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Free and combined amino acids in marine background atmospheric aerosols over the Eastern Mediterranean

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ABSTRACT

During a six-week intensive field campaign at a background marine site of the Eastern Mediterranean, consecutive 24-h air samples were collected and analyzed for combined (CAA) and free amino acids (FAA), as well as for key chemical characteristics of aerosols. The total concentration of CAA (719 ± 326 pmol m^{-3}) was on average four times higher than that of FAA (172 ± 147 pmol m^{-3}), while glycine was the most abundant compound detected in both FAA and CAA. Back-trajectory analysis demonstrated that the geographical origin of the air masses did not have a significant influence on the atmospheric levels of amino acids. Wind speed was found to be the most important meteorological factor and it exhibited a negative correlation with both FAA and CAA. Moreover, FAA and CAA concentrations showed a more pronounced correlation with water-soluble organic carbon (WSOC) than elemental carbon. On average, FAA and CAA accounted for 0.3 ± 0.2% and 1.8 ± 0.8% of WSOC, respectively. The levels of anionic surfactants determined as methylene blue active substances did not show any positive correlation with CAA, while the corresponding correlation with FAA was only of marginal significance. The total protein concentration measured by NanoOrange assay was six times higher compared to that measured through CAA. It is suggested that the results from the application of commercially available protein quantitation kits should always be considered with caution, as these are more prone to matrix-related interferences.

1. Introduction

Organic nitrogen compounds comprise a large fraction of the total nitrogen in aerosols (~20–80%) (Zhang et al., 2002; Zhang and Anastasio, 2001; Mace et al., 2003a,b) and, when deposited on Earth's surface, they can serve as a source of nutrients to marine ecosystems. Among the various chemical species present in atmospheric particles, amino acids are considered to be an important class of nitrogen-containing compounds characterized by high bioavailability (Zhang et al., 2002). A number of previous studies confirmed the ubiquitous presence of free amino acids in aerosols (Zhang and Anastasio, 2001, 2003; Zhang et al., 2002; Mace et al., 2003a,b,c; Yang et al., 2005; Yu, 2002; Matsumoto and Uematsu, 2005; Wedyan and Preston, 2008), while growing evidence indicates that a large amount of these compounds may also exist in the combined form, such as proteins, peptides, amino acid complexes with humic substances, etc. (Yu, 2002; Zhang and Anastasio, 2003; Zhang et al., 2002; Wedyan and Preston, 2008).

Besides their potential role in atmosphere–biosphere nutrient cycling, the presence of amino acids and proteinaceous material in aerosols has received special attention over the last decade for several other reasons. In their theoretical study, Saxena and Hildemann (1996) assumed that amino acids significantly contribute to the water-soluble organic carbon (WSOC) fraction, which is believed to promote mass growth of aerosols via water vapor uptake. More recent experimental studies investigated the potential effect of amino acids (Chan et al., 2005) and proteins (Mikhailov et al., 2004) on the hydroscopic growth of particles. Other studies have postulated that amino acids can contribute to the formation of new particles in the atmosphere (Leck and Bigg, 1999; De Haan et al., 2009). In addition, many proteins that are present in ambient particles are strong allergens and there is a growing concern about the contribution of proteinaceous material on the allergenic potency of aerosols and the effects may induce on human health (Miguel et al., 1999).

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While amino acid-based compounds in aerosols are inferred to be of biological origin (e.g. pollen, spores, plant debris, fungi, bacteria, algae, viruses, remnants of microorganisms or extracellular proteins from cell lysis; Milne and Zika, 1993), the factors controlling the levels of free (FAA) and combined amino acids (CAA) in the atmosphere are not well understood. Indeed, detailed data about the contribution of each individual amino acid are quite limited, especially for CAA (Yu, 2002; Zhang and Anastasio, 2003; Zhang et al., 2002; Wedyan and Preston, 2008).

Up to now, the identification/quantitation of amino acids was based on the derivatization of the target compounds with o-phthalaldehyde (OPA) and the analysis of the derivatives by means of HPLC with fluorescence detection. In a recent article, we showed that derivatization with N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) in conjunction with gas chromatography–mass spectrometry (GC–MS) overcomes some of the problems encountered with the conventional high performance liquid chromatography (HPLC)-fluorescence method and offers an alternative technique for the unambiguous and accurate identification of amino acids in atmospheric particles (Mandalakis et al., 2010). The present study aimed to investigate the levels and speciation of both FAA and CAA in marine background aerosols from the Eastern Mediterranean Sea using the GC–MS based method. We also examined whether total protein quantitation using commercially available kits (e.g. NanoOrange assay) could be used in place of GC–MS analysis of CAA for an easier and more rapid evaluation of the proteinaceous material in aerosols. The obtained results were compared with those reported in the literature and discussed with respect to variations in key chemical characteristics of aerosols and meteorological conditions.

