

A SEARCH FOR EXTRATERRESTRIAL AMINO ACIDS IN CARBONACEOUS ANTARCTIC MICROMETEORITES

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(Received 15 November 1996)

Abstract. Antarctic micrometeorites (AMMs) in the 100–400 μm size range are the dominant mass fraction of extraterrestrial material accreted by the Earth today. A high performance liquid chromatography (HPLC) based technique exploited at the limits of sensitivity has been used to search for the extraterrestrial amino acids α -aminoisobutyric acid (AIB) and isovaline in AMMs. Five samples, each containing about 30 to 35 grains, were analyzed. All the samples possess a terrestrial amino acid component, indicated by the excess of the L-enantiomers of common protein amino acids. In only one sample (A91) was AIB found to be present at a level significantly above the background blanks. The concentration of AIB (~ 280 ppm), and the AIB/isovaline ratio (≥ 10), in this sample are both much higher than in CM chondrites. The apparently large variation in the AIB concentrations of the samples suggests that AIB may be concentrated in rare subset of micrometeorites. Because the AIB/isovaline ratio in sample A91 is much larger than in CM chondrites, the synthesis of amino acids in the micrometeorite parent bodies might have involved a different process requiring an HCN-rich environment, such as that found in comets. If the present day characteristics of the meteorite and micrometeorite fluxes can be extrapolated back in time, then the flux of large carbonaceous micrometeorites could have contributed to the inventory of prebiotic molecules on the early Earth.

1. Introduction

Large micrometeorites ranging in size from 100–400 μm , and dominated by the 100–200 μm size fraction, have been recovered from Antarctic ice during the 1988, 1991 and 1994 field seasons. It has been previously shown that these micrometeorites correspond to the peak in the mass distribution of the micrometeorite flux evaluated in the 50–500 μm size range, which represents by far the main source of extraterrestrial material that survives atmospheric entry (Maurette *et al.*, 1991ab; Hammer and Maurette, 1996; Taylor *et al.*, 1996). Indeed, these micrometeorites deliver to the Earth about 100 times more material than objects found outside this size range, including the much larger meteorites and the smaller interplanetary dust particles collected in the stratosphere.

These large AMMs are related to a relatively rare class of meteorites, the carbonaceous chondrites, and within this group mostly to the CM chondrites (about 2% of meteorite falls). Carbonaceous chondrites such as the Murchison meteorite are rich in organic compounds, including amino acids and their precursors (see

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Kvenvolden *et al.*, 1970; Cronin, 1976; Cronin and Pizzarello, 1983). The α -dialkyl amino acids α -aminoisobutyric acid (AIB) and isovaline are two of the most abundant amino acids present in Murchison, Murray and several Antarctic meteorites (Cronin and Moore, 1971; Shimoyama *et al.*, 1979, 1985; Cronin and Pizzarello, 1983; Cronin *et al.*, 1988). In Murchison, 1% of the total organic carbon consists of amino acids and their acid-labile precursors. AIB comprises 10% of the Murchison amino acids (about 10 ppm), and therefore 0.1% of the total organic carbon in Murchison. The concentration of isovaline is approximately half that of AIB. AIB and isovaline are also significant products of the Miller-Urey experiment (Wolman *et al.*, 1972). AIB has also been synthesized from aqueous cyanide, whereas the yield of isovaline and other amino acids with more than four carbons from HCN is negligible (see Fox *et al.*, 1976; Ferris *et al.*, 1978; and Yuasa *et al.*, 1984).

