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Quantitative enantioseparation of amino acids by comprehensive two-dimensional gas chromatography applied to non-terrestrial samples

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ABSTRACT

This work presents an improved analytical procedure for the resolution and quantification of amino acid enantiomers by multidimensional gas chromatography. The procedure contains a derivatization step, by which amino acids were transformed into *N*(*O*,*S*)-ethoxycarbonylheptafluorobutyl esters. It was optimized for the resolution of non-proteinogenic amino acids in the matrix of complex non-terrestrial samples. The procedure has proven to be highly sensitive and shows a wide linearity range with 0.005–3 pmol detection limits for quantitative determinations. The developed procedure was tested on a sample of the Murchison meteorite, for which obtained chromatograms show excellent peak resolution, minimal co-elution and peak overlap. We conclude that comprehensive two dimensional chromatography, in combination with the optimized derivatization method is a highly suitable technique for the analysis of samples with very limited quantities and containing potentially prebiotic molecules, such as interstellar ice analogs and meteorites.

1. Introduction

The search for prebiotic organic compounds of non-terrestrial origin such as amino acids, is one of the major tasks of numerous space missions [1,2] and meteorite studies [3]. Once found, they open a window into the chemical evolution of organic matter—possible precursors to the origin of life and further biological evolution [4]. It has been shown that some of the key molecules in terrestrial biology, e.g. amino acids [5,6], nucleobases [7–9], and sugar acids [10] are present in ppb to ppm level within a specific class of meteorites—carbonaceous chondrites, thus suggesting an exogenous delivery of prebiotic organic matter to Earth. In this context the enantiomeric resolution of chiral species is of a primary relevance as the ratio between two optical isomers can be viewed as a signature of their biotic versus abiotic origin. Additionally, small enantiomeric excesses (*ee*) are often interpreted as the result of a molecular symmetry breaking event, e.g., driven by a chiral force [11]. The first detection of an enantiomeric enrichment in L-amino acids within the Murchison meteorite opened a debate on the indigeneity of the observed *ee*s and the efficiency of the applied analytical separation technique [12]. Since then, demands for the analytical procedure with high precision *ee* measurement and increased separation power have raised significantly.

Gas chromatography (GC) has a long track record in the analysis of extraterrestrial samples [13]. Given the complex nature of extraterrestrial organic matter and its low available quantity [14], an analytical procedure for the enantioselective analysis of amino acids has to meet certain requirements such as: (i) low detection and quantification limits; (ii) high enantiomeric resolution; (iii) complete absence of racemization following the analytical protocol; (iv) minimal co-elution. While some of the mentioned limitations are linked to the derivatization step (e.g., the degree of racemization) others are mainly related to the separation power of conventional GC. As the monodimensional capillary GC technique has reached its limits for complete analyte separation, further improvement of gas chromatographic resolution has been achieved as a result of the development of comprehensive multidimensional gas chromatography (GC × GC). Since its first introduction in the 1990s [15] the innovative technique has been successfully applied to detect and quantify trace-level constituents and contaminants

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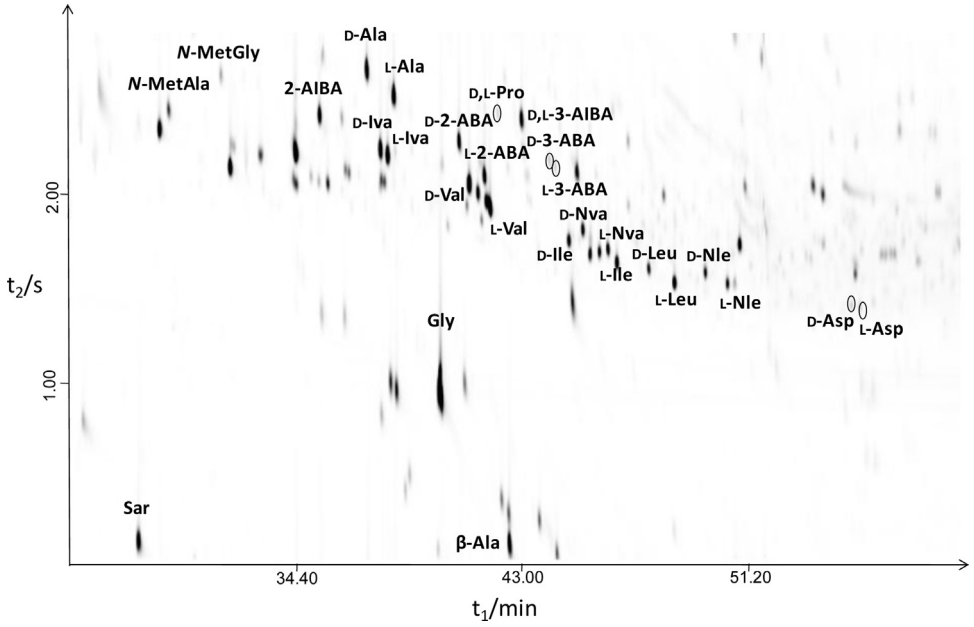

