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Low energy photoelectron resonance capture ionization aerosol mass spectrometry of small peptides with cysteine residues: Cys-Gly, γ-Glu-Cys, and glutathione (γ-Glu-Cys-Gly)

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ABSTRACT

The photoelectron resonance capture ionization (PERCI) of cysteine (Cys) and small gas-phase neutral peptides that contain the Cys residue (Cys-Gly, γ-Glu-Cys, and glutathione (γ-Glu-Cys-Gly)) is reported. At an ionization energy less than 1 eV two types of dissociative electron attachment ionization were observed for Cys: hydrogen atom loss, resulting in formation of the ion $[Cys–H]^-$, and dissociation of the CH_2–SH bond, resulting in formation of the ion $[SH]^-$. The presence of these ions suggests that both the $\pi^*(–CO_2H)$ and $\sigma^*(C–S)$ orbitals can act as low energy electrophores on Cys. This ionization trend was observed for the dipeptides Cys-Gly and γ-Glu-Cys as well as glutathione, with evidence that dissociation of the CH_2–SH bond in these peptides can also result in ions of the form $[M–SH]^-$. Also measured were ions resulting from bond dissociation of the amide linkage as well as for the amide N–C_α bond. In both of these cases the charge is retained on the fragment containing the nitrogen of the amide bond, indicating that ion formation by the PERCI process is directed by the electron affinity (EA) of the fragments. The backbone fragmentation of the PERCI process appears distinct from other low energy processes, including electron detachment dissociation and electron capture dissociation, as evidenced by the lack of amide C_α–C cleavage; the dependence of ion formation on the EA of the fragments, not their •H affinity; and the observation that PERCI is not a directionally restricted mechanism.

1. Introduction

As of late, there has been renewed interest in the ionization of biological molecules by low energy electrons (~0–30 eV). This is largely motivated by the need for a more complete radiobiological description of the role of low energy ionizing radiation in inflicting cellular damage. Electron energies lower than approximately 15 eV facilitate ionization (via resonance electron capture, REC) of many classes of biological molecules [1]. Dissociative electron attachment (DEA) ionization is a mechanism of molecular fragmentation commonly observed in this energy range [2,3], with the transient negative ion (TNI, denoted $[AB^-]$) fragmenting into an anion and a neutral radical (reaction (1a)):

$$AB + e^-(< 15\,eV) \rightarrow [AB^-] \rightarrow A^- + B^\bullet \quad (1a)$$

$$AB + e^-(< 15\,eV) \rightarrow [AB^-] \rightarrow AB^* + e^- \quad (1b)$$

The lifetime of the anion, A^-, may be sufficiently long to facilitate measurement by mass spectrometry. This DEA ionization process competes with autodetachment of an electron by the TNI resulting in the formation of an excited intermediate, AB* (reaction (1b)). Other forms of ionization exist at very low energies, including associative electron attachment [4,5], in which no fragmentation of the molecule occurs in the ionization process.

For many biological molecules and less structurally complex organic proxies, the fragmentation associated with the DEA ionization process occurs near the site of electron capture. This is perhaps most succinctly demonstrated by the ionization of biological molecules that contain the carboxylic acid moiety, –COOH; for example, amino acids and polypeptides, fatty acids and other biologically derived organic acids. In this case, resonance electron capture arises from temporary occupation of a normally empty π* orbital. The formation of the carboxylate ion and atomic hydrogen at electron energies less than about 2 eV appears to be the prevalent ionization trend for both simple organic acids [6,7] and higher molecular weight fatty acids, such as 9-octadecenoic acid (i.e., oleic acid, an 18:1 fatty acid) [8–10].

Amino acids, peptides, and polypeptides represent more complex systems in terms of DEA ionization than structurally simpler organic acids. These molecules possess an empty π* orbital of the –COOH group(s), but can also contain side groups (–R) that may be involved in the REC process [11,12]. For example, certain amino

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acids, including tryptophan, phenylalanine, and cysteine (Cys), have side groups that can support low energy resonances [13]. These complex, low symmetry molecules may support mixing of anionic states (i.e., mixing of the TNI state associated with an electron bound on the –COOH and –R groups) [14]. Mixing of the states may alter the energy and lifetime of the TNI compared to values associated with temporary anionic states of independent or separated functional groups. A biological molecule with a long-lived TNI state may transfer an electron to a site that is distant from the point of attachment, inducing remote bond breaking. This type of ionization process may be the most radiologically significant for the strand breaking mechanisms of DNA [1,14–16].

In the following report, photoelectron resonance capture ionization (PERCI) utilizing very low ionization energies (~0–1 eV) is employed in conjunction with aerosol mass spectrometry (AMS) to investigate the ionization properties of cysteine, two dipeptides that contain the Cys residue (Cys-Gly and γ-Glu-Cys) and the biologically relevant tripeptide, γ-Glu-Cys-Gly (glutathione, GSH). Mechanisms of ion formation and fragmentation are suggested and ion yield profiles are presented. Finally, the ionization of Cys and Cys-derived polypeptides by nominally 0.5 eV photoelectrons is compared to other low energy, electron-based ionization processes (i.e., electron capture and electron detachment dissociation).