In contrast to their likely widespread occurrence in abiotic environments, AIB and isovaline are extremely rare on the Earth. Biogenic AIB is found in only a few fungal peptides (Mathew and Balaram, 1983; Brückner *et al.*, 1989), and non-biogenic AIB and isovaline are hydrolysis products of hydantoins which may be synthesized during coal gasification (Olson, 1992). Mita and Shimoyama (1996) have reported the occurrence of AIB and racemic isovaline in modern sediments from Tokyo Bay which were apparently derived from hydantoins produced from chemical industry pollutants. Analyses of unfiltered Antarctic ice (McDonald and Bada, 1995) and filtered sea water (K. L. F. Brinton and J. L. Bada, unpublished results) have found that if AIB or isovaline are present, their levels are below the analytical method's detection limits (about 1 ppb for sea water and 0.002 ppb for Antarctic ice). The only geologic occurrence reported so far of AIB and isovaline is in sediments associated with the Cretaceous/Tertiary (K/T) boundary (Zhao and Bada, 1989). In this case, these amino acids are considered either to be extraterrestrial in origin and delivered to the Earth by the K/T bolide (Zhao and Bada, 1989), or to have been synthesized during the impact event (Olson, 1992). Thus, on the Earth, it appears that the occurrence of AIB and concomitant isovaline is restricted to deposits associated with extraterrestrial processes or events, or in some minor cases, to industrial processes.

The study of the carbon chemistry of micrometeorites is much more difficult than that of meteorites. This is primarily due to their very small mass, which requires the use of the most sensitive analytical methods. Moreover, micrometeorites can be easily contaminated as a result of their high surface to volume ratio, their marked porosity, and their probable high chemical reactivity when exposed to gases and water. However, with micrometeorites it is not possible to minimize contamination by extracting an internal chunk, which is a current procedure used in studies of large meteorites. Consequently, all steps in micrometeorite studies must be carefully controlled from their collection in the cleanest, most unpolluted spots on Earth (e.g., pre-industrial Antarctic ices), to their preparation and analyses under ultra-clean conditions.

We have searched for amino acids in samples consisting of ~30–35 AMMs with sizes ranging from 100–400 μm , using a method based on HPLC. This technique has been used over the last two decades at the Scripps Institution of Oceanography to analyze amino acids in a variety of extraterrestrial samples (Zhao and Bada, 1989, 1995; McDonald and Bada, 1995; Brinton and Bada, 1996). Our objectives were to determine to what extent the amino acid distribution observed in AMMs reflects similarities and/or differences with that observed in CM chondrites, and to try to assess the contribution of the micrometeorite flux to the delivery of complex organics to the early Earth.

2. Materials and Methods

2.1. COLLECTION, SELECTION, AND PREPARATION OF AMMs, THE MURCHISON METEORITE, AND ANTARCTIC SOIL

The cleanest collection of micrometeorites has been recovered from blue ice fields near the margin of the Antarctic ice sheet during the Antarctic summers of 1987, 1991 and 1994 (Maurette *et al.*, 1991ab, 1994). The technique was to melt in the field about 10 to 15 tons of pre-industrial blue ice during each day of good weather. The filtering of this water on a stack of stainless steel sieves yielded a glacial sand very rich in micrometeorites. At this location, ultra-clean winds blowing almost constantly from the center to the margin of the ice sheet contributed to keeping the ice free of sea spray, a potential source of terrestrial contamination.

The AMMs in this study were preserved since the day of their collection by deep freezing in a small aliquot of their original melt ice water in glass vials. The vials were further wrapped in small Teflon bags in the field. The bag containing a given daily collection was taken out of the freezer and, in a dust free room, a few drops of water loaded with the glacial sand enriched in AMMs are extracted with a glass pipette and deposited for drying on a stack of ash free filters.

In the present work we have used micrometeorites from the 1991 and 1994 collections, and we selected large AMMs with sizes ranging from 100 to 400 μm and dominated by the 100–200 μm size fraction (Hammer and Maurette, 1996; Taylor *et al.*, 1996). We selected five samples using a low-magnification binocular microscope, and according to simple criteria such as color ('dark' without any red spot) and texture (irregular shape without euhedral surface). The samples were designated A91 (50 μg), I91 (175 μg), III91(310 μg), IV94 (166 μg), and V94 (259 μg) and each contained about 30 to 35 micrometeorites.

