

STRATEGIES FOR DISTINGUISHING ABIOTIC CHEMISTRY FROM MARTIAN BIOCHEMISTRY IN SAMPLES RETURNED FROM MARS. D. P. Glavin, A. S. Burton, M. P. Callahan, J. E. Elsila, J. C. Stern, and J. P. Dworkin, NASA Goddard Space Flight Center, Greenbelt, MD 20771, daniel.p.glavin@nasa.gov.

Introduction: A key goal in the search for evidence of extinct or extant life on Mars will be the identification of chemical biosignatures including complex organic molecules common to all life on Earth. These include amino acids, the monomer building blocks of proteins and enzymes, and nucleobases, which serve as the structural basis of information storage in DNA and RNA. However, many of these organic compounds can also be formed abiotically as demonstrated by their prevalence in carbonaceous meteorites [1]. Therefore, an important challenge in the search for evidence of life on Mars will be distinguishing between abiotic chemistry of either meteoritic or martian origin from any chemical biosignatures from an extinct or extant martian biota. Although current robotic missions to Mars, including the 2011 Mars Science Laboratory (MSL) and the planned 2018 ExoMars rovers, will have the analytical capability needed to identify these key classes of organic molecules if present [2,3], return of a diverse suite of martian samples to Earth would allow for much more intensive laboratory studies using a broad array of extraction protocols and state-of-the-art analytical techniques for bulk and spatially resolved characterization, molecular detection, and isotopic and enantiomeric compositions that may be required for unambiguous confirmation of martian life.

Here we will describe current state-of-the-art laboratory analytical techniques that have been used to characterize the abundance and distribution of amino acids and nucleobases in meteorites, Apollo samples, and comet- exposed materials returned by the Stardust mission with an emphasis on their molecular characteristics that can be used to distinguish abiotic chemistry from biochemistry as we know it. The study of organic compounds in carbonaceous meteorites is highly relevant to Mars sample return analysis, since exogenous organic matter should have accumulated in the martian regolith over the last several billion years and the analytical techniques previously developed for the study of extraterrestrial materials can be applied to martian samples.

Analytical Techniques: In order to measure the soluble organic composition (including amino acids and nucleobases) found in terrestrial and extraterrestrial samples, solvent extraction of the powdered sample followed by purification and chemical derivatization is typically required depending on the target compound and analytical technique used. Details of the extraction procedures used for amino acids and nucleobases in meteorites are published elsewhere [4,5]. In brief, for

amino acid analyses, hot water extracts were derivatized with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) and analyzed by liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS); for nucleobases, formic acid extracts were analyzed by liquid chromatography with high resolution ($m/\Delta m \geq 60,000$), accurate mass (< 5 ppm) Orbitrap detection.

Amino Acids and Chirality: The amino acid compositions of carbonaceous chondrites have been characterized using a variety of techniques and some of these meteorites contain a rich structural diversity that is not seen in terrestrial amino acids.

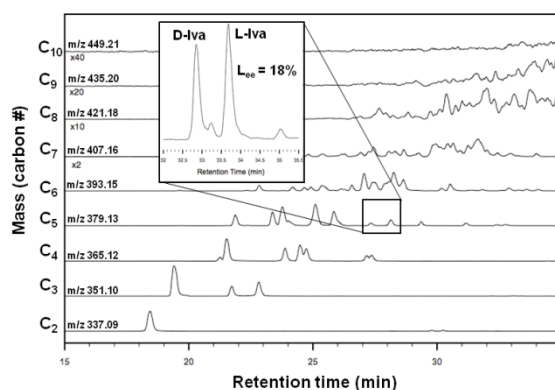


Figure 1. LC-ToF-MS chromatogram of OPA/NAC amino acid derivatives in the Murchison meteorite showing the mass traces corresponding to C₂ to C₁₀ amino acids. A large L-enantiomeric acid (L_{ee}) excess of 18% was measured for the C₅ amino acid isovaline, a terrestrial rare amino acid [4].

For example, over 80 different amino acids have been identified in the CM meteorites Murchison and Murray using gas chromatography mass spectrometry, and they comprise a mixture of two- to eight-carbon cyclic and acyclic monoamino alkanolic and alkandioic acids of nearly complete structural diversity, many of which are completely nonexistent in the terrestrial biosphere [1]. Our recent analyses of the Murchison meteorite using a much more sensitive LC-ToF-MS instrument provide evidence for a much larger diversity of amino acids with masses of up to ten carbons [6], indicating that hundreds of individual amino acids are present (Fig. 1). Many of the amino acids in carbonaceous meteorites are thought to have formed by Strecker synthesis during aqueous alteration on the parent body, although other mechanisms for the formation of amino acids in some more thermally altered meteorites have been proposed [7]. The complex amino acid distribution in Murchison is distinct from present day life where proteins are built from a much more limited set of amino acids. Although it is unknown whether or not a past

martian biota even existed, characterizing and comparing the complete distribution of amino acids in a returned sample to what is commonly produced in abiotic chemistry would provide important constraints on their origin(s).

