PROTOCOL FOR FUTURE AMINO ACID ANALYSES OF SAMPLES RETURNED BY THE STARDUST MISSION.

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Introduction: The delivery of amino acids to the early Earth by interplanetary dust particles, comets, and carbonaceous meteorites could have been a significant source of the early Earth's prebiotic organic inventory [1]. Amino acids are central to modern terrestrial biochemistry as major components of proteins and enzymes and were probably pivotal in the origin of life. A variety of amino acids have been detected in the CM carbonaceous meteorite Murchison [2], many of which are exceptionally rare in the terrestrial biosphere including α-aminoisobutyric acid (AIB) and isovaline. AIB has also been detected in a small percentage of Antarctic micrometeorite grains believed to be related to the CM meteorites [3].

One problem associated with the analysis of micrometeorites is that these particles can be heated to very high temperatures (1000 to 1500°C) during atmospheric entry [4], causing some of the amino acids originally present in the grains to decompose or evaporate into the cold atmosphere [5]. In contrast to large micrometeorites, particles returned from comet 81P/Wild 2 by *Stardust* provide an opportunity to investigate the amino acid content in grains that likely did not experience as extensive heating between their departure from the comet and their delivery to Earth.

We have recently optimized a new liquid chromatography-time of flight-mass spectrometry (LC-ToF-MS) technique coupled with OPA/NAC derivatization in order to detect amino acids in meteorite grain extracts by UV fluorescence and exact mass simultaneously [6]. To validate our technique for amino acid analyses of Stardust material, we analyzed 20 μm sized grains from the Murchison meteorite and 36 different *Stardust* aerogels from both the comet and interstellar collection surfaces. Preliminary results from these analyses are reported here.

Samples and Analytical Techniques: The Murchison meteorite (USNM 6650.2) was crushed with an annealed (500°C overnight) mortar and pestle and fifteen meteorite grains (20 μm dia., total mass ~ 0.15 μg) were hand-picked under an optical microscope and transferred to a clean test tube. Olivine grains from a crushed sample that had been heated at 500°C for several hours were used as a procedural blank. In addition, 36 "*Stardust*-like" aerogel samples and soil and water samples collected from the *Genesis* crash site in the Utah Test and Training Range (UTTR) were analyzed in parallel. The UTTR soil and aerogel samples were crushed with a glass rod inside a test tube.

Each sample was sealed in a glass test tube with 1 ml of double-distilled water for 24 h in a heating block set at 100°C. The ampoules were cracked open and centrifuged to separate out the particulate from the water supernatant. The water supernatant was transferred to a separate test tube, dried under vacuum, hydrolyzed under 6 M HCl vapor at 150°C for 3 h and analyzed directly by OPA/NAC derivatization and LC-ToF-MS with UV fluorescence detection [6]. The UTTR soil and water samples were desalted by using cation exchange resin (Bio-Rad AG50W-X8, 100-200 mesh, H^+ form) prior to OPA/NAC derivatization.

Results and Discussion: A summary of the results of the analyses is shown in Fig. 1. We identified several amino acids in the Murchison meteorite grain extract above background levels including AIB and β-alanine (BALA) by fluorescence retention time and exact mass. The distribution and abundance of amino acids found in the Murchison meteorite grains was similar to analyses of a much larger bulk meteorite that was determined to have a total amino acid concentration of 15 parts-per-million, ppm [6]. Although several amino acids including aspartic and glutamic acids, serine, glycine, alanine and BALA were present in the olivine blank, the total concentration of amino acids in the Murchison grains was a factor of ten higher than background (Fig. 1).

Several protein amino acids were also detected in the *Stardust* aerogel extracts with total concentrations ranging from ~1 to 6 ppm. The concentrations of amino acids in the aerogels were much lower than those detected in Murchison. A sample chromatogram showing the amino acids extracted from 0.3 μg of aerogel material (roughly twice the total mass of the meteorite grains) is shown in Fig. 1. Since these samples were not from the main curatorial archive, but splits stored under non-ideal conditions (S. Sandford, personal communication) it is possible that these contaminants arose from storage after sample preparation. Nevertheless, we did not detect AIB nor isovaline in any of the aerogel extracts above the 0.01 ppm level. Therefore, amino acid contamination from the aerogel will not interfere with the detection of these non-protein amino acids in *Stardust* material.

There should be no contamination of the *Stardust* samples from UTTR soil or water. In the event that it does occur, samples of UTTR air, soil, and water from the Stardust landing site will be collected and archived. We did not detect AIB or isovaline in the *Genesis* UTTR samples.

