids. This observation, coupled with $\delta^{13}C$ isotopic values of −21‰ to −33‰ for glycine, β-alanine, and L-alanine in the Apollo 70011 sample, suggests that terrestrial biological

contamination is a primary source of a large portion of the detected amino acids.

The presence of AIB, an amino acid that is rare in the terrestrial biosphere, and racemic β-ABA, a nonproteinogenic amino acid, suggest potential contributions from exogenous sources such as meteorites, micrometeorites, or IDPs (Thomas-Keprta et al., 2014). These compounds were not present in sufficient abundance to measure their $\delta^{13}C$ isotopic values. Both AIB and β-ABA are found in carbonaceous chondrites, and AIB has also been identified in some micrometeorites, but this is the first report of their detection in lunar regolith samples.

Previous work suggested that amino acid precursors such as HCN could be present in the lunar regolith samples due to contamination from lunar module exhaust or implantation from solar wind. Our results do not show strong contributions from either of these sources. We saw no significant differences in total amino acid content between a sample collected under the lunar module exhaust compared to one collected 6.5 km away, nor did we observe the correlations between sample maturity and amino acid content expected from solar wind contributions.

This work highlights the fact that despite thoughtful and careful contamination control (Flory and Simoneit, 1972) and curation efforts, trace organics in extraterrestrial samples can be overwhelmed by terrestrial sources. Future missions emphasizing organic analysis must consider not only contamination control but witness samples and contamination knowledge efforts to understand the unavoidable contamination background (Sandford et al., 2010; Summons et al., 2014; Dworkin et al., 2015). This work also highlights the lasting value of sample return missions. The techniques used in our study were not yet invented when the samples were collected; curation of the samples for future work allowed us to address the question of the origins of the amino acids detected in lunar regolith samples in ways that the original investigators were unable to resolve.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2015.10.008.

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