Micro-detection system for determination of the biotic or abiotic origin of amino acids

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Final Project Summary

Sublimation (Bada, Glavin, SIO, UCSD): A description of the sublimation based extraction of amino acids from natural samples was published in August 1998 in Analytical Chemistry. Sublimation by itself requires no wet chemical reagents and eliminates the need for cation-exchange chromatography to isolate amino acids. The sublimation-based process provides an efficient way for isolating amino acids from natural samples without altering their original enantiomeric composition (ratio of D- to L-enantiomers). The sublimation-based isolation of amino acids could be especially useful in spacecraft instrumentation, where the use of corrosive liquid reagents required in traditional methods would be impractical. Future in situ amino acid analyses on solar system bodies such as Mars, Europa, asteroids and comets could use sublimation for amino acid isolation.

Further work with the sublimation process has demonstrated that besides amino acids, the nucleobases of DNA and RNA (adenine, cytosine, guanine, thymine and uracil) also readily sublime under reduced pressure at elevated temperatures. In addition, we found that the nucleobases could be sublimed directly from intact DNA, thus allowing the detection for the presence of genetic material in a sample. Thus, sublimation can be used to directly isolate both protein and genetic components from natural samples.

Integrated Microchemical Analysis System (Frank Grunthaner and Paula Grunthaner, JPL): In order to supplement the sublimation release of amino acids, we have also developed a design for an integrated wet chemical analyzer using microchip CE for detection. In this design, after the sample is obtained, it is placed in a crucible and sealed from the ambient. Liquid water is then added and the sample is extracted at 145°C for approximately 5 to 7 minutes. A portion of this water extract is transferred directly to the CE system for analysis of free amino acids, while the remainder is evaporated to dryness. This residue is hydrolyzed in HCl at elevated temperatures for a few minutes.
The acid is removed by evaporation and the sample then transferred into a microfluidics processing cell for desalting, labeling and concentrating. Finally, the sample is injected into the microfabricated CE system (see below) for separation of amino acids which are detected using UV fluorescence.

**CE experiments (Mathies, Hutt, UCB):** The overall purpose of this project is the development of a bench top, microfabricated device capable of amino acid composition analysis and quantitative determination of their enantiomeric ratios. Our initial efforts focused on experimentating with all of the parameters which affect chiral separations of amino acids in microfabricated capillary electrophoresis (CE) channels. Such parameters include the magnitude of the applied electric field across the separation channel, the temperature at which the runs were conducted, and the length and geometry of the channels. With respect to the composition of the run buffer, all of the following variables were explored: type and concentration of cyclodextrin (the chiral discriminator molecule), buffer pH, and addition of sodium dodecyl sulfate (SDS) micelles.

The test system used consisted of a twice-folded, 19.0 cm long, 50 mm by 20 mm channel that was photolithographically fabricated in a 4" diameter glass wafer. A standard containing D/L Ala, D/L Asp, D/L Glu, D/L Ser, D/L Val, a-aminoisobutyric acid (AIB), and Gly was derivatized with fluorescein isothiocyanate (FITC) overnight and run at an approximate concentration of 100 nM. Detection of the labeled amino acids was by laser excited (488 nm) confocal fluorescence that provides attomole sensitivity. Using a 12 mM SDS, 5 mM g-cyclodextrin, 10 mM carbonate, pH 10.0 buffer and a separation voltage of 550 V/cm at 10°C, baseline resolution of the amino acids along with some of the D/L pairs was achieved.

To further test our microchip CE device, an amino acid extract taken from the outside of the Murchison meteorite was derivatized with FITC and analyzed. Enantiomeric ratios for Asp and Glu were determined by comparing the relative peak areas and correcting them with the appropriate calibration curves. The resulting D/L values were in close agreement with those obtained at SIO by the HPLC based method. A manuscript describing these results has been submitted for publication in *Analytical Chemistry*.

**Publications:**

Bada, J. L., “What compounds do we look for in Martian (SNC) meteorites for evidence of abiotic and/or biotic chemistry on Mars?” Amer. Chem. Soc. National


Micro-Detection System for Determination of the Biotic or Abiotic Origin of Amino Acids

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The research carried out under this PIDDP grant involved the development of a breadboard version of a spacecraft based system for the detection of amino acid chirality (handedness) on solar system bodies. The design concept has three distinct components: a sublimation chamber for the release of amino acids from an acquired sample; a microchip based capillary electrophoresis (CE) chip for the separation of amino acids and their enantiomers; and a fluorescent based detection system. In addition, we have investigated the use of a microfluidics system for the extraction of amino acids in samples in which sublimation has proven to be problematic. This is a joint project carried out at the Scripps Institution of Oceanography (SIO), University of California at San Diego; the Jet Propulsion Laboratory (JPL), Pasadena; and the Department of Chemistry, University of California, Berkeley.

Subject Terms:
amino acid chirality, capillary electrophoresis, biosignatures