Our current best detection limit for amino acids using standard LC-ToF-MS and UV fluorescence detection is 10^{-15} to 10^{-16} mol. Therefore, assuming the extractable concentration of amino acids in *Stardust* material is similar to the amino acid concentration of Murchison, a minimum of ten *Stardust* grains would be required to detect an abundant amino acid like AIB in 10 μm sized grains using our current instrumentation (Table 1). However, if *Stardust* grains more closely resemble the Tagish Lake meteorite, the equivalent mass of more than 2500 grains would be required for the analysis of amino acids. We are currently integrating a UV laser induced fluorescence (LIF) detector into our LC-ToF-MS analytical system. The sensitivity of the LIF detector for amino acids is ~1000 times better than standard UV fluorescence [7], therefore in most cases a single 10 μm sized particle should allow us to detect amino acids with LIF well above the detection limit (Table 1).

Conclusions: We have demonstrated that LC-ToF-MS coupled with UV fluorescence detection is a powerful tool for the detection of amino acids in meteorite extracts. Using this new analytical technique we were able to identify the extraterrestrial amino acid AIB extracted from *fifteen* 20 μm sized Murchison meteorite grains.

We found that the amino acid contamination levels in *Stardust* aerogels was much lower than the levels observed in the Murchison meteorite. In addition, the α-dialkyl amino acids AIB and isovaline which are the most abundant amino acids in Murchison were not detected in the aerogel above blank levels. We are currently integrating LIF detection capability to our existing nanoflow LC-ToF-MS for enhanced sensitivity required for the analysis of amino acids in *Stardust* samples.

References: [1] Ehrenfreund P. et al. (2001) *PNAS*, 98, 2138-2141. [2] Botta O. and Bada J. L. (2002) *Surv. Geophys.*, 23, 411-467. [3] Matrajt G. et al. (2004) *Meteorit. Planet. Sci.*, 39, 1849-1858. [4] Toppani A. et al. (2001) *Meteorit. Planet. Sci.*, 36, 1377-1396. [5] Glavin D. P. and Bada J. L. (2001) *Astrobiology*, 1, 259-269. [6] Glavin D. P. et al. (2006) *LPSC XXXVI*, Abstract #xxxx, LPI, Houston (CD-ROM). [7] Mank A. J. and Yueng E. S. (1995) *J. Chromatogr. A*, 708, 309-321. [8] Pizzarello S. et al. (2001) *Science*, 293, 2236-2239.

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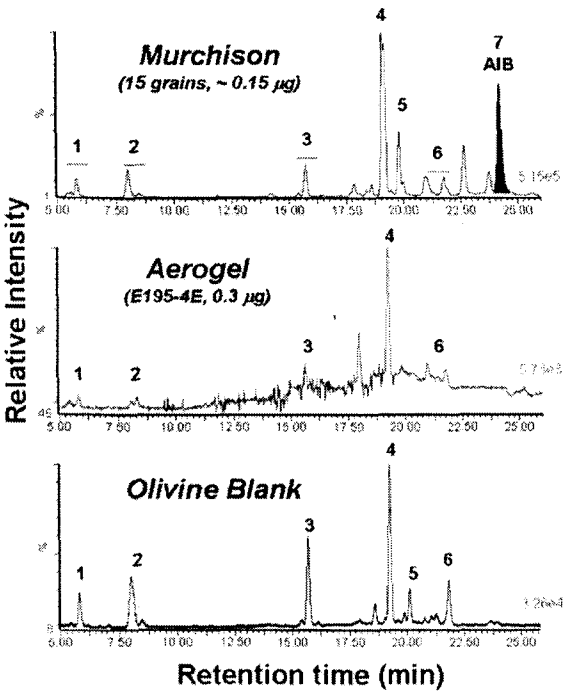

Figure 1 (left): HPLC separation of OPA/NAC derivatives with UV fluorescence detection of amino acids in the 6 M HCl-hydrolyzed, hot-water extracts. Peaks were identified by comparison of the retention time to those in a standard run in parallel and by exact molecular mass as follows: 1, D+L-aspartic acid; 2, D+L-glutamic acid; 3, D+L-serine; 4, glycine; 5, β-alanine (BALA); 6, D+L-alanine; and 7, α-aminoisobutyric acid (AIB).

Table 1: Predicted Detection Limits for Amino Acids in Meteorite Grains.

Meteorite sample (amino acid)	Total (ppm)	No. of 10 μm grains for LC-ToF-MS analysis *standard*	*with LIF*
Murchison* (AIB)	6	10	1/80
Orgueil[1] (β-ALA)	2	30	1/30
AMMs[3] (AIB)	0.2	300	1/3
Orgueil[1] (AIB)	0.04	2000	2
Tagish Lake[8] (AIB)	< 0.03	>2500	>2

* Average from literature.