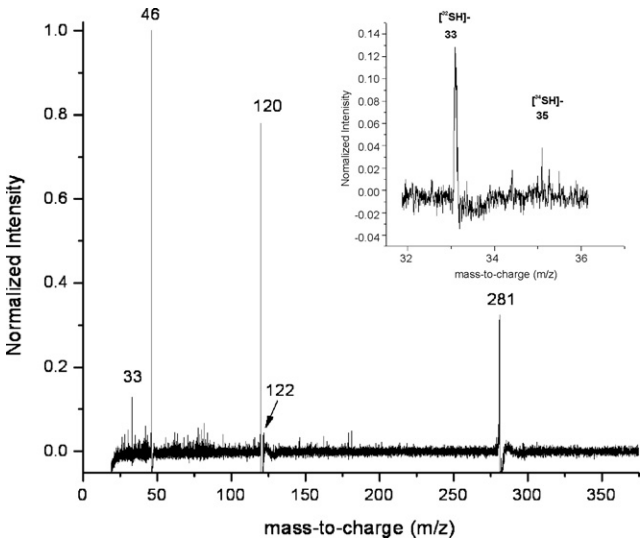


Fig. 1. PERCI mass spectrum of Cys + OL ionization by 0.47 eV photoelectrons. Mass spectrum is an average of 200 laser shots to aerosol particles that were flash vaporized at 400 °C. The concentration of OL and Cys in the aerosol particles, in mole fraction (χ) were: $\chi_{OL} = 0.63$ and $\chi_{Cys} = 0.37$.

2. Experimental

2.1. Photoelectron resonance capture ionization aerosol mass spectrometry (PERCI AMS)

Detailed descriptions of PERCI AMS have been presented in earlier reports [5,9,10], so only key features and modifications relevant to the following experiments will be described. Polydisperse aerosols were generated using a glass, concentric pneumatic nebulizer (J.E. Meinhard Associates, Santa Ana, CA) and the solvent was removed by passing the aerosol through a ~1 m long columnar diffusion dryer packed with silica and activated charcoal.

Particles were sampled through a 260 μm critical orifice with a flow rate of 0.5 L min^{-1} and were introduced into the PERCI source of the mass spectrometer through a differentially pumped particle inlet and focused into a beam using an aerodynamic lens [17]. The focused particle beam was targeted onto a coiled Nichrome filament that was heated resistively and maintained at a constant temperature of 400 °C. The vaporized organics are ionized by photoelectrons 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 aluminum photocathode that is in close vicinity to the vaporization filament. The photoelectron energy (which for these experiments is nominally 0–1 eV) is the difference in energy of the incident photon and the photoelectric work function of the metal (φ_{Al} = 4.3 eV) [18].

Anion mass spectra were recorded with a TOF mass spectrometer (R.M. Jordan Inc., Grass Valley, CA) operating in reflectron mode. Data was acquired at 1 GS/s on a digital oscilloscope (WavePro 7000, LeCroy, Chestnut Ridge, NY). The working mass range of the instrument at this sampling rate was 0–500 m/z. The measured mass spectral resolving power ($m/\Delta m$) was 510 at 187 m/z and 310 at 281 m/z.

2.2. Reagents

GSH (L-glutathione reduced, ≥99%, Sigma–Aldrich), Cys (≥97%, Sigma–Aldrich), Cys-Gly (≥85% by TLC, Sigma–Aldrich), and γ-Glu-Cys (≥80% by HPLC, Sigma–Aldrich) were all used without additional purification. All the results reported herein are for internally mixed aerosol particles, with the main component of the particles (i.e., "particle matrix") being oleic acid (~99% by GC, Sigma–Aldrich), which was used without further purification. Solutions were prepared in ethanol or methanol (≥99.5%, Sigma–Aldrich) and deionized water (18 MΩ water, Milli-Q, Model Gradient A10, TOC <5 ppb), typically at 3:1 by volume, respectively for amino acid solutions and 70%:30% by volume for oleic acid solutions. USP medical air (UN1002, Airgas East, Williston, VT, USA) was used as the carrier gas.

3. Results

3.1. Cysteine + oleic acid

The PERCI mass spectrum for mixed particles composed of Cys and oleic acid (OL) (Fig. 1) recorded at an intermediate value of the photoelectron energy (nominally 0.5 eV) employed for ionization in these studies shows an ion signal at 281 m/z due to the DEA ion of OL [8,9], and this assignment is consistent for all analytes. There is no evidence of any alternate modes of ion formation by OL from 0–1 eV. Conversely, Cys shows evidence of two modes of ionization. The 120 m/z ion signal arises from DEA ionization, most likely from loss of hydrogen from the carboxyl group, which has an unoccupied π^* orbital and subsequently acts as the site of REC:

$$SHCH_2CH(NH_2)CO_2H + e^- \rightarrow SHCH_2CH(NH_2)COO^- + H^\bullet \quad (2)$$

There is also evidence of another DEA ion formation channel of Cys in this energy range, as indicated by the 33 m/z ion signal (Fig. 1 inset), likely arising from the stable anion, SH^-:

$$SHCH_2CH(NH_2)CO_2H + e^- \rightarrow {}^\bullet CH_2CH(NH_2)CO_2H + SH^- \quad (3)$$

Ionization most likely proceeds via photoelectron attachment to the σ^*(C–S) orbital. It should be noted also that there is no evidence of the carbanion being formed (i.e., $[CH_2CH(NH_2)CO_2H]^-$, 88 m/z). Interestingly, the energy range of the photoelectrons used in this study is below the vertical attachment energy (VAE, i.e., the energy to attach an electron to the LUMO of a molecule in the gas phase when the neutral molecule is in its equilibrium geometry) of the σ^*(C–S) orbital of Cys. The VAE distributions of Cys [13] and CH_3SH [19] have been measured by electron transmission spectroscopy to be very weak and broad with maxima at 3.59 and 2.85 eV respectively. Most likely the weak ion signal for $[SH]^-$ in the PERCI

