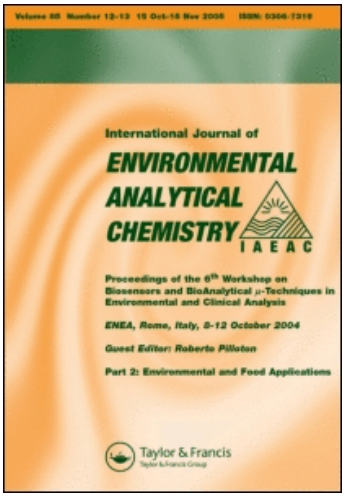

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Detection of free amino acids in proxies of marine aerosol by photoelectron resonance capture ionization aerosol mass spectrometry

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Photoelectron resonance capture ionization aerosol mass spectrometry (PERCI-AMS) has been applied to the analysis of proxies for marine aerosols with and without ozone; proxies used were mixed oleic acid–amino acid particles. The mechanism of ion formation for serine (104 m/z), glutamic acid (146 m/z), and phenylalanine (164 m/z) was dissociative electron attachment. This corresponds to loss of the hydrogen atom only, allowing for straightforward identification of the free amino acids. No ozonolysis products for the free amino acids were observed, even at high concentrations of ozone (500 ppm for 19 s). The direct detection of a novel gas-phase hydrated anion, $[\text{serine} + H_2O\text{–}H]^-$, is described. These preliminary results suggest that PERCI-AMS may provide an effective, simple and direct online method for the detection of organic nitrogen from free amino acids for future field studies of the marine troposphere.

Keywords: Marine aerosols; Amino acid detection; Mass spectrometry; Organic particles

1. Introduction

Marine aerosols are multicomponent admixtures containing both inorganic salts and organic compounds that are formed primarily by a bubble bursting mechanism [1, 2] at the air–water interface. The formation of these aerosols is a critical step in the mass transport of matter from the ocean to the troposphere and of central importance in several biogeochemical cycles, including the nitrogen cycle. Organic nitrogen (ON) makes a significant contribution to the total nitrogen in marine aerosol [3], as well as in rain [3–5] and fog water [6, 7]. While most inorganic nitrogen is found in coarse- and accumulation-mode particles [8, 9], the majority of the ON is in the fine mode [3] and is a quantitatively substantial component of nitrogen deposition. ON contributes to the water-soluble organic carbon content of fine-mode particles [10], and it has been suggested that ON compounds may act as cloud condensation nuclei [11].

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While there is a need for the speciation and quantification of ON in tropospheric particles, to date there are a limited number of studies of amino acids, especially free amino acids, in marine aerosols and marine rain. Free amino acids have been detected in marine rain [5, 12] and tropospheric aerosols in remote oceanic regions [13, 14] and from coastal waters [12, 15]. Recently, Kuznetsova *et al.* [15] showed that free and combined amino acids (i.e., peptides and proteins and other macromolecules) were enhanced in aerosols 1.2–20 times that of bulk sea water they sampled. Marine rain is rich in free amino acids, with Mace *et al.* [12] determining that in coastal marine rain at Cape Grim, Tasmania, Australia, free amino acids contributed ~53% of ON, but were less significant in the sampled marine aerosol. Matsumoto and Uematsu [13] recently reported the geographical distribution of two size segregated marine aerosols from the western North Pacific Ocean. They reported 1.5–30.8 pmol Nm^{-3} of total dissolved free amino acids in marine aerosols with higher concentrations in the northern Pacific compared with the subtropical and tropical ocean. Dissolved free amino acid concentrations in fine particles in that region correlated with anthropogenic particles, suggesting long-range transport from continental regions to the remote ocean [13].

