Structure and Reactivity of the Glutathione Radical Cation: Radical Rearrangement from the Cysteine Sulfur to the Glutamic Acid α-Carbon Atom


In memory of Detlef Schröder

A gas-phase radical rearrangement through intramolecular hydrogen-atom transfer (HAT) was studied in the glutathione radical cation, [γ-ECG]⁺⁺, which was generated by a homolytic cleavage of the protonated S-nitrosoglutathione. Ion–molecule reactions suggested that the radical migrates from the original sulfur position to one of the α-carbon atoms. Experiments on the radical cations of dipeptides derived from the glutathione sequence, [γ-EC]⁺⁺ and [CG]⁺⁺, pointed to the glutamic acid α-carbon atom as the most likely site of the radical migration. Infrared multiple-photon dissociation (IRMPD) spectroscopy was employed to generate complementary information. IRMPD of [γ-ECG]⁺⁺ in the approximately 1000–1800 cm⁻¹ region was inconclusive owing to the relatively broad, overlapping absorption bands. However, the IRMPD spectrum of [γ-EC]⁺⁺ in this region was consistent with the radical migrating from the sulfur to the α-carbon atom of glutamic acid. IRMPD in the 2800–3700 cm⁻¹ region performed on [γ-ECG]⁺⁺ is consistent with a mixture of both the original sulfur-based radical and the resulting glutamic acid α-carbon-based species. Comparisons are made with previously published condensed and gas-phase studies on intramolecular HAT in glutathione.

Introduction

Reactive oxygen species (ROS) and other free radicals can cause damage to biomolecules present in cells if left unchecked, which can ultimately lead to diseases such as cancer.[1] A key cellular defense mechanism is the use of antioxidants, which function by either inhibiting oxidation or by repairing free-radical intermediates.[2] Of the multiple types of antioxidants, the tripeptide glutathione with the sequence α-Glu-Cys-Gly (referred to in this study as γ-ECG) plays a key role owing to a number of factors including its high concentrations in cells, which can reach as high as 10 μM.[3,4] It acts as an antioxidant by directly attacking ROS and by acting as a substrate for glutathione peroxidases, which catalyze the reduction of peroxides. Glutathione also has the ability to function as an electron “sink”, in which it removes radicals from proteins by hydrogen-atom transfer (HAT) placing the radical on the sulfur moiety of the glutathione (Scheme 1).[5,6] This reaction is possible because the S–H bond, contained in the side chain of cysteine, has a much lower bond dissociation energy (BDE) than most of the other bonds found in the amino acid side chains or the peptide backbone.[7,8]

A number of solution-based techniques have been employed to study radical processes in glutathione or other peptides and proteins.[6,9–15] The two most common methods for these types of studies have been electron paramagnetic resonance (EPR) spectroscopy and fluorescence spectroscopy.[6,9–15] In addition, deuterium labeling in conjunction with NMR spectroscopy or mass spectrometry (MS)-based methods have been used to infer the sites of HAT. Although the reactions of the glutathione thiol radical, including intramolecular hydrogen-atom transfer, have been studied for decades,[6,16–23] as Hofstetter et al. recently noted, “The story of thiol radicals in general, and of the GS in particular, does not appear to be finished yet”.[16] This is due to the fact that solution studies can be chal-
Radicals in solution are often prone to a range of competing reactions (in the case of thiyl radicals these include intermolecular and intramolecular HAT, deamination, or desulfurization) and direct spectroscopic characterization of radicals often require them to be long lived and typically at high concentrations. For these reasons, gas-phase studies (often utilizing mass spectrometry) present an attractive alternative since the competing reactions can be minimized. For example, intermolecular and intramolecular HAT can be differentiated readily.

Several methods have been developed to selectively generate peptide radicals in the gas phase. Siu’s group pioneered the use of collision-induced dissociation (CID) of a metal–peptide complex formed in solution to fragment the metal–peptide bond. Other methods of generating peptide radicals in the gas phase involve chemically modifying a functional group on one of the amino acids to form a weak covalent bond. Then CID is performed to homolytically cleave the weak bond, thereby generating the radical. Examples include forming radicals on the N terminus or lysine side chain by introducing a peroxycarbamidyl derivative to the amine at these positions using serine nitrate esters to form carbon-centered radicals and introducing thyl radicals from nitrosylated cysteine precursors. A third method often used is the UV photodissociation of weak bonds in a peptide such as iodotyrosine.

CID has been widely used to study radical migration in gas-phase ions. Recently, we used two different approaches to probe radical migration of the radical cation of the tripeptide GCR: 1) S–NO chemistry was used to generate the radical at the sulfur atom of cysteine; 2) oxidative dissociation of both [Cu(terpy)(GCR)]$^{2+}$ (terpy = 2,2′,6′,2″-terpyridine) and [Fe(salen)(GCR)]$^+$ (salen = 2,2′-ethylenbis(nitrilomethylidene) diphenol) complexes was carried out, which is known to often produce α-carbon-based radicals. We observed that the CID spectra of GCR$^+$ were identical and thus independent of the mode of formation. This was attributed to HAT between the cysteinyl radical and an α-carbon atom under CID conditions. A more detailed study of isomeric regiospecifically generated radicals (i.e., G'C$^+$ vs. GC(S)$^+$ or G'C'G$^+$ vs. GC(S)'G$^+$) confirmed that this type of side-chain-to-backbone HAT is very common. Our explanation was that associated with such HAT needed to be competitive (i.e., 105–170 kJ mol$^{-1}$) with the typical even-electron processes (charge-directed) for the isomerization experimentally observed.

