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SHORT COMMUNICATION

S-to-αC Radical Migration in the Radical Cations of Gly-Cys and Cys-Gly

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Abstract

The radical cations of Cys-Gly and Gly-Cys were studied using ion-molecule reactions (IMR), infrared multiple-photon dissociation (IRMPD) spectroscopy, and density functional theory (DFT) calculations. Homolytic cleavage of the S–NO bond of nitrosylated precursors generated radical cations with the radical site initially located on the sulfur atom. Time-resolved ion-molecule reactions showed that radical site migration via hydrogen atom transfer (HAT) occurred much more quickly in Gly-Cys$^{\bullet+}$ than in Cys-Gly$^{\bullet+}$. IRMPD and DFT calculations indicated that for Gly-Cys, the radical migrated from the sulfur atom to the α-carbon of glycine, which is lower in energy than the sulfur radical (–53.5 kJ/mol). This migration does not occur for Cys-Gly because the glycine α-carbon is higher in energy than the sulfur radical (10.3 kJ/mol). DFT calculations showed that the highest energy barriers for rearrangement are 68.2 kJ/mol for Gly-Cys and 133.8 kJ/mol for Cys-Gly, which is in agreement with both the IMR and IRMPD data and explains the HAT in Gly-Cys.

Key words: Radical cations, Ion-molecule reactions, IRMPD spectroscopy, DFT calculations, Radical migration, Hydrogen atom transfer

Introduction

When tamed within the active sites of enzymes, protein based radicals play key roles in transforming substrates [1–6]. In contrast, when randomly generated under conditions of oxidative stress, radical generation can be destructive to proteins [7–12], ultimately leading to loss of function. The S based thiyl radicals of the side chains of cysteine are one class of protein radicals that have attracted considerable recent attention through studies of model systems in both the condensed [12] and gas phases [13–15]. Of particular interest have been the structural requirements for intramolecular hydrogen atom transfer (HAT) from the α-carbon position to the sulfur in peptide thiyl radicals, which is slightly exothermic because of the enhanced stability of peptide backbone radicals, particularly those located at the α-carbon of glycine [11]. This process is quite slow for the amino acid cysteine, both in solution [9] and in the gas phase [15], due to the high energy of the four-membered ring transition state required for this intramolecular HAT, but becomes much faster for peptides where HAT can occur from adjacent α-carbon positions, thus alleviating the steric constraints of the transition state. For instance, α-carbon-to-sulfur HAT in glutathione (γ-Glu-Cys-Gly) radical is almost 10 times faster compared with that in the Cys radical [9].

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Infrared multiple photon dissociation (IRMPD) spectroscopy is a potent tool for determining the radical site, as first demonstrated for the histidine radical cation [16]. Recently, our group has taken advantage of S-nitroso chemistry to generate radical cations of cysteine [17], and used IRMPD spectroscopy to study the structures of the radical ions of cysteine methyl ester [14] and *N*-acetyl cysteine [13]. It was shown for *N*-acetyl cysteine radical cation that rearrangement from the S radical to the α-carbon radical occurred [13], as confirmed by ion-molecule reactions [13]. Here we turn our attention to HAT within the radical cations of the dipeptides Gly-Cys and Cys-Gly. The aim of this work is to see if the less strained transition state of moving the radical from the cysteine sulfur to the glycine α-carbon leads to HAT and if the location of glycine at the N- or C-terminus affects the HAT.

Experimental

All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used without further purification.

Ion-molecule reactions were performed on a modified Bruker Esquire 3000 quadrupole ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) described previously [18]. Generation of radical cations from nitrosylated precursor has been previously discussed [13, 14]. Briefly, the nitrosylated protonated precursor was formed by electrospray ionization and fragmented by collision-induced dissociation (CID) in the ion trap Equation (1).

$$RSNO^{+} \rightarrow RS^{\bullet+} + \bullet NO \quad (1)$$

The resulting radical cation was mass-selected and allowed to react with a neutral reagent (allyl iodide) introduced into the trap via a leak valve. The pressure of neutral was about 1×10^{-7} Torr in the ion trap region as estimated by fast ion-molecule reactions. The scan delay was varied from 0 to 5000 ms.