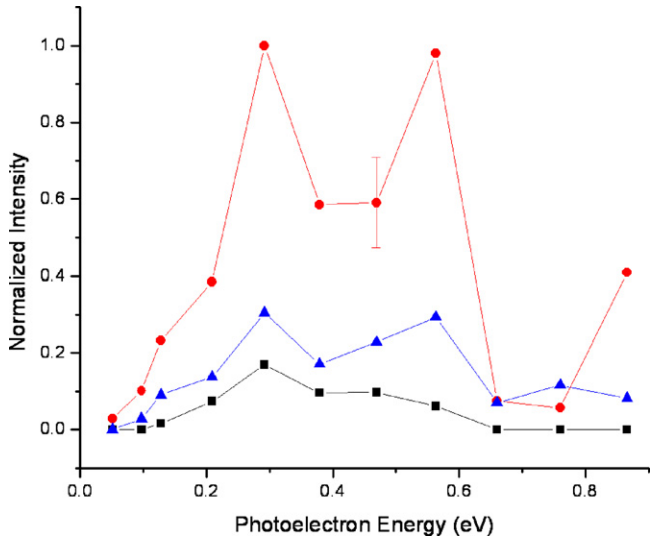


Fig. 2. Ion-yield curves of major ions of Cys + OL. These are averaged ion intensities for the main ions for Cys + OL particles: (■) denotes $[SH]^-$ (33 m/z); (▲) denotes $[OL–H]^-$ (281 m/z); (●) denotes $[Cys–H]^-$ (120 m/z). The aerosol sampling and ionization conditions are identical to conditions noted in Fig. 1. The concentration of OL and Cys in the aerosol particles, in mole fraction (χ) were: $\chi_{OL} = 0.63$ and $\chi_{Cys\text{-}Gly} = 0.37$. Ion yields were renormalized for small differences in laser energy across the wavelengths employed.

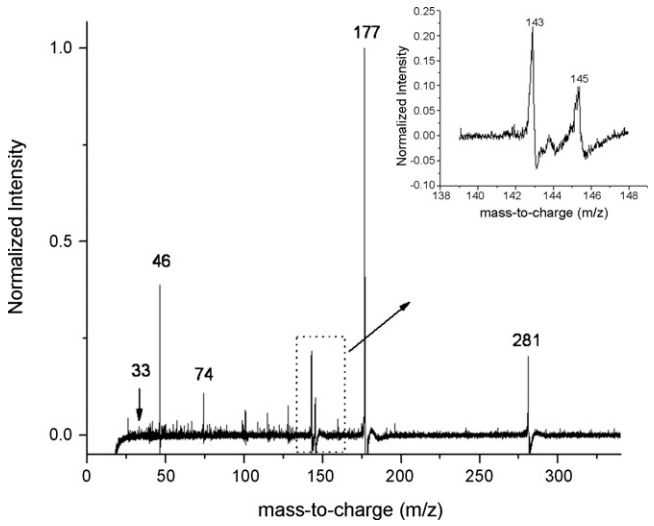
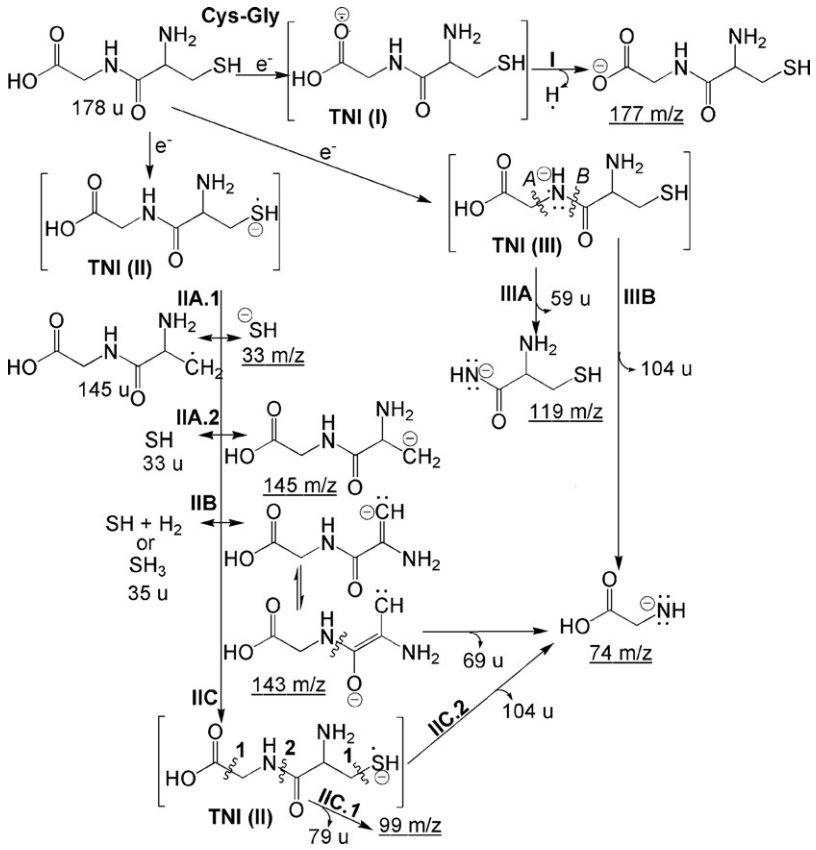


Fig. 3. PERCI mass spectrum of Cys-Gly + OL. Ionization by 0.47 eV photoelectrons. Mass spectrum is an average of 200 laser shots to aerosol particles that were flash vaporized at 400 °C. The concentration of OL and Cys-Gly in the aerosol particles, in mole fraction (χ) were: $\chi_{OL} = 0.69$ and $\chi_{Cys\text{-}Gly} = 0.31$.

mass spectrum arises from a low photoelectron capture cross-section in our energy range which is on the wing of the resonances reported.

