Journal of Chromatography A, 1332 (2014) 30–34

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Amino acid analysis in micrograms of meteorite sample by nanoliquid chromatography–high-resolution mass spectrometry

Michael P. Callahan[a,*], Mildred G. Martin[b], Aaron S. Burton[c,1], Daniel P. Glavin[a], Jason P. Dworkin[a]

[a] Solar System Exploration Division, NASA Goddard Space Flight Center, Greenbelt, Maryland 20771, USA
[b] Catholic University of America, Washington, D.C. 20064, USA
[c] NASA Postdoctoral Program Fellow, NASA Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

ARTICLE INFO

Article history:
Received 13 November 2013
Accepted 10 January 2014
Available online 18 January 2014

Keywords:
Amino Acids
astrobiology
high-resolution mass spectrometry
Murchison meteorite
nanoliquid Chromatography
orbitrap

ABSTRACT

Amino acids and their enantiomers in a 360 microgram sample of Murchison meteorite were unambiguously identified and quantified using chemical derivatization and nanoliquid chromatography coupled to nanoelectrospray ionization high resolution orbitrap mass spectrometry techniques. The distribution and abundance of amino acids were similar to past studies of Murchison meteorite but the samples used here were three orders of magnitude lower. The analytical method was also highly sensitive, and some amino acid reference standards were successfully detected at a level of ~200 attomoles (on column). These results may open up the possibility for investigating other less studied, sample-limited extraterrestrial samples (*e.g.*, micrometeorites, interplanetary dust particles, and cometary particles) for biologically-relevant organic molecules

Published by Elsevier B.V.

1. Introduction

Most meteorites are fragments of asteroids; therefore, laboratory analyses of meteorites can provide a window into the extraterrestrial organic chemistry that took place during the formation of the solar system. Carbonaceous chondrites, a rare class of chondritic meteorites, have been found to be the most complex in terms of organic composition. Among the organics in carbonaceous chondrites, a diverse suite of over 100 amino acids have been detected [1,2]. Amino acids, the monomers of peptides and proteins, are essential in modern life and were most likely critical for nascent biochemistry. Additionally, nitrogen heterocycles [3–5], sugar-related organic compounds [6], and (potential) metabolic precursors [7,8] have also been identified in carbonaceous chondrites. The detection of numerous biologically-relevant organic compounds in carbonaceous chondrites has led to the assertion that these meteorites delivered important organics for the origin of life on the early Earth (and elsewhere). However, this belief has been criticized by many researchers based on estimates of carbonaceous meteorite flux (being relatively low compared to other extraterrestrial material) [9] coupled to the observations of low abundances of meteoritic organic compounds—typically in the parts-per-billion (ppb) to parts-per-million (ppm) concentration range [10].

Micrometeorites ($<10^{-4}$ g) and interplanetary dust particles (IDPs) ($<10^{-6}$ g) represent the present day dominant source of extraterrestrial material delivered to Earth [11,12], and these sources were more likely to have provided a steady-state flux and more significant quantities of prebiotic reagents to the early Earth compared to carbonaceous chondrites [9]. Unfortunately, there have been limited studies examining their organic composition [13–20], especially with regard to biologically-relevant molecules that may have been important for the origin of life [21,22], due to the miniscule size of these samples. Thus, it would be highly desirable to have analytical instrumentation and methods that could address these issues. Here, we demonstrate the chiral separation, identification, and quantitation of amino acids in an extraction of 360 micrograms of the well-characterized Murchison meteorite, which serves as an analog for future micrometeorite/IDP studies. This was achieved by using a nanoliquid chromatograph (nano-LC) coupled to a linear ion trap–orbitrap hybrid mass spectrometer *via* a nanoelectrospray ionization (nano-ESI) source. These instruments represent the current state-of-the-art for laboratory analytical science but have been underutilized in the analysis of organics in extraterrestrial materials.

In this study, the nano-LC primarily serves two purposes. The first is to perform efficient separation of chiral amino acids. To achieve this, we employ a precolumn chiral derivatization of primary amines using *o*-phthaldialdehyde/*N*-acetyl-L-cysteine

* Corresponding author: Tel.: +1 301 286 1548; fax: +1 301 286 1683.
E-mail address: michael.p.callahan@nasa.gov (M.P. Callahan).
[1] Present address: Astromaterial Research and Exploration Science Directorate, NASA Johnson Space Center, Houston, TX 77058, USA.

0021-9673/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.chroma.2014.01.032

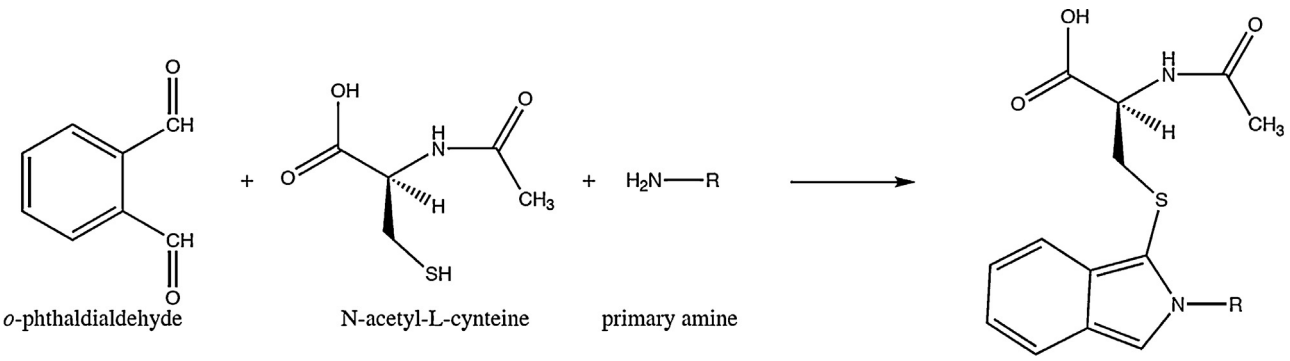

Figure 1. The chemical derivatization scheme to produce OPA/NAC amino acid derivatives.