IRMPD spectroscopy studies were carried out at the *f*ree *e*lectron *l*aser for *i*nfrared e*x*periments (FELIX) facility [19]. S-nitrosylated peptides were introduced into an FT-ICR mass spectrometer [20] via electrospray ionization and radical cations were formed Equation (1) by in-source CID. Experimental details were recently described [13, 14].

DFT calculations were done at the B3LYP/6-311++G(d,p) level of theory as described previously [13, 14]. All minima and infrared spectra were optimized using this basis set. For the comparison of DFT spectra to IRMPD spectra, the computed frequencies were scaled by a factor of 0.98.

Results and Discussion

Figure 1 shows the kinetic plots for the time-dependent ion-molecule reactions of Cys-Gly$^{\bullet+}$ (Figure 1a) and Gly-Cys$^{\bullet+}$ (Figure 1b) with allyl iodide. In both cases, the reaction of the radical cation (*m/z* 178) with allyl iodide shows the addition of allyl (41 Da) producing a peak at (*m/z* 219), and the addition of iodine (127 Da) producing a peak at (*m/z* 305).

$$M^{\bullet+} + CH_2{=}CH-CH_2-I \rightarrow [M-CH_2-CH=CH_2]^{+} + \bullet I \quad (2a)$$

$$\rightarrow [M-I]^{+} + \bullet CH_2-CH=CH_2 \quad (2b)$$

It has been shown by Kenttamaa et al. that allyl iodide is quite reactive towards sulfur radicals [21], and we used this volatile neutral to detect sulfur radicals in cysteine-containing systems as well [13, 14]. Since our initial radical is located at the sulfur (Equation (1)), occurrence of reactions 2a-b are expected.

If there is migration of the radical from the sulfur atom to an α-carbon position, the reactivity should cease. Previous studies have shown that α-carbon radicals are not reactive towards allyl iodide [22].

The kinetic plots shown in Figure 1 highlight that the reactivity towards allyl iodide is different for the two peptide radical cations. For Cys-Gly$^{\bullet+}$ (Figure 1a) the reactivity follows a typical pseudo-first order kinetics. On the other hand, Gly-Cys$^{\bullet+}$ (Figure 1b) stops reacting with allyl iodide after about 800 ms. This suggests that HAT occurs in Gly-Cys$^{\bullet+}$ but not in Cys-Gly$^{\bullet+}$.

Gly-Cys and Cys-Gly have two available α-carbon radical sites with some of them lower in energy than those of the sulfur radicals (Figures 2 and 3). In order to confirm whether radical migration was occurring and to which α-carbon position the radical was moving, the IR spectra of these gas-phase radical cations were obtained through the use of IRMPD spectroscopy.

Figure 2 shows the comparison of the experimentally obtained IR spectrum of Cys-Gly$^{\bullet+}$ with the calculated IR spectra of the sulfur radical (Figure 2a), glycine α-carbon radical (Figure 2b), and cysteine α-carbon radical (Figure 2c). Although the α-carbon radical of cysteine is lower in energy than the sulfur radical by 34.1 kJ/mol, the experimental spectrum best matches the theoretical spectrum of the sulfur radical. The cysteine α-carbon radical, however, has two intense bands at 1740 and 1595 cm^{-1} in the calculated spectra that do not match the experimental IR spectrum. This suggests that the sulfur radical is the only species present. This is an interesting finding since theoretical calculations predict that the cysteine α-carbon radical is much lower in energy than the radical on the sulfur or the α-carbon of glycine. The pathway calculations, however, revealed that there are high energy barriers associated with radical migration from the sulfur to the cysteine α-carbon either directly via a strained four-membered ring transition state (161.8 kJ/mol; Figure S1) or by the way of the glycine α-carbon (168.2 kJ/mol; Figure S2). These barrier values are