More detailed chemical and mineralogical studies showed that about 80% of the grains selected using these simple criteria are chondritic micrometeorites mostly related to CM carbonaceous chondrites such as Murchison, which was used as a standard in this work. The other particles are mostly rust grains, formed and then heated on the surface of the 20 meter long tubing of the steam generators used to melt the ice. These rust particles were used as blanks. For more detailed

information regarding the collection and selection of AMMs, see Kurat *et al.* (1994) and Maurette (1997).

Two samples of Murchison, 5 g and 5 mg, were each selected from an internal portion of the meteorite (5 g sample provided by Dr. John Kerridge; 5 mg sample provided by Dr. Michel Maurette).

A 5 g portion of Antarctic soil was taken from sample #726 provided by Dr. Chris McKay at NASA Ames Research Center. The soil was one of the original samples used to test the GC/MS system of the Viking Mars lander.

2.2. AMINO ACID EXTRACTION AND ANALYSIS

All glassware was cleaned in Chromerge and annealed at 500°C for 3 h. The Murchison and micrometeorite extracts were prepared by adding double-distilled water to each sample and heating for 24 h at 100°C in a sealed glass tube. The supernatant was decanted and dried and the residue hydrolyzed in double-distilled 6 N HCl for 24 h at 100°C in a sealed glass tube. The Antarctic soil was hydrolyzed directly in double-distilled HCl without previous hot water extraction. Each hydrolyzed residue was then dried, redissolved in double-distilled water and passed through a desalting column containing AG 50W-X8 resin (Bio-Rad). The amino acid fraction was eluted with aqueous NH₄OH. The eluate was dried, resuspended in aqueous 0.4 N borate buffer pH 9.4 and dried again to remove ammonia. The amino acid/borate buffer residue was then redissolved in double-distilled water and derivatized by the OPA/NAC (*o*-phthaldialdehyde/N-acetyl L-cysteine) technique (OPA and NAC both from Fisher). The derivatives were separated by reverse phase HPLC with fluorescence detection and identified by comparison of retention times with standards (Zhao and Bada, 1989, 1995; Brinton and Bada, 1996). AIB was identified according to both its retention time and how quickly it reacted with the OPA/NAC derivative. The reaction of α -dialkyl amino acids with the OPA/NAC requires longer to reach completion, thus providing us with an additional way to confirm the identification of these amino acids (Zhao and Bada, 1989, 1995). Control blanks were processed and analyzed concurrently with all samples.

In order to evaluate the ratio of free amino acids to precursors in the sample we typically analyze the hot-water extract prior to hydrolysis. However, we were unable to do so in this study because of the unusually small sample size. The sample size and the total amounts of amino acids typically detected in the AMM samples (roughly 10⁻⁷ g amino acids per sample) prevented the use of other techniques such as GC/MS to provide additional confirmation of our results.

3. Results and Discussion

The results of our search for amino acids in the ~200 μ m-sized AMMs are given in Table I. Because we were analyzing such small amounts of material and despite

Table I

Summary of amino acid analyses of Antarctic micrometeorites (reported in ppm). The mass of each sample corresponds to the sum of a random selection of ~30 to 35 micrometeorites. Sample A91 is dominated by the smallest grains (~100 μm) of this size fraction, while the grains of the other samples have larger sizes averaging about 200 μm . The enantiomers of D- and L-glutamate were not resolved, and so the values reported represent the sum of both. D- and L-isovaline were not detected in any of the samples. The values given for D-alanine in IV94 and V94, for AIB in IV94, and for isovaline in all the samples are upper limits based on the detection limit calculated for each. All the amino acid values are averages of the 1- and 15-minutes OPA/NAC derivatizations, and all values are blank-corrected. The blank used to correct A91 was an extract of ~35 rust grains, while the blanks used for the remaining samples contained no extractable material.