Ion-yield curves (Fig. 2) for the three ions show the strongest PERCI ion signal for $[SH]^-$ at ~0.3 eV. This is about 0.5 eV lower than the calculated DEA ionization energy of 0.8 eV for reaction (3). The nominal DEA ionization energy is taken as the difference in the C–S bond energy (~3.12 eV in Cys [20]) and the electron affinity of SH (between 2.2 and 2.3 eV, see Ortiz [21] and references therein). Generally, the ion-yield curves for the DEA ions formed by loss of hydrogen from the carboxyl groups, i.e., $[OL–H]^-$ and $[Cys–H]^-$, have bimodal structure, with $[Cys–H]^-$ having stronger ion intensity at most ionization energies compared to $[OL–H]^-$, despite the higher concentration of OL in the particles (i.e., the respective



Scheme 1. Transient negative ions (TNI) and fragmentation of Cys-Gly. Ion masses underlined were measured in the PERCI mass spectra.

mole fractions, χ, were: $\chi_{OL} = 0.63$ and $\chi_{Cys} = 0.37$). This apparent enhancement of H–loss DEA ionization for Cys may arise from mixing of multiple anionic states (i.e., π^* and σ^*(C–S)), increasing the lifetime of the TNI (i.e., decreasing the rate of autodetachment and increasing the cross-section) compared to OL that has only one temporary anionic state (i.e., π^*).

3.2. Cys-Gly + oleic acid

Cys-Gly (L-cysteinyl-L-glycine) and γ-Glu-Cys (γ-L-glutamyl-L-cysteine) represent ideal probes for elucidating the low energy ionization mechanisms of small peptides that have more than one functional group, which may undergo REC and facilitate mixing of multiple anionic states. Both of these dipeptides as well as GSH contain a Cys residue allowing for attachment of very low energy photoelectrons to the unoccupied σ^*(C–S) orbital as well as to the unoccupied π^* orbital of the carboxyl group(s). γ-Glu-Cys represents a somewhat more complex electrophore than Cys-Gly, bearing the Cys functionality along with two carboxyl groups, as well as the unusual γ-peptide linkage between the amine group of Cys with the carboxyl moiety of the glutamate R-group.

The PERCI mass spectrum of Cys-Gly + OL (Fig. 3) shows no evidence of reaction between OL and Cys-Gly. As shown in Scheme 1 (Cys-Gly-Channel I), the base peak at 177 m/z corresponds to the ion $[Cys\text{-}Gly\text{-}H]^-$, most likely formed from dissociation of the hydrogen atom of the carboxyl group of the glycine residue, which has an unoccupied π^* orbital and subsequently acts as the site of REC. The weak ion signal at 33 m/z is evidence of another channel of DEA ionization, namely the dissociation of $[SH]^-$. Cys-Gly-Channel IIA.1 (Scheme 1) shows formation of the thiol ion, $[SH]^-$, and the corresponding neutral radical (145 u).

The inset of Fig. 3 highlights one of the most revealing features of the Cys-Gly + OL PERCI mass spectrum, with the doublet at 143 and 145 m/z. As shown in Scheme 1, Cys-Gly-Channel IIA.2 is a competitive ionization branch to Channel IIA.1 and suggests the route to formation of the highest m/z of this doublet, the 145 m/z ion. In Cys-Gly-Channel IIA.2, bond dissociation of Cys-Gly TNI (II) forms the ion, $[Cys\text{-}Gly\text{–}SH]^-$ (145 m/z), and the thiol group leaves as the thiyl radical neutral fragment, $^\bullet SH$. The 143 m/z ion signal most likely derives from fragmentation of Cys-Gly TNI (II). The formation of this ion suggests the loss of a 35 u neutral fragment, which is proposed to be either $^\bullet SH_3$ or $^\bullet SH + H_2$, as indicated by Cys-Gly-Channel IIB. As depicted in Scheme 1, the relatively high intensity of the 143 m/z ion signal may arise from resonance stabilization. Fragmentation of the 143 m/z ion at the amide linkage may be a route to formation of the observed moderate-intensity 74 m/z ion, along with a 69 u neutral fragment. Additionally, there are other routes arising from Cys-Gly TNI (II) that may lead to formation of the 74 m/z ion (see Scheme 1); for example, fragmentation about the amide linkage of Cys-Gly (Cys-Gly-Channel IIIB), which would also lead to a 104 u neutral radical fragment. There is also evidence of fragmentation of the N–C_α bond (Cys-Gly-Channel IIIA), which leads to a very weak 119 m/z ion and a 59 u neutral radical. As shall be explained, this common motif of fragmentation about the amide bond suggests an intermediate in which the negative charge of the TNI is localized about the amide linkage (i.e., Cys-Gly TNI (III)). In Cys-Gly-Channel IIC.2 the thiol radical anion makes a nucleophilic attack on C-4 (carbonyl group) giving the 74 m/z anion and a 104 u neutral fragment radical. The 99 m/z product ion is observed in the PERCI mass spectrum, suggesting loss of the 79 u neutral, SH_2CO_2H. This mechanism is similar to that of the highly reactive thiyl radical (i.e., $RS^\bullet$), and is well-documented in peptides and proteins with the Cys residue(s) (see the review by Stubbe and van der Donk [22], and references therein), including GSH. It is important to note however that the present work contrasts with the former in terms of gas-phase ions, *sans* solvent, and rearrangements that occur via a radical thiol *anion* (*cf.* the thiyl radical). Further support for this and other mechanisms will be given in Sections 3.3 and 3.4 on the PERCI ionization of γ-Glu-Cys and GSH.