Herein, we report preliminary results on the sampling and detection of free amino acids from synthetic mixed organic aerosols (i.e., free amino acids + oleic acid). These mixed particles represent the first stages of the development of proxies to the organic component of marine fine mode particles for direct, online mass spectral analysis. Photoelectron resonance capture ionization aerosol mass *s*pectrometry (PERCI-AMS) is employed as a soft ionization method with a demonstrated high sensitivity to oxygenated organics, particularly acids and diacids [16], which suggests applicability towards the analysis of free amino acids in aerosols. PERCI affords minimal fragmentation of the analyte, typically with only the loss of hydrogen [17, 18], which allows for straightforward identification of molecular compounds. This method has played an important role in elucidating the heterogeneous reactions of fine mode particles composed of fatty acids [18–20] and fatty acid derivatives [21] and complex oil-based media [22] with ozone. In particular, PERCI-AMS has played an important role in the elucidation of the heterogeneous processing of the monounsaturated fatty acid, oleic acid (18 : 1) [18–20]. As recently reviewed [23], this fatty acid is ubiquitous in the aerosols of both the continental and marine troposphere and is a logical choice for a component in mixed particles. We are now extending the use of PERCI-AMS to the analysis of mixed particles containing ON. As noted, there is a very limited number of studies of free amino acids in marine aerosols [12–15], and subsequently there is little known about the effects of oxidative and photochemical processing of this potentially important source of ON in mixed marine particles. In solution chemistry, the amine group is known to react with ozone [24–27], which is an important tropospheric oxidant; however, no studies of free amino acid containing proxies with mixed fine mode particles have been reported to date. Hence, we investigate the effects of the heterogeneous reaction of ozone with the oleic acid-free amino acid mixed particles.

Herein, we demonstrate that free amino acids are readily detected in mixed particles with minimal fragmentation by PERCI-AMS. We demonstrate the direct determination of simple solutions of single amino acids serine, phenylalanine, glutamic acid, and a mixture of the three with oleic acid. Future studies and potential applicability to field measurements form the conclusion of this work.

2. Experimental

2.1 *Reagents*

Oleic acid was purchased from Mallinckrodt Baker and used without further purification. Three species of amino acids were used without further purification in the two- and four-component particles: glutamic acid (Glu; 99%+, Sigma-Aldrich), serine (Ser; 99%, Sigma-Aldrich), and phenylalanine (Phe; 98%, Sigma-Aldrich). The aerosols were formed by pneumatically nebulizing ethanol/water solutions as described below using 100% ethanol (Pharmco, CT), and 18 MΩ water (Milli-Q, model gradient A10, TOC < 5 ppb).

2.2 *Principles of PERCI-AMS*

A complete schematic of the PERCI-AMS system is depicted in a prior work [22]. Briefly, it consists of three main components: (1) aerosol generation and sampling, (2) PERCI source, and (3) time-of-flight mass spectrometer. PERCI employs low-energy photoelectrons, with energies of about 0 eV [16–18] used for ionization in this study. The photoelectrons are generated by focusing a low-energy (sub-mJ) pulsed (10 Hz), tunable (235–300 nm) ultraviolet laser (Opotek, Carlsbad, CA) onto the surface of a pure aluminium photocathode, generating a short (5 ns) burst of photoelectrons. In this study, a wavelength of 270 nm was employed. The photoelectron energy (~0 eV) is nominally equal to the difference between the incident photon energy (i.e. for $\lambda = 270$ nm, $E_{\text{photon}} = 4.59$ eV) and the photocathode metal work function ($\phi = 4.08$–4.28 eV for aluminium) [17]. The measured quantum efficiency has been determined as 6×10^{-4} photoelectrons/incident photon [17] with flux densities as high as 10^{21} cm^{-2} s^{-1} [18], subsequently resulting in the high sensitivity of this photoionization method to organic analytes [16–18].

2.3 *Particle generation, vaporization, and ozone generation*

Solutions of amino acids with concentrations of approximately 150 ppm of each free amino acid and oleic acid at 890 ppm were prepared in a 15% water/ethanol mixture. We assayed two component systems (single amino acid + oleic acid) and a four-component system (Ser + Glu + Phe + oleic acid). These mixtures were aerosolized with a glass, concentric pneumatic nebulizer (J. E. Meinhard Associates, Santa Ana, CA). The solvated particles passed through a diffusion dryer to remove the residual water/ethanol solvent. The particles were then introduced into a custom-made flow reactor (2.54 cm i.d.) via a glass-tube injector (0.32 cm i.d.) centred within the flow reactor. The mixed particles were assayed with and without exposure to ozone. When needed, ozone was generated by passing USP medical air (Airgas Inc., Salem, NH) though a commercial ozone generator (Ozone Services, Yanco Industries, Ltd, Burton, BC, Canada). The ozone concentration, when employed, was ~500 ppm. The ozone exposure time of the particles is dictated by the position of the glass tube injector at a given flow rate of the particle and gas phases. The flow rate in the reactor for these experiments was held constant at 0.5 L min^{-1} with corresponding reaction times from 1 to 24 s.