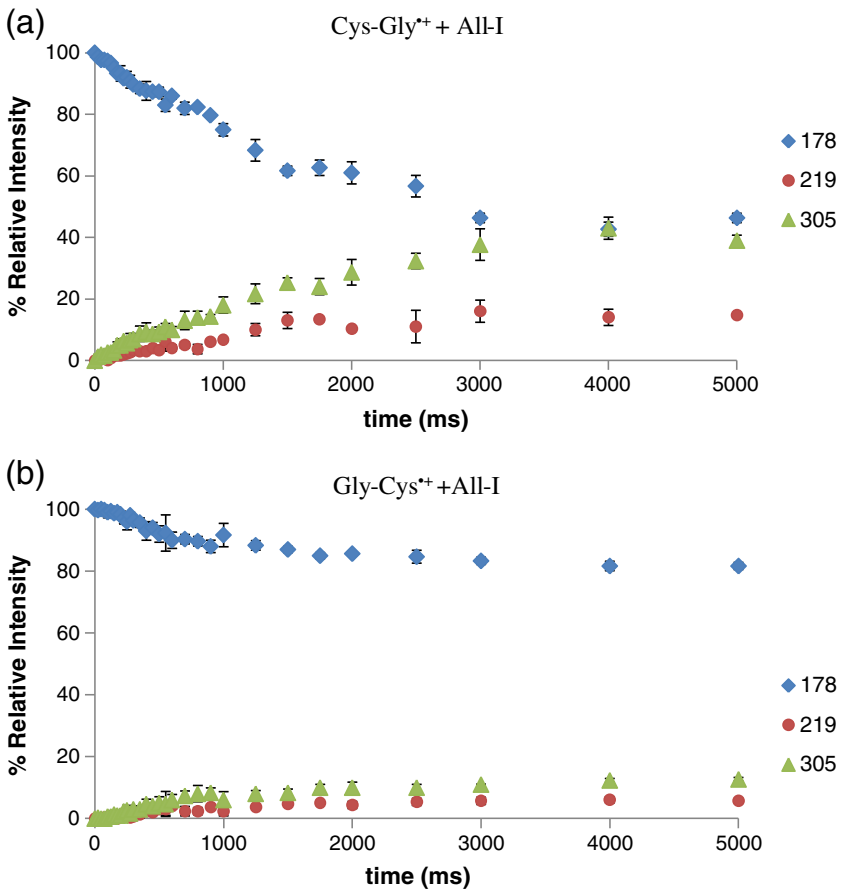

Figure 1. Kinetic plots of the radical cation of **(a)** Cys-Gly and **(b)** Gly-Cys formed via CID reacting with allyl iodide, showing the disappearance of the radical cation (*m/z* 178, diamonds), the appearance of the products corresponding to the iodine abstraction (*m/z* 305, triangles), and the allyl abstraction (*m/z* 219, circles). Pressure of allyl iodide was ca. 10^{-7} Torr