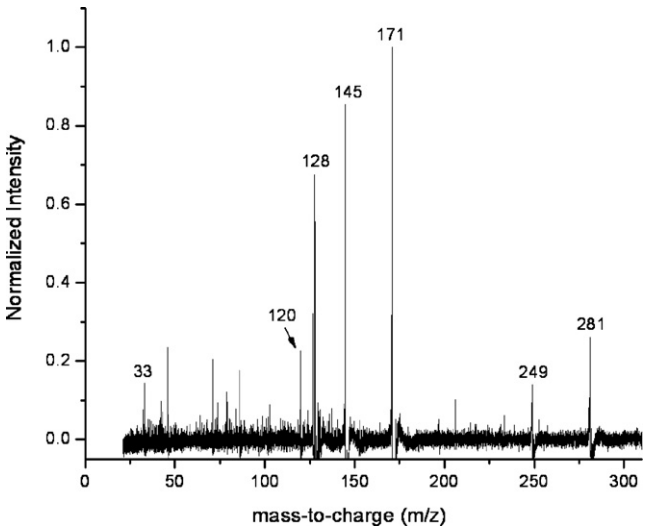


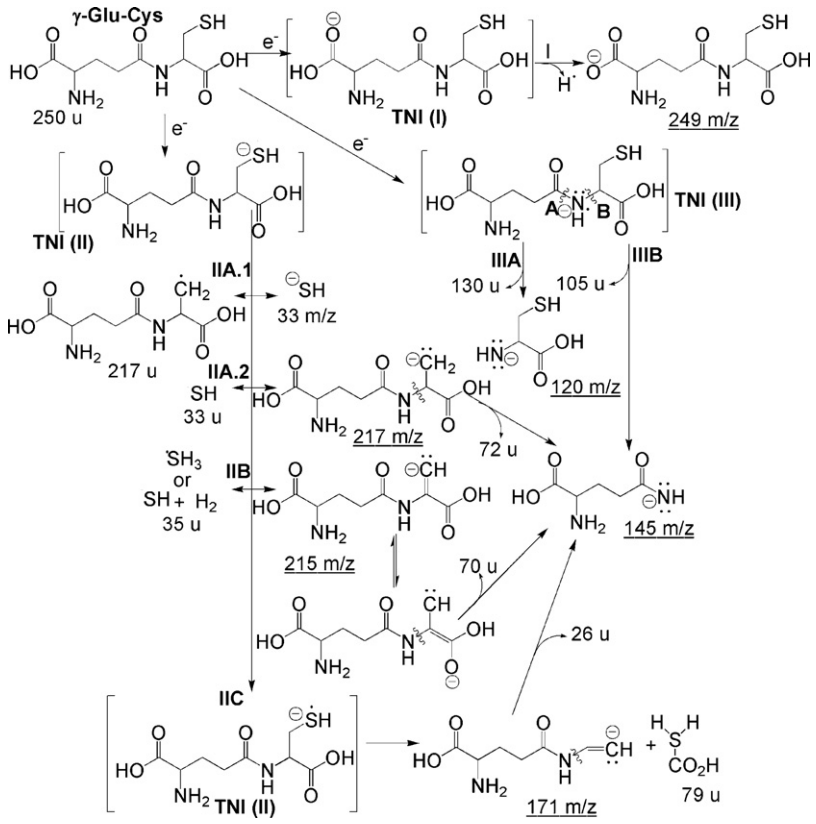
Fig. 4. PERCI mass spectrum of γ-Glu-Cys + OL. Ionization by 0.47 eV photoelectrons. Mass spectrum is an average of 200 laser shots to aerosol particles that were flash vaporized at 400 °C. The concentration of OL and γ-Glu-Cys in the aerosol particles, in mole fraction (χ) were: $\chi_{OL} = 0.81$ and $\chi_{\gamma\text{-Glu-Cys}} = 0.22$.

3.3. γ-Glu-Cys + oleic acid

The 249 m/z ion in the PERCI mass spectrum of γ-Glu-Cys + OL (Fig. 4) is assigned to $[\gamma\text{-Glu-Cys–H}]^-$. Furthermore, the observed mass spectrum is consistent with the formation of a TNI with the charge localized about the thiol group (i.e., γ-Glu-Cys TNI (II), Scheme 2). γ-Glu-Cys-Channel IIA.1 shows the formation of the $[SH]^-$ ion, which is clearly evident in Fig. 4. The two most pronounced ion signals at 145 and 171 m/z may be rationalized by fragment formation through γ-Glu-Cys TNI (II). Also, as in the case of Cys-Gly, there is support for bond dissociation of the amide linkage and the amide N–C_α bond. Fragmentation of the amide N–C_α bond (γ-Glu-Cys-Channel IIIB) may lead to the observed 145 m/z ion along with a 105 u neutral radical. Dissociation of the amide bond is consistent with the formation of the observed 120 m/z ion and suggests the formation of a 130 u neutral radical (γ-Glu-Cys-Channel IIIA).