Amino acid	A91 (50 μg)	I91 (175 μg)	III91 (310 μg)	IV94 (166 μg)	V94 (259 μg)
D-Aspartate	10	1	18	20	20
L-Aspartate	34	1	30	40	100
L-Serine	85	73	100	7	100
D,L-Glutamate	280	170	40	40	70
Glycine	260	250	210	5	40
D-Alanine	570	73	37	<0.1	<0.6
L-Alanine	620	310	180	60	60
AIB	280	78	22	<0.1	20
D,L-Isovaline	<13	<3	<20	<0.1	<1

the sensitivity of our method (detection limit roughly 10^{-14} moles), we found that most of the samples yielded amino acid concentrations barely distinguishable from our blanks. In samples I91, III91 and V94 a peak corresponding to the retention time of AIB was observed, and this peak did increase in size with the 15 minute derivatization. Thus AIB may be present in these samples, but its actual concentration is highly uncertain; the values we give in Table I should be considered very approximate and are probably upper limits. In addition, the low D/L alanine ratios in these samples indicate that substantial terrestrial contamination is present (McDonald and Bada, 1995).

In contrast to these samples, A91 yielded AIB well above the blank levels (see Figure 1a). The concentration of AIB in sample A91 was estimated to be ~280 ppm, which is more than an order of magnitude greater than the AIB level of 10 ppm present in the Murchison meteorite (Cronin and Pizzarello, 1983). Surprisingly, no isovaline was detectable in any of the samples; upper limits based on the detection limit of the technique are shown in Table I in lieu of actual measured values.

Since the concentration of AIB is highly variable in the five samples, it could be that AIB is concentrated in rare families of AMMs amounting to at most a few percent of the total grains. Consequently they can be missed in a random selection of 30 to 35 AMMs. If this is the case, then these rare grains would be