Fig. 1. Two dimensional surface plot of GC × GC-TOFMS for the Murchison extract. Selected atomic masses are: 102, 116, 130, 142, 144, 158, and 342.

in various types of samples [16]. In this study we emphasize the advantages of enantioselective multidimensional gas chromatography applied to samples of astrobiological relevance, including a sample of the Murchison meteorite. The developed analytical method is compared to previously reported HPLC, GC–MS and GC × GC–MS methods [17–21] for the separation of amino acid enantiomers.

Furthermore, the classical optimization and validation of analytical procedures for amino acid separation is generally limited to 20 genetically encoded units, keeping a much larger group of non-proteinogenic amino acids out of the picture. However, the enantiomeric separation of non-proteinogenic amino acids gains more and more attention in a number of fields of bioanalytical chemistry, metabolomics, medicinal chemistry, and pharmacology [22]. Regarding an outer space origin of these molecules, the newly developed analytical procedure is of primary interest in meteoritic and planetary research. In spite of their importance to the field, only a few researchers have studied their resolution and analytical response [23,24]. Thus, this work contains an elaborated list of amino acids taking into account their relevance for the chemical evolution of organic matter in solar system objects. Additionally, high precision measurements of amino acid *ees* in a Murchison meteorite extract were performed.

2. Experiments

2.1. Chemicals, reagents and tools

Different groups of amino acids were analyzed: proteinogenic amino acids (Gly, Ala, Ser, Asp, Val, Pro, Glu, Met, Leu, Ile, and Phe), α-non-proteinogenic amino acids (2-aminobutyric acid, norvaline, norleucine, and *tert*-leucine), β-amino acids (β-alanine, 3-aminobutyric acid, 3-aminoisobutyric acid, 3-aminopentanoic acid, and β-leucine), a γ-amino acid (4-aminobutyric acid), α-dialkylated amino acids (2-aminoisobutyric acid, isovaline, and 2-methylglutamic acid), *N*-alkylated amino acids (sarcosine, *N*-methylalanine, and *N*-ethylglycine), an iminodiacid (iminodiacetic acid), and diamino acids (2,3-diaminopropanoic acid and 2,3-diaminobutanoic acid). All the chemicals used in this study were purchased from Sigma–Aldrich, Fluka, or Acros Organics. To prepare the amino acid stock solution (1.25×10^{-3} M), the individual 30 amino acids were weighted, mixed, and dissolved in 0.1 M HCl solution prepared by dilution of 6 M HCl (Fluka) in ultrapure water (HPLC grade). Serial dilutions (from 10^{-5} M to 10^{-9} M) were prepared for the generation of the calibration curves (Supplementary information Fig. S1 (1–8)). Due to their low response, two amino acids—isovaline and α-aminoisobutyric acid—were prepared separately with serial dilution from 10^{-3} M to 10^{-6} M. All glassware used was wrapped in aluminum foil, and heated at 500 °C for at least 3 h to eliminate possible terrestrial contamination.

2.2. Derivatization

Diluted series of the standard solution were transformed into *N*(*O*,*S*)-ethoxy-carbonylheptafluorobutylester derivatives (ECHFBE) according to the modified standard procedure of Abe et al. [25] described elsewhere [19,20]. Arguments for the choice of the derivatization method were reviewed by Meinert and Meierhenrich [26]. The reagents were added into a reaction vial in the following order: 25 μL of a 2,2,3,3,4,4,4-heptafluoro-1-butanol/pyridine mixture (3:1, v/v) followed by 5 μL of ethyl chloroformate. The reaction mixture was vigorously shaken for 10 s to form the ECHFBE derivatives. The amino acid derivatives were extracted from the reaction mixture by adding 40 μL of chloroform and 10 μL solution of pyrene in chloroform (2 μg mL^{-1}), the last serving as an internal standard (IS). The organic phases were withdrawn and transferred into 1 mL GC vials equipped with 100 μL inserts for enantioselective GC × GC analyses.

This derivatization method was also applied to an extract of the Murchison meteorite. The detailed extraction procedure is described in the Supplementary information and can be found in the literature [6].

2.3. GC × GC-TOFMS

The enantioselective multidimensional analysis was carried out by a GC × GC Pegasus IV D instrument coupled to a time-of-flight mass spectrometer (LECO, Michigan, USA). The MS system operated at a storage rate of 150 Hz, with a 50–400 amu mass range,