3.4. Ionization of glutathione (GSH) + OL

The 306 m/z ion in the PERCI mass spectrum of GSH + OL (Fig. 5), consistent with the ionization trends of the dipeptides Cys-Gly and γ-Glu-Cys, arises from loss of the hydrogen atom from either of the two carboxyl groups (for brevity these two possible TNIs are collectively referred to as TNI (I) in Scheme 3). The ion-yield curves, normalized to laser energy, for some of the major ions in the GSH + OL system (Fig. 6) show a bimodal ionization trend for the 306 and 281 m/z ions, both arising from the dissociation of the hydrogen atom from the carboxylic acid moiety. The $[SH]^-$ ion signal (33 m/z) at this ionization energy (0.47 eV) is very weak for GSH (Fig. 5); however, the other channels associated with GSH TNI (II) are evident. GSH Channels IIA.2 and IIB show formation of the observed 274 and 272 m/z ions, respectively, consistent with those observed in the Cys-Gly and γ-Glu-Cys systems. Similarly, the strong ion signal at 171 m/z most likely arises from a channel analogous to that shown in Scheme 2 for γ-Glu-Cys, TNI (II), i.e., due to similar reactivities.



Scheme 2. Transient negative ions (TNI) and fragmentation of γ-Glu-Cys. Ion masses underlined were measured in the PERCI mass spectra.

GSH has two amide linkages, with TNI (III) and TNI (IV) in Scheme 3 used to denote TNIs with charge localized about the amide linkage of the Glu and Cys residues (the "Glu-side" amide bond) and the Cys and Gly residues (the "Gly-side" amide bond), respectively. Based on the ionization trends for the dipeptides, for TNI (III) the 177 *m*/*z* ion most likely arises from dissociation of the Glu-side amide bond (GSH Channel IIIA). The 145 *m*/*z* ion may arise from dissociation of the N–C_α bond of the Glu-side amide (GSH Channel IIIB). Channel (IV) shows the dissociation of the Gly-side amide linkage and the N–C_α bond respectively leading to the observed 74 and 248 *m*/*z* ions. It should be noted that the mechanisms presented in Schemes 1–3 do not imply the exclusion of TNI (III) arising from the transfer of an electron from TNI (II) to the amide group. Future studies will investigate the PERCI ionization trends of structural analogues to these Cys-derived peptides in which the thiol group is displaced from the amide linkage.

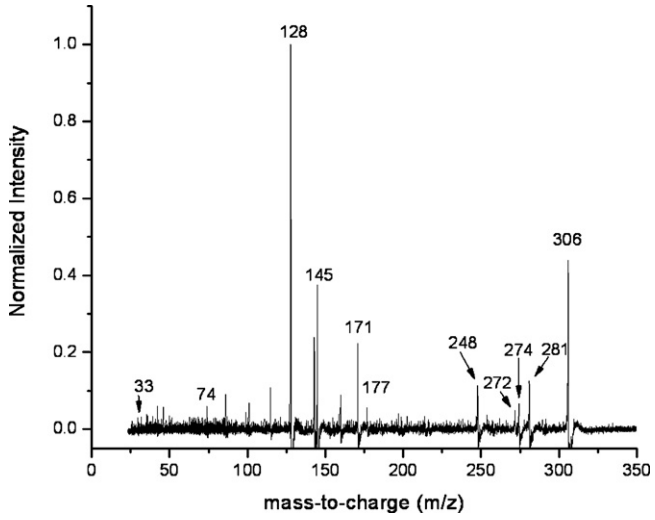
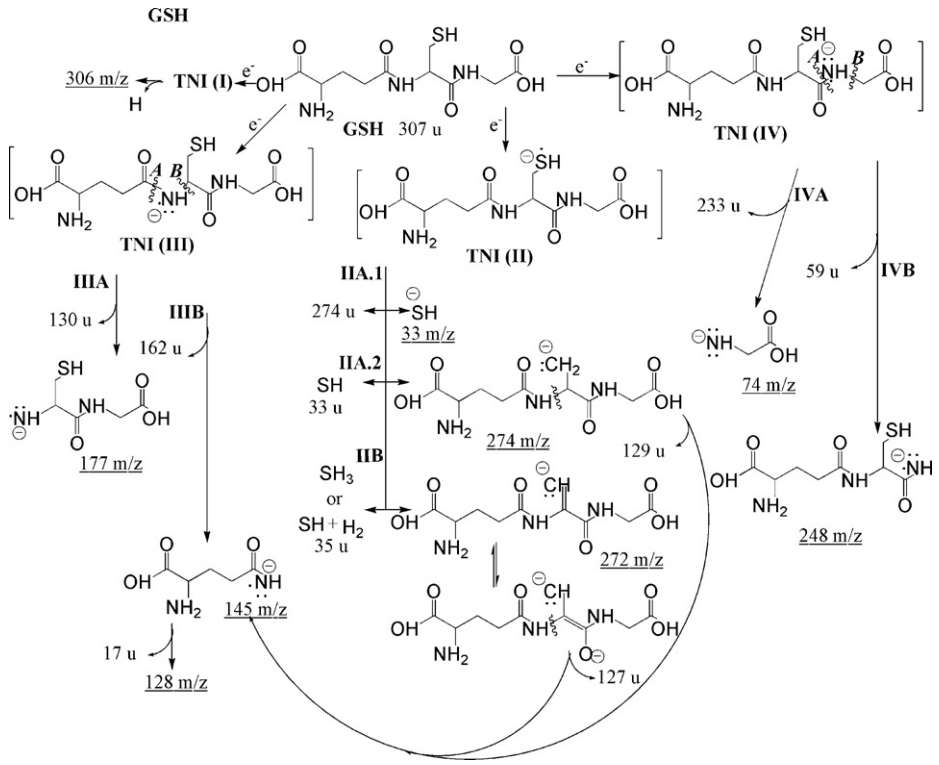


Fig. 5. PERCI mass spectrum of glutathione + OL. Ionization by 0.47 eV photoelectrons. Mass spectrum is an average of 200 laser shots to aerosol particles that were flash vaporized at 400 °C. The concentration of OL and GSH in the aerosol particles, in mole fraction (χ) were: χ_{OL} = 0.80 and $\chi_{\gamma\text{-Glu-Cys}}$ = 0.20.