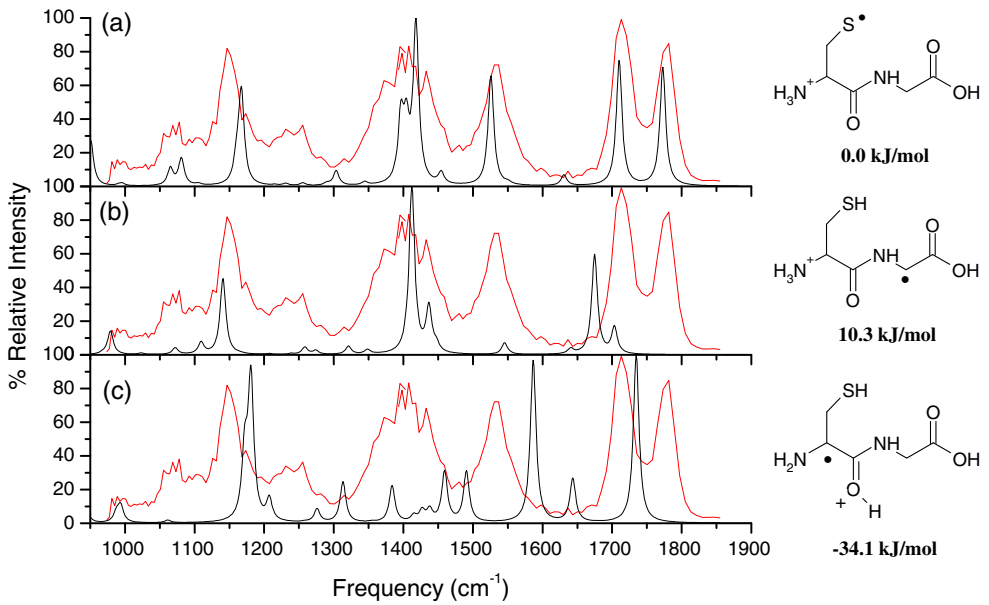

Figure 2. Comparison between experimental and theoretical IR spectra for Cys-Gly radical cation: **(a)** theoretical sulfur radical spectrum (0.0 kJ/mol), **(b)** theoretical glycine α-carbon radical spectrum (10.3 kJ/mol), and **(c)** theoretical cysteine α-carbon radical spectrum (–34.1 kJ/mol). The experimental spectrum (red trace) is overlaid on the theoretical spectra. DFT-calculated structures of the isomers are shown in Figures S3–S5

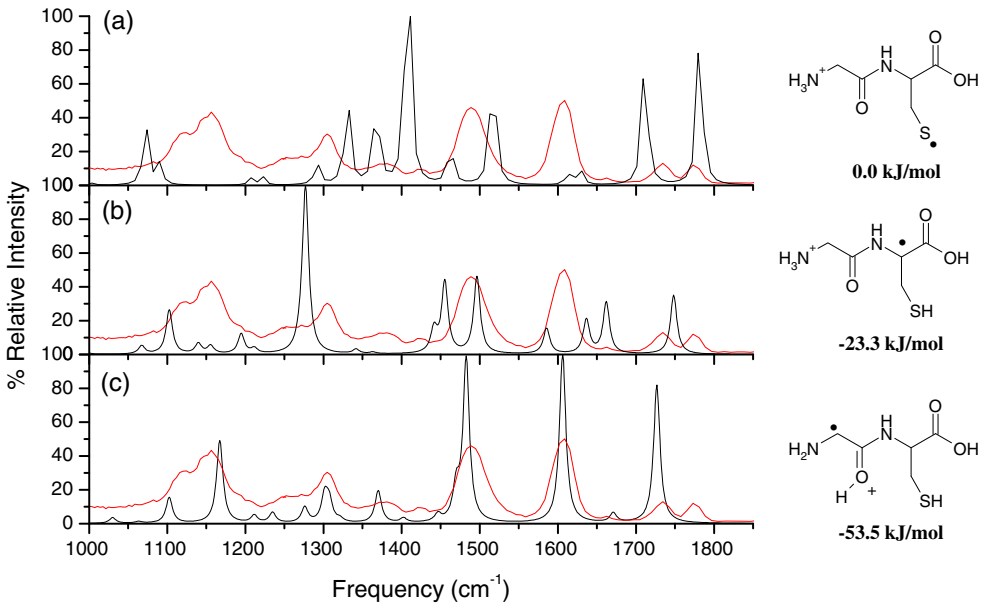

Figure 3. Comparison between experimental and theoretical IR spectra for Gly-Cys radical cation: **(a)** theoretical sulfur radical spectrum (0.0 kJ/mol), **(b)** theoretical cysteine α-carbon radical spectrum (–23.3 kJ/mol), and **(c)** theoretical glycine α-carbon radical spectrum (–53.5 kJ/mol). The experimental spectrum (red trace) is overlaid on the theoretical spectra. DFT-calculated structures of the isomers are shown in Figures S6–S8

