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Free and combined amino acids in size-segregated atmospheric aerosol samples

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HIGHLIGHTS

- Distribution of free and combined amino acids in atmospheric sizesegregated particles.
- Amino acid and total proteinaceous material concentrations in NIST SRM 1649b.
- Protein bioaerosol concentrations in ultrafine fraction were studied.

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ABSTRACT

Concentrations of free and combined amino acids in an urban atmosphere and their distributions in size-segregated particles were investigated in the cold and warm seasons. In particular this article provides the first investigation of protein bioaerosol concentrations in ultrafine fraction ($PM_{0.1}$) of particulate matter.

In addition the present work provides amino acid and total proteinaceous material concentrations in NIST SRM 1649b, useful as reference values. The reference material was also used to build matrix matched calibration curves.

Free amino acid total content in winter and summer $PM_{0.1}$ was respectively 48.0 and 94.4 ng m^{-3}, representing about 0.7 and 7.4% by weight of urban particulate matter in the two seasons.

Total airborne protein and peptide concentrations in the same ultrafine fractions were 93.6 and 449.9 ng m^{-3} respectively in winter and in summer, representing 7.5 and 35.4% w/w of $PM_{0.1}$, and demonstrating an exceptionally high percentage in summer ultrafine fraction.

The significant potential adverse health effects of ultrafine particulate matter include allergies mainly caused by protein particles and we assumed that in summer 162 ng h^{-1} of proteinaceous material, by means of ultrafine particles, can penetrate from the lungs into the bloodstream.

1. Introduction

Bioaerosols are atmospheric aerosol particles of biological origin (Després et al., 2012), including bacteria, fungi, spores, viruses, debris, by-products of biological activities, pollen, cells, cell fractions or organic matter of animal and plant.

Besides contributing to alteration of cloud coverage and hence to the global climate, biological aerosols can induce genotoxicity, cardiovascular effects, and allergic, toxic, and infectious responses in exposed individuals (Ariya and Amyot, 2004; Chan et al., 2005; Menetrez et al., 2007a; Deguillaume et al., 2008; Després et al., 2012).

Although it is recognized that exposures to complex mixtures of toxins and allergens cause high prevalence of respiratory symptoms and airway inflammation with major public health impact, knowledge of the total amount of bioaerosols in the air is difficult to achieve.

Some authors have identified the presence of pollen, bacteria and fungal spores in atmospheric particulate matter, through the detection of chemical biomarkers or through traps or viable microorganism sampling, growing and count, or molecular biology techniques (Ong et al., 1995; Bauer et al., 2002; Womiloju et al.,

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2003; Ho et al., 2005; Lee et al., 2006; Bauer et al., 2008a,b; Di Filippo et al., 2013).

Difficult to apply, biological methods also give partial information, because limited to the biological component under study.

Amino acids and proteins, both important constituents of bioaerosol, can be an index of its occurrence in atmosphere (Poruthoor et al., 1998; Czerwieniec et al., 2005).

Free amino acid contribution to inhalable airborne particles is due to direct emission from plants and animals and from biomass burning, or may be issued by degradation products of peptides and proteins through enzymatic or photo-catalytic reactions in atmosphere (for example, degradation of organic litter) (Gorzelska et al., 1992; Lobert and Warnatz, 1993; Milne and Zika, 1993; Melillo et al., 1989).

Concerning atmospheric proteins, specific outdoor and indoor airborne proteins deriving from bacteria, fungi, house dust mites, multiple pollens, animal dander, molds, and fragments of animals, insects and plants are implicated in severe lung diseases, causing respiratory disorders, asthma and chronic obstructive pulmonary disease to exposed individuals (Miguel et al., 1996; Burge and Rogers, 2000; Douwes et al., 2003; Adhikari et al., 2006; Georgakopoulos et al., 2009; Dockery and Pope, 1994).

Within the scarce literature concerning protein occurrence in atmosphere, the available studies are concerned with proteins originating from pollens of plants, especially anemophilous ones, from bacterial and fungal spores, from fragments of human skin or fragments of animals, insects and plants (Burge and Rogers, 2000; Miguel et al., 1999; Schappi et al., 1997; Zhang and Anastasio, 2003).

Among the most common natural atmospheric occurring proteins are the expansins, glycoproteins of about 30 kDa found in large quantities on the outer pollen wall of almost all species of grasses (corn, wheat, maize, barley and oats) (Grobe et al., 1999). Similarly the small, acid-soluble spore proteins (SASP), abundant proteins in spores of Bacillus species (Hathout et al., 2003; Fergenson et al., 2005; van Wuijckhuijse et al., 2005), can be found in atmosphere. Finally, some literature is available regarding the birch and turnip pollens in atmosphere, causing immune response with production of IgE antibodies (Knox and Suphioglu, 1996; Platt-Mills et al., 1998; Burge and Rogers, 2000; Schappi et al., 1997).

Free and combined amino acids are water-soluble organic compounds (WSOC). In 1996, Saxena and Hildemann (Saxena and Hildemann, 1996) reported a list of water-soluble atmospheric organic compounds; among them amino acids emerged as likely to contribute to the water soluble fraction. In 2005, Chan (Chan et al., 2005) reported that free and combined amino acids were about 10% of atmospheric water-soluble organic carbon (WSOC) in fine aerosol, and suggested that aerosols containing free amino acids may retain water at low RH, and serve as efficient cloud condensation nuclei.