The base peak at this photoelectron energy is 128 *m*/*z* and this is so in all cases except at the near 0 eV limit of ionization energy. This ion signal, like the 145 *m*/*z* ion, is strong for GSH and γ-Glu-Cys and apparent, albeit weaker, in the Cys-Gly PERCI mass spectrum. This tentatively suggests that the 128 *m*/*z* ion is derived from the 148 *m*/*z* ion or has the same TNI parent as the 145 *m*/*z* ion. The ion-yield curve (Fig. 6) supports this conclusion with the same pattern of ion intensity evinced for the 145 and 128 *m*/*z* ions. The even mass-to-charge ratio of this 128 *m*/*z* ion suggests that it has an odd number of nitrogen atoms. The absence of a 130 *m*/*z* ion (Fig. 5) suggests that there is no sulfur in this ion, which suggests that this ion may be formed by loss of ammonia (NH_3, 17 u) from the 145 *m*/*z* ion, resulting in the anion $[C_5H_6O_3N]^-$ (Scheme 3). A similar pathway may exist for the formation of this ion from the 145 *m*/*z* ions for γ-Glu-Cys and Cys-Gly.

3.5. Comparison to other low energy ionization processes: EDD and ECD

In this report we present the first application of PERCI AMS to peptides containing the Cys residue. Several motifs of fragmentation were observed, including: (1) loss of a hydrogen atom from the carboxyl group, forming the $[M–H]^-$ ion; (2) fragments that likely arise from a TNI with an electron attached to the thiol of the Cys residue; (3) fragmentation at the amide linkage, with the amide nitrogen-containing fragment retaining the negative charge;



Scheme 3. Transient negative ions (TNI) and fragmentation of glutathione (GSH). Ion masses underlined were measured in the PERCI mass spectra.

(4) fragmentation of the amide N–C_α bond, again with the amide nitrogen-containing fragment retaining the negative charge. Following, the ion-forming pathways observed in the PERCI mass spectrum are compared to other extant methods of ion formation employed in the MS of peptides and polypeptides, with emphasis on resonance processes.

In electron detachment dissociation (EDD) [23–27] the fragmentation of deprotonated peptides predominantly leads to cleavage of the peptide about the C_α–C backbone giving $a^\bullet$ and x fragment ions, along with lower intensity c and y ions [28]. The 0–1 eV ionization afforded by PERCI bears some similarities as well as some notable differences to EDD, specifically in regards to fragmentation about the amide linkage. The fragmentation of the amide linkage induced by EDD shows strong evidence of being a directionally restricted mechanism (i.e., unidirectional fragmentation occurs). In EDD there is strong evidence that the C_α–C bond rupture propagates from the radical site toward the N-terminus [27] (i.e., in EDD α-cleavage leads to the radical on the α-carbon, giving the radical $a^\bullet$ ion). In PERCI, the ionization trends of Cys-Gly, γ-Glu-Cys and GSH show strong evidence of fragmentation about the amide and amide N–C_α linkages, with the charge being retained by the nitrogen of the amide. The first case corresponds to the observed ion being the C-terminus fragment (y ion) and the latter being the N-terminus fragment (c ion), i.e., in the bond dissociation process the charge is retained on the nitrogen of the amide, but not at a specific terminus. Note, the PERCI mass spectrum of these small peptides shows no evidence of amide C–C_α bond dissociation, which is a predominant fragmentation mode observed in EDD [23,27]. These key differences between EDD and PERCI in terms of the backbone fragmentation in peptides probably arise from the higher ionization energy of the former method: EDD typically involves irradiation of peptides and polypeptides with electron energy greater than ca. 10 eV, while in this study the photoelectrons used where ca. 0–1 eV in energy. Secondly, EDD methodology is typically applied to gas-phase polyanionic polypeptides, not peptides without (initially) any net charge as is the case in the PERCI ionization. PERCI is a one-electron ionization process starting with a gas-phase neutral peptide: thus for a given bond dissociation, to a first approximation, the resulting fragment with higher electron affinity (EA) will retain the negative charge and the other fragment will remain a neutral. For fragmentation between the amide and the α-C, the EA of the amidyl radical fragment (i.e., $RC(O)N^\bullet$) for these small peptides is likely greater than 2.5 eV (for example, the recently reported benzylamidyl radical has an EA = 2.70 ± 0.17 eV [29]). This is presumably