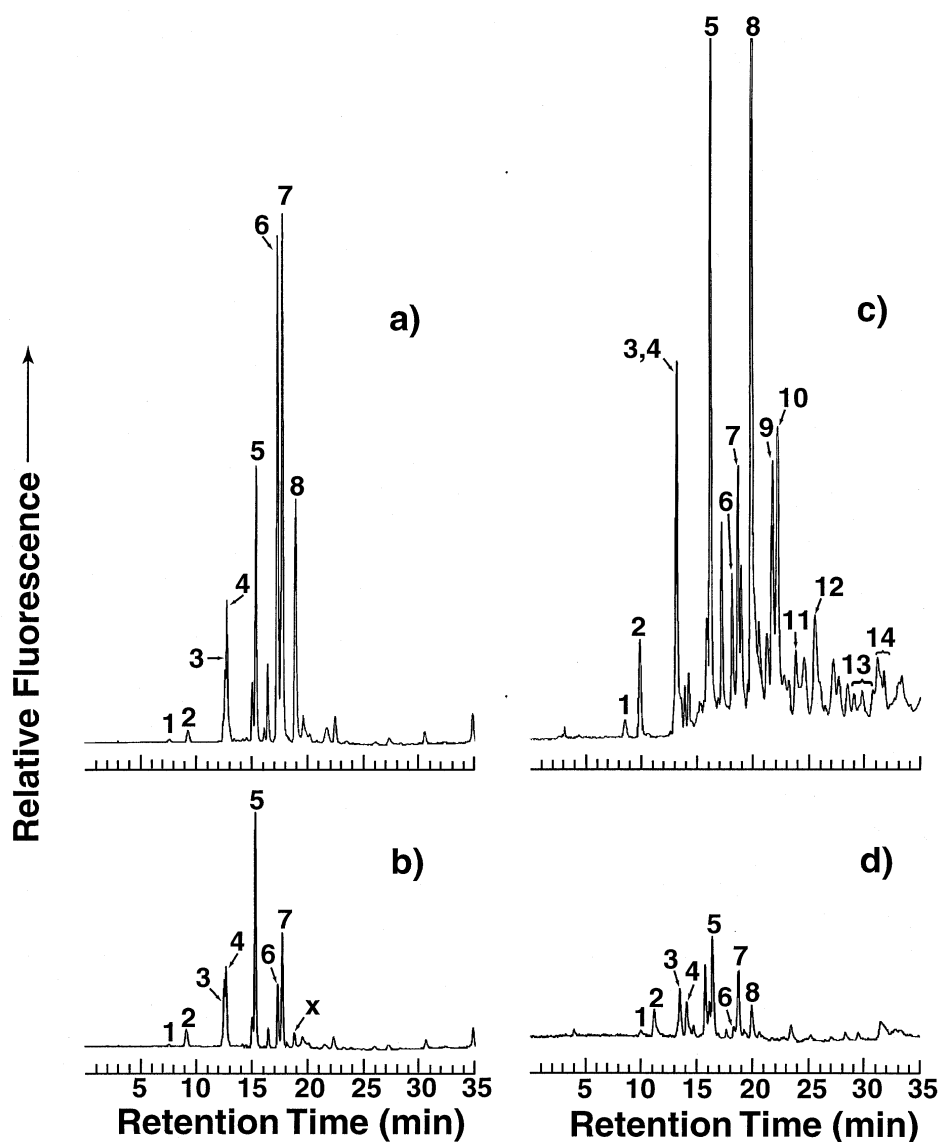


Figure 1. HPLC chromatograms of the amino acids found in **a)** 20 μg of sample A91, **b)** 20 μg of rust grains (used as a blank for A91), **c)** 10 mg of Murchison (taken from the 5 g sample), and **d)** 100 μg of Murchison (taken from the 5 mg sample). Because only a portion of the total sample extract was injected into the HPLC, the mass values given in Figure 1 correspond to the actual injected amounts. The chromatograms show amino acids derivatized with OPA/NAC for 15 minutes prior to analysis by HPLC. Peak identifications are based on retention times in comparison to a standard run the same day. The identification of AIB and isovaline are by retention times as well as by peak growth with longer derivatization. The major amino acids are identified as follows: 1) D-aspartate; 2) L-aspartate; 3) D,L-serine; 4) D,L-glutamate; 5) glycine; 6) D-alanine; 7) L-alanine; 8) AIB; 9, 10) D- and L-isovaline; 11) L-valine; 12) D-valine; 13) D,L-isoleucine and D,L-alloisoleucine; 14) D,L-leucine. The peak labeled X in Figure 1b is an unknown compound with the same retention time as AIB, but which did not grow with longer derivatization. In Figure 1c, the Murchison D/L ratios of enantiomeric pairs of biogenic amino acids are much less than 1. This indicates that during the Murchison's residence time on Earth, despite careful storage and handling, it has become contaminated. Control blanks showed that little contamination was introduced during the analytical procedure.

extremely rich in amino acids. Furthermore, the AMMs selected for sample A91 were at the smaller end of the size range, and it may be that smaller micrometeorites experience a lower temperature upon entry into the atmosphere, resulting in greater preservation of their organics. A related possibility is that AIB in AMMs has been synthesized by a process where slight changes in conditions might lead to large variations in the amino acid yields and distributions.

While sample A91 is notable for its AIB content (~ 280 ppm), which is unprecedented in any sample of solar system material analyzed so far, it is perhaps equally interesting for what it lacks: isovaline. Isovaline is abundant in Murchison and yet was not detected in A91. This implies that the AIB to isovaline ratio is much larger (> 10) than in other carbonaceous chondrites. Furthermore, relative to Murchison, A91 is depleted in all of the amino acids with 5 or more carbon atoms.

In addition to AIB, A91 contains a simple suite of protein amino acids, consisting largely of the L-enantiomers, with the exception of alanine, which is nearly racemic. The smaller, more hydrophilic amino acids are favored, and the distribution closely parallels that of the Antarctic ice (McDonald and Bada, 1995). Typically, D/L ratios are used to distinguish amino acids produced by abiotic processes, which are racemic, from terrestrial biogenic amino acids, in which the L-enantiomers predominate (Bada and McDonald, 1996). However, one of the two A91 blanks (possessing no extractable material) inexplicably contained glycine and racemic alanine in excess of either A91 or the other blank, perhaps due to incomplete removal of ammonia in the blank, which could have led to the deacetylation of NAC. Brinton and Bada (unpublished results) have previously observed that both old NAC and cysteine produce glycine and racemic alanine during derivatization (see Bada, 1991 for discussion of this type of decomposition pathway). Therefore, we hesitate to assert that the racemic alanine in sample A91 is indigenous and of extraterrestrial origin.