In addition, many amino acids are structurally chiral. Life on Earth is dominated by L-amino acids, while all known abiotic syntheses of amino acids result in racemic mixtures of amino acids (i.e. L = D) in the absence of a chiral driving force. Therefore, chirality can be an important tool to help discriminate between amino acids of abiotic or biotic origins. However, even chirality measurements of amino acids found in a martian sample would need to be interpreted with caution, since many amino acids common to life on Earth will racemize on geologically short timescales changing their original enantiomeric ratios. In addition, large L-enantiomeric excesses of isovaline found in Murchison and other aqueously altered meteorites [6] demonstrate that abiotic mechanisms for producing enantiomeric biases exist. Ideally, detection of a large excess of D-amino acids in a martian sample would provide compelling evidence for an origin of life independent from Earth.

Nucleobases: Until recently, the origin of nucleobases identified in some carbonaceous meteorites [8] had not been firmly established. One of the issues was that all of the purines (adenine, guanine, hypoxanthine, and xanthine) and one pyrimidine (uracil) detected are biologically common and could be explained as the result of terrestrial contamination.

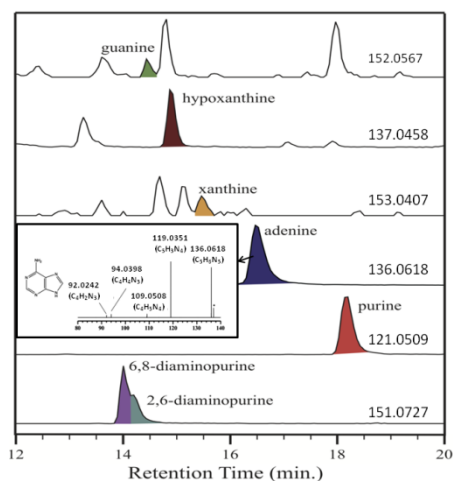


Figure 2. Single ion accurate mass chromatograms of a formic acid extract of a meteorite analog (HCN polymer) obtained using liquid chromatography Orbitrap mass spectrometry. Purines were identified by their monoisotopic parent masses (± 5 ppm) and multiple fragment masses (inset) and chromatographic retention time [5].

Using liquid chromatography Orbitrap mass spectrometry, a diverse suite of purines were unambiguously identified by accurate mass in Murchison [5]. In addition

to the purines previously identified in Murchison [8,9], three unusual and terrestrially rare nucleobase analogs were also found: purine, 6,8-diaminopurine, and 2,6-diaminopurine. An identical suite of nucleobases and nucleobase analogs were produced in reactions of ammonium cyanide (Fig. 2), which provides a plausible mechanism for their synthesis and strongly supports an extraterrestrial origin for these compounds in meteorites. The discovery of new nucleobases in meteorites expands the prebiotic molecular inventory available for constructing the first genetic molecules on Earth and potentially on Mars.

Due to the extremely low abundances (< 250 ppb) of nucleobases in meteorites [5], determination of their C, N, and H isotopic ratios has been extremely challenging. Martins *et al.* reported non-terrestrial carbon isotope values for xanthine and uracil in Murchison [9], however this measurement alone required ~ 15 grams of bulk sample. Compound-specific stable isotopic analysis of organic compounds in samples returned from Mars will be extremely important in establishing their origin, especially if the molecules identified are similar to those found in life on Earth.

Conclusions: Ultimately, the search for indisputable chemical evidence of life on Mars may require measurements that even go beyond current laboratory analytical capabilities, including molecular *spatial resolution* of amino acids, nucleobases, carboxylic acids, and other organic molecules important to life. Currently, the spatial distribution of these key organic compounds found in meteorites is not understood. In the future, instruments with much lower detection limits combined with new sample handling/extraction technologies will enable detection of these organic compounds in individual micron sized grains. In addition, other non-destructive analytical techniques need to be developed to enable spatially resolved measurements of these organic compounds. Even with improvements in instrument and sample handling technologies, identifying molecular biosignatures from an extinct martian biota in a returned sample that was significantly altered over time in the harsh radiation and oxidizing martian surface environment will be challenging.

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