Aerosols (geometric mean diameter = 82 nm; geometric standard deviation = 1.7) were introduced into the mass spectrometer through a differentially pumped inlet and focused into a beam using an aerodynamic lens [28, 29]. A 260-μm critical orifice at the entrance of the inlet keeps the sampling flow rate constant at 0.70 L min^{-1}, with further characterization reported elsewhere [30].

A vaporization probe is placed in close proximity to the photocathode and intercepts the particle beam. Details on the construction and temperature programming of the vaporization probes were given in a prior work [16]. For the studies of mixed particles of free amino acids with oleic acid, particles were deposited on the vaporization probe for 150 s at room temperature, and then this probe was ramped to 400°C in 10 s and maintained at that temperature for 50 s. Approximately 1 μg of aerosol was deposited on the vaporizer for each trial.

3. Results and discussion

Two modes of ionization have been described with low-energy photoelectron attachment to organic molecules, namely associative (or non-dissociative) [17, 31] and dissociative electron attachment [17, 32, 33] (AEA and DEA, respectively). In AEA, a low-energy photoelectron attaches to the molecule without any fragmentation of the analyte in the ionization process. In DEA, the loss of an atomic or molecular fragment is concomitant to ionization of the analyte. In our studies with fatty acids with ~0 eV photoelectrons [16, 18–20] we have noted that the DEA is the predominant mode of ionization, with a loss of hydrogen, and we have not observed the AEA mode with this class of organics. Being organic acids, the free amino acids were expected to show a similar ionization trend. DEA appears to be the major mode of ionization of the free amino acids assayed. Figure 1 shows the PERCI mass spectrum of Ser + oleic acid.

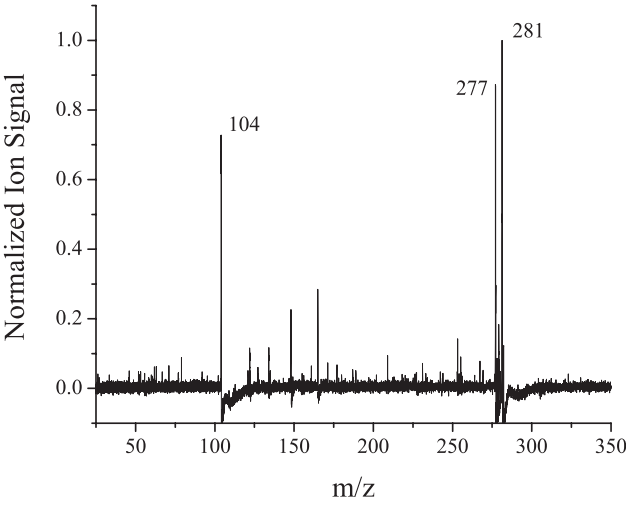

Figure 1. PERCI mass spectrum of aerosol particles generated from a solution of serine (1.30 mM) and oleic acid (1.58 mM) in 15% water/ethanol. The spectrum represents the average of 10 individual mass spectra.

The base peak at 281 m/z is the DEA ion of oleic acid [18, 19]. Also evident is a strong linolenic acid DEA ion signal at 277 m/z. Linolenic acid is an impurity in the oleic acid stock but presents no difficulty in the subsequent spectral interpretation. Also detected were palmitic and palmitoleic acid impurities, respectively, at 255 and 253 m/z. The strong ion signal at 104 m/z is the DEA ion of Ser. DEA ionization was observed with Glu and Phe in the two-component mixed particles with oleic acid, with ions at 146 and 164 m/z, respectively. Figure 2 shows the PERCI mass spectrum of the four component particles (Ser + Glu + Phe + oleic acid), which shows all four DEA ions at 104, 146, 164, and 281 m/z, respectively. The minimal fragmentation afforded by PERCI-AMS along with the apparent sensitivity towards carboxyl groups common to both fatty and amino acids is a motivation in adapting this method to the analysis of marine aerosol. Aerosols contain both classes of compounds, but great uncertainty presently exists regarding speciation and quantity of the amino acids.