consistent with previous studies. For the cysteine methyl ester, the sulfur-to- α-carbon (cysteine) energy barrier was calculated at 183 kJ/mol [14]. Siu and co-workers calculated that the barrier for an HAT from an α-carbon to an adjacent α-carbon in Gly-Gly-Gly$^{\bullet+}$ is ≥187 kJ/mol [23].

Figure 3 shows the comparison of the experimentally obtained IR spectrum of Gly-Cys$^{\bullet+}$ with the calculated IR spectra of the sulfur radical (Figure 3a), the cysteine α-carbon radical (Figure 3b), and the glycine α-carbon radical (Figure 3c). There is an excellent match between the experimental and theoretical IR spectra of the glycine α-carbon radical. The cysteine α-carbon radical can be excluded entirely, although it is –23.3 kJ/mol lower in energy than the S-radical. The peaks at 1750, 1450, and 1275 cm^{-1} in the calculated spectra do not match the features of the experimental spectrum. The sulfur radical may have a small residual contribution based on the peak at 1755 cm^{-1}. It is clear, however, that the majority of the radical ions present have the glycine α-carbon radical structure. This is in agreement with the calculated energies as the glycine α-carbon radical is –53.5 kJ/mol lower in energy than the sulfur radical. The calculated structures for Cys-Gly$^{\bullet+}$ and Gly-Cys$^{\bullet+}$ and their geometries are available in the Supplemental Information (Figures S3–S8).Thus, ion-molecule reactivity observations are consistent with the IRMPD data. For Gly-Cys$^{\bullet+}$ the IR spectrum shows that the majority of the ions has the radical located on the glycine α-carbon and only a minor amount of the radical is sulfur-based (Figure 3). Conversely, for Cys-Gly$^{\bullet+}$ the IR spectrum shows that the vast majority of the ions has the radical located on the sulfur (Figure 2).

In order to understand the difference in behavior of these two radical cations, the lowest energy pathways for HAT resulting in the radical site migration from sulfur to the glycine α-carbon were calculated for Gly-Cys$^{\bullet+}$ (Figure S9a) and for Cys-Gly$^{\bullet+}$ (Figure S9b). The highest energy barrier for Gly-Cys$^{\bullet+}$ is only 68.2 kJ/mol, whereas the highest energy barrier for Cys-Gly$^{\bullet+}$ is 133.8 kJ/mol. This readily explains the difference in HAT between these two dipeptide radical cations. The structures for each step of both pathways are contained in the Supplemental Information (Gly-Cys$^{\bullet+}$ Figure S10, Cys-Gly$^{\bullet+}$ Figure S11).

In conclusion, HAT in the thiyl radical cations of Cys-Gly and Gly-Cys was experimentally and theoretically studied. Time-dependent ion-molecule reaction studies and IRMPD spectroscopy revealed that HAT is observed in Gly-Cys$^{\bullet+}$ but does not proceed in Cys-Gly$^{\bullet+}$. This was supported by DFT calculations, which showed a much higher barrier for the sulfur-to-α-carbon radical migration in Cys-Gly$^{\bullet+}$ versus Gly-Cys$^{\bullet+}$ (133.8 and 62.8 kJ/mol, respectively), and the fact that the sulfur radical is 10.3 kJ/mol lower in energy than the glycine α-carbon radical in Cys-Gly$^{\bullet+}$. This has potential implications for larger peptide systems, where the position of the cysteine could play a role in the direction of HAT along the peptide backbone.

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