2. Materials and methods

2.1. Sample collection and processing

Forty-six ($N = 46$) aerosol samples were collected between June 25 and August 9, 2007 from the marine background station of Finokalia (35°19′N, 25°40′E), located at the northeastern coast of Crete island, Greece. For amino acid measurements, sampling was conducted using a high-volume air sampler that pumped ambient air through a 20 × 25 cm glass fiber filter (GFF) at a flow rate of 30 m^3 h^{-1}. Collection time was 24 h giving a total air volume of ~720 m^3 per sample. For measurements of elemental carbon (EC), organic carbon (OC), WSOC and methylene blue active substances (MBAS), parallel aerosol samples were collected on 20 × 25 cm quartz fiber filters (QFF) using an identical sampling system. Preceding sampling, GFFs and QFFs were heated at 450 °C for 5 h to remove any traces of organic compounds, wrapped in pre-combusted aluminum foil and sealed in plastic bags. After each deployment, the filters were collected, resealed, and stored at −18 °C until analysis. The mass of collected particles was analyzed gravimetrically as the difference between pre- and post-sampling filter weight.

2.2. Sample analysis

2.2.1. Analysis of free and combined amino acids

Procedures for the extraction and analysis of water-soluble FAA and CAA in aerosol samples are described in detail elsewhere (Mandalakis et al., 2010). In brief, a portion of GFF (corresponding to 220 m^3 of air) was spiked with 1500 ng of 2-aminobutyric acid (recovery standard) and FAA were extracted twice with 12 ml of water/isopropanol mixture (1:1, v/v), using a sonication bath (60 °C for 20 min). The extract was centrifuged (2800 × g for 20 min) to remove suspended debris and filter particles, and the supernatant was evaporated to 0.2 ml using a Martin Christ (Osterode, Germany) rotational vacuum concentrator.

For CAA, a much smaller portion of filter (corresponding to 60 m^3 of air) was ultrasonically extracted twice with 6 ml of water. The extract was evaporated to 0.2 ml, transferred into a Pyrex glass hydrolysis tube and evaporated to dryness. After the addition of 250 μl of HCl 6 M and 25 μl of ascorbic acid (20 μg μl^{-1}), the tube was flushed with argon, tightly sealed and the sample was hydrolyzed for 24 h at 110 °C.

The concentrated extract of FAA and the hydrolysate of CAA were transferred to separate vials, spiked with 1500 ng of norvaline and 2-aminopimelic acid (internal standards) and evaporated to dryness. Then, 60 μl of MTBSTFA and 10 μl of dimethylformamide were added and the vials were sealed with PTFE-lined caps. Finally, the samples were heated at 70 °C for 20 min to achieve the chemical derivatization of amino acids. The analysis of amino acid derivatives was conducted on an Agilent 6890 gas chromatograph equipped with an on-column injector and interfaced with an Agilent 5973 mass spectrometer operating under electron impact ionization conditions and in the selected ion monitoring mode. Analytes were separated on a 15 m DB5-MS capillary column (Agilent, phenyl arylene polymer, 0.25 mm i.d, 0.25 μm film thickness) operating with helium carrier gas (constant velocity 46 cm s^{-1}) under the following temperature program: from 120 to 150 °C at 120 °C min^{-1} (5 min hold), to 240 °C at 7 °C min^{-1} and finally to 295 °C at 20 °C min^{-1} (16 min hold). A total of 19 amino acids were regularly detected and quantified using the internal standard method. The concentration of CAA in each aerosol sample was calculated by subtracting the quantity of FAA from that measured after acid hydrolysis. The results for both FAA and CAA were blank-subtracted and recovery corrected. Further details on quality assurance and control (blank levels, recoveries and method detection limits of amino acids) have been reported elsewhere (Mandalakis et al., 2010).

2.2.2. Analysis of key chemical characteristic of aerosols

Elemental and organic carbon were analyzed on parallel aerosols samples collected on QFFs (punch of 1.5 cm^2) using a thermal–optical carbon analyzer (Sunset Laboratory Inc., Oregon) (Birch and Cary, 1996). For the analysis of WSOC, a punch of QFF (~4.5 cm^2 of filter) was ultrasonically extracted for 20 min using 10 ml of water. Suspended particulate matter was filtered out of the extract using a PTFE-membrane syringe filter (13 mm in diameter, pore size 0.45 μm) and the solution was analyzed using a Shimadzu TOC liquid analyzer (model TC5000A). The concentration of anionic aerosol surfactants was determined as MBAS, by extracting a punch of the QFF (~15 cm^2) and using the method described by Latif and Brimblecombe (2004). The sampling volume corresponding to the filter size used for the analysis of elemental/organic carbon, WSOC and MBAS was 2.0, 6.5 and 21.5 m^3, respectively.

For total protein analysis, a portion of each GFF (~90 cm^2; corresponding to 130 m^3 of air) was ultrasonically extracted with 10 ml of water and the extract was centrifuged at 2800 × g for 20 min. Subsequently, protein was measured fluorometrically in the supernatant using the Nano-Orange Protein Quantification Kit (Molecular Probes, Eugene, OR) following the manufacturer's instructions (Jones et al., 2003). Fluorescent signal was measured using a Hitachi F-2000 spectrofluorometer (excitation 485 nm, emission 590 nm) and the quantity of total protein in aerosol samples was determined using a standard calibration curve of bovine serum albumin.

2.2.3. Back-trajectories calculation and clustering

Backward air trajectories arriving at Finokalia site (at 0.25 km above ground level) were computed using the Hybrid Single