The amino acid distribution in A91 differs from CM meteorites in that it shows only a few peaks corresponding to a simple mixture of small amino acids. The comparison of the chromatograms reported in Figure 1 illustrate the greater complexity of the mixture of amino acids observed in Murchison.

The problem of contamination greatly complicates the detection of extraterrestrial amino acids in micrometeorites. It is unlikely that we can further reduce this terrestrial contamination, as Antarctic micrometeorites were already recovered from the cleanest zones of the Earth, although with larger samples and the reduction of blank levels we may be able to use mass spectrometric techniques in order to evaluate the terrestrial component.

The distribution of amino acids observed in sample A91 was compared with that (see Figure 2) measured in a sample of Antarctic ice collected in the Allan Hills area (McDonald and Bada, 1995). With the exception of AIB and alanine, the two distributions bear a clear similarity. The low D/L ratios of the amino acids with chiral carbons in the ice indicate that the dominant source is terrestrial and is not amino acids leached from AMMs themselves (Bada *et al.*, 1996), despite the

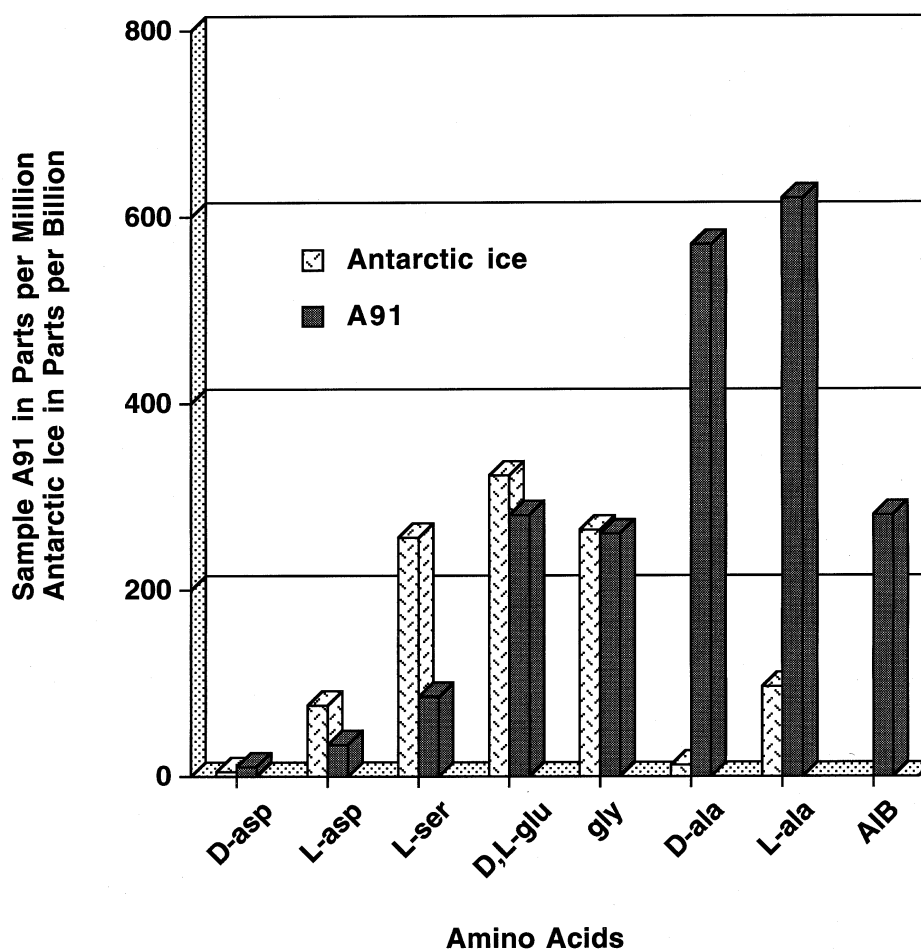


Figure 2. Comparison of the distributions of the amino acids found in sample A91 (light shaded histogram), and in ice collected in Antarctica (dark shaded histogram; ice values are taken from McDonald and Bada, 1995).