Zhang in 2003 showed that the average concentrations of free amino compounds (amino acids and alkyl amines) were generally 4–5 times lower than those of combined amino compounds (proteins and peptides); in 2005, Jaenicke reported that cellular protein-containing particles were a major fraction of atmospheric aerosols (Zhang and Anastasio, 2003; Jaenicke, 2005).

The study of peptides and proteins in atmosphere is particularly complex, for their presence in low concentration and in composite mixtures. Moreover they can be modified by chemical and physical processes in the atmosphere (Milne and Zika, 1993; McGregor and Anastasio, 2001; Franze et al., 2005; Haan et al., 2009). Therefore some authors only studied the total protein contribution through bicinchoninic acid assay (Micro BCA Protein Assay Kit) for the colorimetric detection and quantitation of the total proteins (Kang et al., 2012; Chen and Hildemann, 2009); or using the Nano-Orange Protein Quantification Kit (Molecular Probes, Eugene, OR) (Menetrez et al., 2007b; Menetrez et al., 2009; Mandalakis et al., 2011) or through water-soluble organic nitrogen analysis (Zhang et al., 2002). A method of determining combined amino acids in bioaerosols is the hydrolysis of peptides and proteins, followed by analysis of total amino acids (Samy et al., 2011).

Table 1
Names and abbreviations of the studied amino acids and their respective internal standards.

Name	Abbreviation	Internal standard
Glycine	Gly	labeled Glycine ($^{15}N_{13}$CGly)
Alanine	Ala	deuterated Alanine ($^{2}H_{4}$Ala)
Valine	Val	deuterated Valine ($^{2}H_{8}$Val)
Leucine	Leu	deuterated Leucine ($^{2}H_{3}$Leu)
Isoleucine	Ile	deuterated Isoleucine ($^{2}H_{1}$Ile)
Methionine	Met	deuterated Proline ($^{2}H_{7}$Pro)
Proline	Pro	deuterated Proline ($^{2}H_{7}$Pro)
Phenylalanine	Phe	labeled Phenylalanine ($^{13}C_{6}$Phe)
Tryptophan	Trp	deuterated Tryptophan ($^{2}H_{3}$Trp)
Serine	Ser	deuterated Serine ($^{2}H_{3}$Ser)
Threonine	Thr	deuterated Threonine ($^{2}H_{2}$Thr)
Asparagine	Asn	deuterated Serine ($^{2}H_{3}$Ser)
Glutamine	Gln	deuterated Serine ($^{2}H_{3}$Ser)
Tyrosine	Tyr	labeled Tyrosine ($^{13}C_{6}$Tyr)
Cysteine	Cys	deuterated Citrulline ($^{2}H_{2}$Cit)
Lysine	Lys	deuterated Ornithine ($^{2}H_{2}$Orn)
Arginine	Arg	labeled Arginine ($^{2}H^{13}$CArg)
Histidine	His	deuterated Proline ($^{2}H_{7}$Pro)
Aspartic acid	Asp	deuterated Serine ($^{2}H_{3}$Ser)
Glutamic acid	Glu	deuterated Serine ($^{2}H_{3}$Ser)
Hydroxyproline	Hyp	deuterated Proline ($^{2}H_{7}$Pro)
Ornithine	Orn	deuterated Ornithine ($^{2}H_{2}$Orn)
Citrulline	Cit	deuterated Citrulline ($^{2}H_{2}$Cit)
γ-aminobutyric acid	GABA	labeled Glycine ($^{15}N_{13}$CGly)

Bioaerosol toxicity is also dependent on its particle size distribution. Since amino compounds adsorbed on particles can reach the systemic circulation, across the pulmonary alveoli, on the basis of particle size, their distribution into different size fractions of airborne particulate matter is an interesting approach to assess atmospheric allergenicity.

Although the particle size of pollen and fungal spores has been considered too large to penetrate the lower airways, it has been proved that small parts of them have been found adsorbed on small diesel exhaust particles (Busse et al., 1972; Suphioglu, 1998; Burge and Rogers, 2000; Penn et al., 2005; Shimada et al., 2006).

In 2009, Menetrez studied an urban site with a 15-stage particle impactor and the collected particulate was analyzed for mass and proteins (Menetrez et al., 2009).

In the present work, we optimized a method for the determination of free amino acids and proteins in particulate matter, improving a method previously published (Buiarelli et al., 2013). An atmospheric particulate material, collected in the Washington, DC area and passed through a 63 μm (230 mesh) sieve, Urban Dust NIST SRM 1649b, was used as a complex matrix for the optimization of the method.

Next, we characterized the amino acid and protein content in thirteen sub-fractions of urban particulate matter of 10 μm in aerodynamic diameter (PM_{10}) including the ultrafine particles ($PM_{0.1}$), in summer and winter.

As additional result, we obtained concentrations of free and combined amino acids in NIST SRM1649b.

2. Experimental

2.1. Investigated compounds

Twenty-four amino acid concentrations were determined using isotopically labeled analogues as internal standard, in the Standard