Ozone is an important trace oxidant in the troposphere. Primary amines have been shown to undergo oxidation with ozone in solution [24]. Bailey proposed a general mechanism for the ozonation of amines that is initiated by an electrophilic attack on the amine by ozone. Subsequent competing fates for this amine-ozone adduct include: (1) the loss of an oxygen and the formation of an amine oxide product or intermediate; (2) intramolecular side-chain oxidation; and (3) dissociation into cation and anion radicals. In the studies of the ozonation of two primary amines, *n*-butylamine and isopropylamine, it was found that the amine oxide route was favoured [24]. There are very few studies of the effects of ozone on free amino acids and none, to our knowledge, on free amino acids in particles. Le Lacheur and Glaze [27] investigated the effects of ozone and hydroxyl radical reactions with aqueous phase serine. Under radical promoting conditions, they found that Ser ruptured on the carbon backbone. In the presence of hydroxyl radical scavengers, nitrate and nitrite along with carbonyl and carboxylic acid by-products were the major products [27]. Figure 3 is the PERCI mass

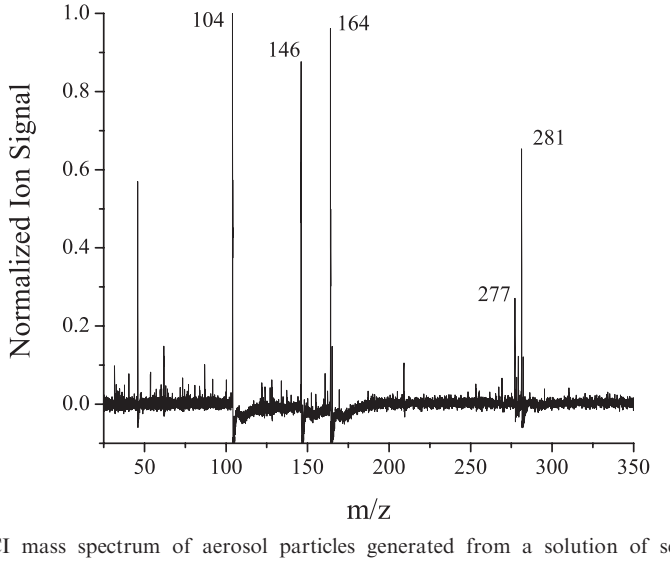

Figure 2. PERCI mass spectrum of aerosol particles generated from a solution of serine (1.30 mM), phenylalanine (0.73 mM), glutamic acid (0.99 mM), and oleic acid (3.16 mM) in 15% water/ethanol. The spectrum represents the average of 10 individual mass spectra.

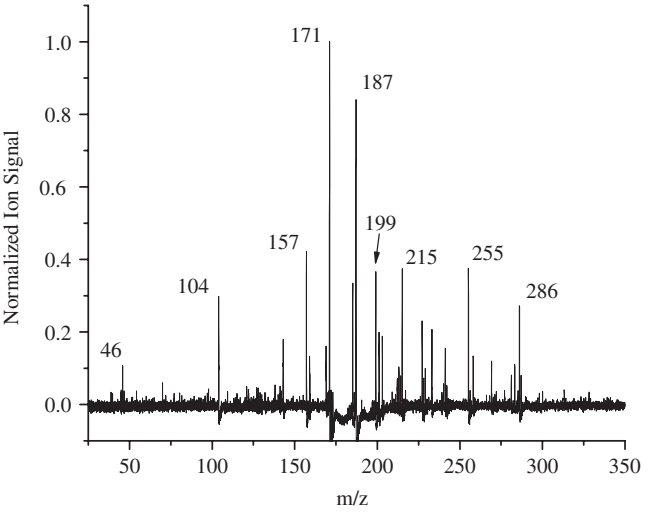

Figure 3. PERCI mass spectrum of mixed particles of serine and oleic acid after heterogeneous reaction with ozone (500 ppm ozone, 19 s reaction time). The spectrum represents the average of 10 individual mass spectra.