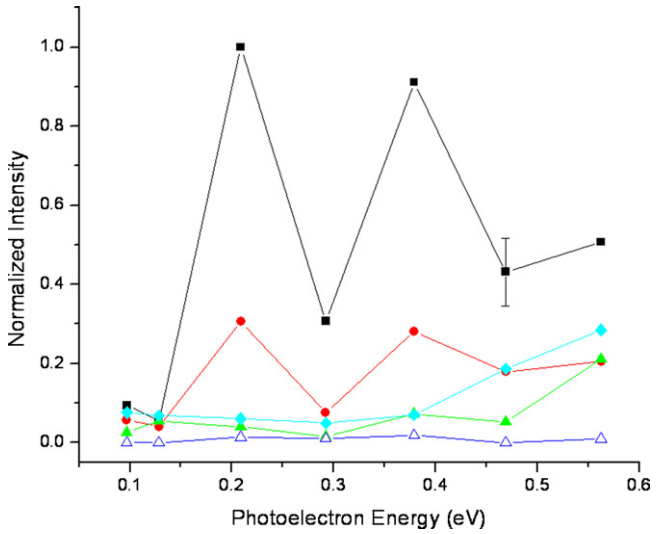


Fig. 6. Ion-yield curves for some of the ions of GSH + OL. These are the averaged ion intensities for GSH + OL particles: (Δ) denotes $[SH]^-$ (33 m/z); (▲) denotes $[OL–H]^-$ (281 m/z); (♦) denotes $[GSH–H]^-$ (306 m/z); (■) and (●) respectively denote the 128 and 145 m/z ions, please refer to Scheme 3 for proposed structures. The aerosol sampling and ionization conditions are identical to conditions noted in Fig. 5. The concentration of OL and GSH in the aerosol particles, in mole fraction (χ) were: $\chi_{OL} = 0.80$ and $\chi_{\gamma\text{-Glu-Cys}} = 0.20$. Ion yields were renormalized for small differences in laser energy across the wavelengths employed.

a much greater EA than that of the complimentary fragment, which likely has an EA comparable to a methylene fragment ($RCH^{\bullet}$, e.g.), due to the lower electronegativity of C compared to N. For the case of fragmentation of the amide bond itself, similar arguments can be made for the observation of the ion corresponding to the ionization of the aminyl radical (i.e., $RNH^{\bullet}$ to RNH^{-}) vs. the radical (i.e., $R\dot{C}(O)$), which remains a neutral.

Recent evidence suggests that DEA ionization at higher energies (i.e., >4 eV) than used in this work can excite core resonances [30], which results in the reversal of ionization trends exhibited at lower energies. Notably, DEA ionization of organic acids (RCOOH) at energies between ca. 0 and 1.5 eV results predominately in the formation of the $RCOO^{-}$ ion (with the hydrogen atom as a neutral fragment) [6,7,9]; whereas energies >4 eV may result in the formation of H^{-} as the predominant ion, as illustrated in the DEA ionization of gas-phase acetic and formic acids [30]. The sharp contrast in these ion-forming processes with relatively simple analytes illustrates how resonance processes at different excitation energies can result in the reversal of ionization trends.

Electron capture dissociation (ECD) [31–36] is a low energy (ca. $\leq$2 eV) ionization technique that employs electrons generated by thermionic emission. ECD typically involves the fragmentation of multiply charged polypeptides in the gas phase, typically resulting in backbone dissociation and the formation of c and $z^{\bullet}$ ions (major) and minor amounts of $a^{\bullet}$ and y ions. The ionization process involves the release of an energetic hydrogen atom, which is subsequently collisionally cooled until intramolecularly captured leading to non-ergodic dissociation [31–33]. In regards to backbone dissociation, the key difference in the formation of the major ions observed in ECD and PERCI is that the former case is dependent on the $H^{\bullet}$ affinity of the functional group that captures this atom:

$$RC(O)NHCHR' + {}^{\bullet}H \leftrightarrow RC^{\bullet}(OH)NHCHR' \rightarrow RC(OH){=}NH\,(c) + {}^{\bullet}CHR'(z^{\bullet})$$

Conversely in PERCI, the ionized products correspond to the fragments with greater electron affinity:

$$RC(O)NHCHR' + e^{-} \rightarrow\rightarrow RC(O)NH^{-} + {}^{\bullet}CHR' \quad \text{(neutral)}$$

or

$$RC(O)NHCHR' + e^{-} \rightarrow\rightarrow RC^{\bullet}(O) \quad \text{(neutral)} + {}^{-}NHCHR'$$

It should be noted that in ECD the electron shows evidence of being captured initially by a diffuse Rydberg-like orbital [35] of a charged group forming a hypervalent radical (i.e., $-CH_2NH_3^{+} + e^{-} \rightarrow -CH_2NH_3^{\bullet}$) that acts as the source of the ejected hydrogen atom. Hence in ECD the state of charge of the polypeptide and probably solvation is central to the ionization process. This is probably not the case with PERCI, where the observed ions are best rationalized by the EA of the radical fragments associated with a given bond dissociation. In PERCI, the fragment with the greater EA forms the anion while the other fragment remains neutral.

4. Conclusion

Low energy photoelectron resonance capture ionization aerosol mass spectrometry (PERCI AMS) of small cysteine containing peptides has shown two sites of low energy electron capture: the $\pi^{*}(-CO_2H)$ and the $\sigma^{*}(C–S)$ orbital. In the dipeptides, Cys-Gly and γ-Glu-Cys, as well as the tripeptide GSH, there was a consistent observation of negative ions that could be rationalized by bond dissociation of the amide linkage as well as the amide $N–C_{\alpha}$ bond, with the ion/neutral formation correlating well with the EA of these fragments. Whether this fragmentation is from photoelectron attachment directly to the vicinity of the amide bond or charge transfer from the $\pi^{*}(-CO_2H)$ and/or the $\sigma^{*}(C–S)$ orbital is not known and will be the subject of future studies. Other future studies will place emphasis on comparing the results of this study of Cys-containing peptides with the ionization of peptides consisting of amino acid residues that have side groups that have not been observed to undergo DEA ionization [13] at very low energies. These studies, along with a wider range of analytes are expected to aid elucidation of low energy electron mediated biological damage mechanisms.

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