(OPA/NAC) so that the resulting diastereomer products (Figure 1) can be separated using a nonchiral chromatography column [23]. The second purpose is to deliver the necessary low flow rates required for the efficient operation of a nano-ESI source.

Since its introduction, nano-ESI has enabled extremely high sensitivity mass spectrometry (in part) due to the reduced droplet size compared to conventional electrospray ionization [24–27]. This high sensitivity along with the excellent mass accuracy (<2 ppm), high mass resolution (>30,000 m/Δm), and MS/MS data afforded by the linear ion trap–orbitrap mass spectrometer significantly aids in the accurate identification and quantitation of targeted and unknown molecules in complex samples such as meteorites.

Our analytical method was first optimized using selected amino acid standards before being applied to an acid-hydrolyzed, hot water extract of a powdered sample of the Murchison meteorite.

2. Experiments

2.1. Chemicals and reagents

All of the chemicals used in this study were purchased from Sigma-Aldrich, Fisher Scientific, and Acros Organics. To prepare amino acid stock solutions (~10^{-3} M), individual compounds were dissolved in ultrapure water (18.2 MΩ·cm, < 3 ppb total organic carbon provided by a Millipore Milli-Q Integral 10 system). These solutions were then combined to enable their measurement in a single chromatographic separation. Serial dilutions (10^{-6} M to 10^{-10} M) were prepared for generation of the calibration curves. Ultrapure water was used exclusively for this study.

The OPA/NAC reagent used for amino acid derivatization was prepared by mixing 300 μL 0.01 M OPA (in methanol) with 15 μL 1 M NAC and 685 μL 0.1 M sodium borate buffer (pH 9) [28]. Solutions of sodium borate were prepared from solid sodium tetraborate decahydrate (Sigma Ultra 99.5–100% purity) that was heated in air at 500 °C for 3 h to remove any organic contaminants prior to dissolution in water. A 0.1 M hydrazine (NH_2NH_2) solution was prepared by double vacuum distillation of anhydrous hydrazine (98% purity) and subsequent dilution in water. The 6 M HCl solution for the acid hydrolysis procedure was also prepared by double vacuum distillation and subsequent dilution in water. For the LC–MS analyses, ammonium formate buffer was prepared by NH_4OH titration of a 10 mM formic acid solution to pH 8.3 and then methanol was added to a final concentration of 5% (v/v). Methanol was Optima® grade from Fisher Scientific.

2.2. Sample handling and extraction procedures

Several interior pieces of the Murchison meteorite (USNM 5453) were provided by the Smithsonian National Museum of Natural History. No fusion crust was observed on these meteorite samples. Sample-handling tools, ceramics, and glassware were all rinsed with ultrapure water, wrapped in aluminum foil, and heated in air at 500 °C for 24 h to remove any organic contaminants. The Murchison meteorite was crushed into a fine powder using a clean mortar and pestle in a Class 100 laminar flow hood (Labconco) under high-efficiency particulate air filtered positive pressure.

We weighed 360 micrograms (arbitrary mass, but in the range of an individual micrometeorite) of Murchison meteorite into a tared glass ampoule using a Mettler Toledo XP56 microbalance. We added 1 mL water to the glass ampoule before it was flame-sealed and placed in an oven set at 100 °C for 24 h. After extraction, the ampoule was cooled, centrifuged for 5 min (Labconco CentriVap) to separate the solid particulate from water supernatant, and then opened. The water supernatant was transferred into a separate glass tube, dried under vacuum and subjected to acid hydrolysis under 6 M HCl vapor at 150 °C for 3 h to liberate any "bound" amino acids. The acid-hydrolyzed extract (representing the total amino acid content) was dried under vacuum (LabConco Centrivap) and redissolved in 1 mL of water. The extract was then desalted by cation exchange column (AG 50W-X8, 100–200 mesh, hydrogen form, BIO-RAD) using water followed by 2 M NH_4OH. The NH_4OH eluate was dried under vacuum and redissolved in 100 μL water. This meteorite extract was stored in a -20 °C freezer until precolumn derivatization and LC–MS analysis.

2.3. Chemical derivatization

For OPA/NAC amino acid derivatization, 10 μL meteorite extract (or amino acid standard solution) was mixed with 10 μL 0.1 M sodium borate buffer (pH 9) and derivatized with 5 μL OPA/NAC in an HPLC vial. The derivatization reaction was then quenched after 15 min at room temperature with 75 μL 0.1 M hydrazine.

2.4. Nano-LC-high resolution MS analysis

OPA/NAC amino acid derivatives were analyzed using a Waters nano-ACQUITY Ultra Performance LC (UPLC) coupled to a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer. Amino acid separation was achieved with a Waters nano-Acquity UPLC column (150 μm × 100 mm, 1.7 μm BEH130 C18) maintained at 30 °C. The mobile phase consisted of (A) 10 mM ammonium formate buffer with 5% methanol, pH 8.3 and (B) methanol. The composition of the mobile phase changed by adding increasing proportions of (B) as follows: 0–2 min 5% B, 2–10 min 5–10% B, 10–50 min 10–50% B, 50–51 min 50–5% B, 51–70 min 5% B. A flow rate of 1.5 μL/min was used. Samples were injected into a 2 μL loop. During initial method development for the chromatographic separation of amino acids, we coupled the nano-LC to a laser induced fluorescence (LIF) detector (Picometrics ZetaLIF, excitation wavelength 355 nm, total emission collection) because the OPA/NAC amino acid