spectrum of Ser + oleic acid under very strong oxidizing conditions (500 ppm ozone, 19 s reaction). The ions were collected during the ramping of the vaporization probe (10 s) and while the temperature of the probe was maintained at its maximum (400°C) for an additional 50 s. The Ser DEA ion is still evident (104 m/z) along with DEA ions that our group and others have assigned in previous studies to ozonolysis products of oleic acid [23]. These ions include: nonanoic acid (157 m/z), 9-oxononanoic acid (171 m/z), and azelaic acid (187 m/z). The smaller signal at ~46 m/z is found in the PERCI mass spectra both with and without the presence of ozone and is most likely the AEA ion of residual ethanol, rather than the nitrite ion, which has an identical nominal mass-to-charge ratio. Other ions including those indicated on figure 3 (199, 215, 255, and 286 m/z) were present in the ozonized control (oleic acid) and most likely do not arise from the oxidation of serine. There is no evidence of amide formation between serine and oleic acid or any of the *in situ* generated acids, namely nonanoic and azelaic acids. It should be noted that our failure to observe ozonolysis products of free amino acids in no way contradicts the prior studies on primary amines [24] or free amino acids [27]. Bailey's ozonation experiments were conducted in chlorinated solvent, while the ozonolysis of Ser was performed in aqueous solutions [27] as opposed to the heterogeneous ozonolysis of oleic acid-free amino acid particles in our case. In future experiments, we will look at more realistic internally mixed particle proxies for marine aerosols that contain sodium chloride, water, fatty acids, and free amino acids to see if oxidation of the latter class of compounds occurs in the presence of ozone.

There has been recent interest in the detection and analysis of solvated amino acids [34–37]. Figure 4 shows a strong signal at 122 m/z for the Ser + oleic acid mixed particles for solutions with increasing concentration of water relative to ethanol. We assign this ion signal to the hydrated DEA molecular ion, $[Ser + H_2O–H]^-$. This hydrated amino acid may be formed in the gas phase, as evidenced by a persistent signal that grew for several minutes after the vaporization cycle. To the best of our knowledge,

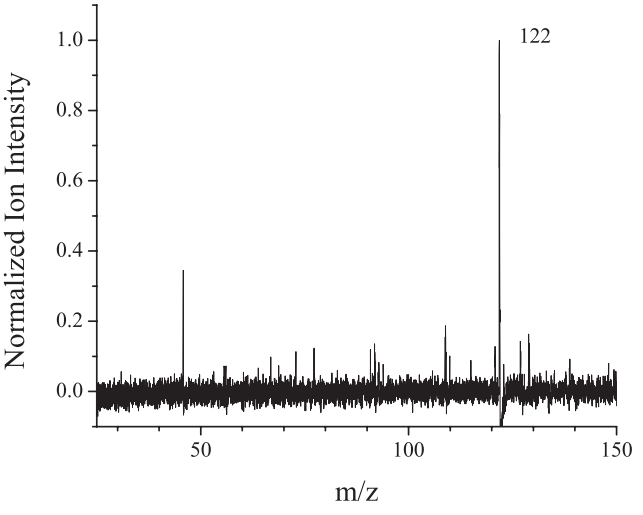

Figure 4. PERCI mass spectrum of mixed particles of serine and oleic acid, showing an ion signal at 122 m/z, which is proposed to be the hydrated molecular ion $[Ser + H_2O–H]^+$. The spectrum represents the average of 10 individual mass spectra.

$[Ser + H_2O–H]^-$ has not been reported, but there are reports of hydrated gas-phase amino acids including valine [35], tryptophan [34, 36, 37], tyrosine [34, 36], and Phe [36].

4. Conclusion and future direction

We have shown that PERCI-AMS has the potential for straightforward analysis of mixed particles of free amino and fatty acids. The only observed mode of ionization is DEA, with loss of hydrogen concomitant to ionization. This presents simplified mass spectra that are advantageous for the detection of free amino acids in real tropospheric marine aerosols. As a proof of principle, we showed the unambiguous formation of the DEA molecular ions of three amino acids in the presence of oleic acid without fragmentation. We are currently working towards establishing the limits of detection of free amino acids in mixed particles containing lipids to assess the applicability of PERCI-AMS to on-line, real-time field measurements of free amino acids in marine aerosols. We are also measuring the sensitivity to naturally occurring free amino acids with vapour-pressure-dependent desorption of deposited particles, and looking at the thermal vaporization cycles employed [16]. By understanding how thermal desorption affects sensitivity, we may be able to overcome isobaric interferences that may occur in actual mixed particles in the marine troposphere. This important analytical feature, along with the limits of detection, will be discussed in an upcoming work.

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