fact that the AMMs are 6 orders of magnitude more concentrated in amino acids than the surrounding ice. That AIB is not detectable in Antarctic ice and yet is so abundant in A91 leads us to rule out the possibility that the AIB is derived from Antarctic ice itself.

Studies of the mineralogy, chemistry and isotopic composition of AMMs indicate that they are related to carbonaceous chondrites. Nevertheless there are major differences between these two classes of solar system objects, with AMMs showing in particular higher carbon contents, a very strong depletion of chondrules, and pyroxene to olivine ratios about 10 times higher than in carbonaceous chondrites (for a recent review see Maurette, 1997).

Despite many structural and chemical similarities between the bulk of the Antarctic micrometeorites and carbonaceous chondrites, the amino acid chemistry of at least a small class of the micrometeorites may be strikingly different. Samples I91, III91, IV94, and V94 contain few amino acids and differ only slightly from the control blank and the small sample of Murchison we analyzed. In contrast, as we discussed previously, sample A91 is very rich in the hydrophilic protein amino acids and AIB. The amount of AIB in A91 (~ 280 ppm) is unprecedented in any other known solar system object. Since the micrometeorites in A91 were selected from the small end of the size fraction ($\sim 100 \mu\text{m}$) it may be that these smaller micrometeorites have a different source, and therefore a different chemistry than the larger micrometeorites, or it may be that the survivability of AIB during atmospheric entry is greater in the smaller micrometeorites. More studies are needed to distinguish between these possibilities.

In the remaining micrometeorite samples (I91, III91, IV94, and V94), as well as in the 5 mg Murchison sample, isovaline was below the detection limit. Nevertheless, given how little AIB was detected, we were unable to determine whether the ratio of AIB to isovaline in these samples differs from what has been previously observed in Murchison. However, sample A91 is unique. AIB is so abundant in A91 that, if its amino acid distribution were similar to that of Murchison and other CM chondrites, isovaline should have been detectable.

The analysis of Antarctic soil was done in order to rule out the possibility that the AIB found in A91 was derived from gasification of coal dust from Antarctic soil (Olson, 1992). Antarctic soil contains 0.03% organic carbon (Cameron, 1971). The analysis of Antarctic soil, after blank correction, yielded only ~ 10 ppb aspartic acid; no other amino acids were detected. The detection limit (~ 1 ppb) of AIB and isovaline is 5 orders of magnitude below the level of AIB observed in A91. If the entire A91 sample consisted of Antarctic soil, no AIB would have been observed.

In Murchison the distribution of amino acids and the presence of α -hydroxy acids indicate that the amino acids were predominantly synthesized by a Strecker-type process (Wolman *et al.*, 1972; Peltzer *et al.*, 1984; Lerner *et al.*, 1993) during aqueous alteration of the parent body. On the other hand, the amino acids in A91 follow a distribution similar to that observed in cyanide synthesis. The aqueous synthesis of amino acids from cyanide has been demonstrated (for example see Fox *et al.*, 1976; Ferris *et al.*, 1978; and Yuasa *et al.*, 1984). Synthesis of amino acid precursors occurs rapidly at 100°C and pH 8.8, and then subsequent hydrolysis at low pH yields free amino acids. Glycine and aspartic acid are the most abundant products, but AIB is also present and lower temperatures may favor AIB production (Brinton *et al.*, in preparation). No significant amount of isovaline or other amino acids with 5 or more carbon atoms has been obtained from aqueous cyanide. Cyanide is known to be an important organic constituent of comets, and so our results, although extremely preliminary, suggest that at least some of these micrometeorites may be cometary in origin.

Direct measurements (Maurette *et al.*, 1991ab; Hammer and Maurette, 1996; Taylor *et al.*, 1996) of the flux of micrometeorites reaching the Earth's surface, and comparison with their pre-atmospheric flux at 1 au (Love and Brownlee, 1993), indicate that micrometeorites in the 50–500 μm size range deliver to the Earth's surface about 100 times more material ($\sim 2 \times 10^{10}$ grams per year for the whole Earth) than smaller micrometeorites and much larger meteorites put together. Moreover, most of the mass would be delivered by micrometeorites with sizes $\sim 200 \mu\text{m}$, such as those investigated in the present work. Although it was predicted that $\geq 99\%$ of micrometeorites around a size of $\sim 100 \mu\text{m}$ were completely melted upon atmospheric entry (Brownlee, 1985), direct examination of Antarctic micrometeorites has shown that the proportion of unmelted micrometeorites was much larger than predicted by the models of frictional heating upon atmospheric entry (see Maurette, 1997, for a discussion of frictional heating models).

If we assume, based on the measurements made in this study, that only about 5% (i. e., the% of the weight of the A91 sample compared to the total weight of all the AMM samples analyzed) of large micrometeorites have a substantial AIB content (~ 300 ppm), then we estimate that the present day flux of exogenous AIB from this source is roughly 3×10^5 grams per year. On the early Earth this extraterrestrial micrometeorite source of AIB would have been several orders of magnitude higher (perhaps $\sim 10^8$ grams per year; see Chyba and Sagan, 1992). However, if this were the dominate source of AIB on the primitive Earth, in order for the early oceans to have an AIB content even in the 10^{-6} M range, then accumulation and survival times of millions of years would be required.

4. Conclusions

AIB has been tentatively identified, using both HPLC retention and reactivity with OPA/NAC, in AMMs with sizes of $\sim 200 \mu\text{m}$ at concentrations that are about 10 times higher on average than in CM chondrites. The much higher ratio of AIB to isovaline found in AMMs suggests that they may have been synthesized in an HCN-rich environment typical of comets by a process different from the Strecker-type synthesis of amino acids in CM chondrites during an aqueous alteration of their parent asteroids.

The distribution of the other amino acids in the micrometeorites bears similarities with that observed in Antarctic ice, which is unique in only containing a few amino acids. This contamination, which has a widespread occurrence in both the Greenland and the Antarctic ice sheet, was likely absorbed from the ice by AMMs.

The results we have obtained in this investigation support the suggestion that large micrometeorites could have played a major role in the exogenous organic delivery scenarios on the prebiotic Earth (Maurette *et al.*, 1995). If the present day characteristics of the micrometeorite and meteorite fluxes can be extrapolated back to ~ 4 bya, we have shown that micrometeorites could have played a role in seeding

the early Earth with complex organics, in particular in delivering amounts AIB, and probably other amino acids as well, in more significant quantities than other exogenous sources such as asteroid or comet impacts.

Despite the rarity of AIB in the terrestrial environment, particularly in Antarctic ice, we recognize the preliminary nature of our results and again emphasize that they are tentative and need confirmation. Nevertheless, the possibility of a new type of solar system object containing an unprecedented concentration of AIB as well as other amino acids and organic compounds clearly merits further study. It is our hope that this report will serve to encourage future work in this area.

Acknowledgements

This work was supported by a NASA Specialized Center of Research and Training in Exobiology at UCSD in the USA, and by Programme d'Exobiologie of the French Space Agency (CNES). The help of INSU and IN2P3 in France is also acknowledged. The recovery of micrometeorites in Antarctica was funded by the Institut Français de Recherches et de Techniques Polaires.

The helpful comments of editor J. Ferris and the reviewers are gratefully acknowledged.

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