Similar to our approach outlined above, Zhao and co-workers have recently studied two isomeric forms of [γ-ECG]$^+$, one generated through CID of [Cu(terpy)(γ-ECG)]$^{2+}$, and the other through homolytic cleavage of the S–NO bond of the protonated S-nitroso derivative, thereby leading to γ-EC(S)G$^+$. The CID spectra of the two species were identical, and the authors suggested that the species formed through CID of the [Cu(terpy)(γ-ECG)]$^{2+}$ complex would be either the canonical structure (one electron removed from the sulfur atom) or one of the carboxy radicals. All of these three species can rearrange without a barrier, or alternatively through a very low (less than 20 kJ mol$^{-1}$) barrier to the sulfur radical γ-EC(S)G$^+$. They also calculated the barriers for the side-chain-to-backbone HAT to be in the range 76–118 kJ mol$^{-1}$ for the three possible α-carbon radicals. The most stable of those, the γ-EC$^+$ isomer, was found to be about 21 kJ mol$^{-1}$ lower in energy than γ-EC(S)G$^+$ and had the lowest rearrangement barrier from the sulfur radical species.

Although CID is often successful in providing valuable information about radical migrations, these experiments impart significant energy into the ions, making comparison to processes in solution difficult. Ion–molecule reactions in the gas phase can be used as an alternative tool for studying radical rearrangements, as this technique proceeds under thermal or near-thermal conditions. For the radical migration from the cysteine sulfur to the backbone α carbon this approach is rather straightforward as sulfur-based radicals display reactivity quite different from that of α-carbon-based ones. In cases where more than one isomer of α-carbon radicals can be formed, infrared multiple photon dissociation (IRMPD) spectroscopy can be used to aid in identifying the structure of the final product(s). We have recently used such a combination of ion–molecule reactions and IRMPD to determine the gas-phase structure of several cysteine derivatives as well as to monitor side-chain-to-backbone HAT in radical cations of the dipptides GC and CG. Herein, we apply this approach to study the radical migration from the sulfur atom in the radical cation of glutathione, and compare our results to solution-phase studies.

**Results and Discussion**

**Radical-cation formation**

Formation of the radical cation is achieved by homolytic cleavage of the S–NO bond of the nitrosylated cysteine-containing peptide (R) by mean of CID in the ion trap [Eq. (1)]:

$$\text{RS–NO}^+ \rightarrow \text{RS}^+ + \text{NO}$$

**Scheme 1.** Hydrogen-atom transfer occurring between glutathione and an α radical located on a glycyl residue in a protein.
present using low-energy CID.\textsuperscript{[34]} This bond cleavage has also been observed using IRMPD spectroscopy.\textsuperscript{[65, 66]}

Glutathione radical cation

**Ion–molecule reactions**

Figure 1 shows the mass spectra for the ion–molecule reactions (IMR) of $\gamma$-EC(S)G\(^{+}\) with allyl iodide (Figure 1a) and dimethyl disulfide (Figure 1b). For the reaction with allyl iodide, the radical cation \((m/z\ 307)\) reaction products are formed at \((m/z\ 348)\) and \((m/z\ 434)\), which correspond to the addition of an allyl group and iodide, respectively [Eqs. (2) and (3)]:

\[
RS^+ + \text{All} \rightarrow RS\text{–All}^+ + I \tag{2}
\]

\[
RS^+ + \text{All} \rightarrow RS\text{–All}^+ \tag{3}
\]

The reaction with dimethyl disulfide led to the addition of 47 mass units, which produced a peak at \((m/z\ 354)\) (Figure 1b). This peak corresponds to the addition of a SCH\(_3\) group [Eq. (4)]:

\[
RS^+ + \text{CH}_3\text{S–SCH}_3 \rightarrow RS\text{–SCH}_3^+ + \text{SCH}_3 \tag{4}
\]

It has been shown in previous studies that the thyl radicals react readily with allyl iodide and dimethyl disulfide.\textsuperscript{[61–63,67,68]} Owing to the fact that initially the radical is formed on the sulfur of glutathione the reactivity observed in Equations (2)–(4) was expected. Indeed, the glutathione radical cation was found to react with allyl iodide and dimethyl disulfide. To confirm that the reactions proceeded by reaction at sulfur, the product of Equation (3), [\(\gamma\)-ECG+I\(^{+}\) \((m/z\ 434)\), was subjected to CID to “sequence” the modified peptide. The resultant spectrum is shown in Figure S1 in the Supporting Information and the two modified b\(_{2}^{+}\) \((m/z\ 359)\) and y\(_{1}^{+}\) \((m/z\ 305)\) sequence ions observed are consistent with the iodine attachment to the cysteine residue.

Radical rearrangement can occur between the sulfur radical and an \(\alpha\)-carbon atom in an adjacent amino acid by means of hydrogen-atom transfer (HAT), as a result of the cysteine S–H bond having a BDE of about 368 kJ mol\(^{-1}\)\textsuperscript{[7]} and the \(\alpha\)-carbon C–H bond of most amino acids being only \(\lesssim 356\) kJ mol\(^{-1}\)\textsuperscript{[6]}\textsuperscript{[8]} Thus the hydrogen-atom transfer is thermodynamically favorable. It has been shown that \(\alpha\)-carbon radicals are not reactive towards the neutral reagents used in this study and instead react with dioxygen, NO, and NO\(_2\).\textsuperscript{[59, 60]} Hence, it is possible to detect radical rearrangement by monitoring the reaction kinetics. We have utilized this approach for the radical cation of the dipeptide GC(S\(^{+}\)), which showed a kinetic profile that suggested a loss of reactivity after some time.\textsuperscript{[63]} This reactivity loss was attributed to radical migration to the nonreactive \(\alpha\)-carbon atom.

In this study, we performed similar experiments by reacting allyl iodide and dimethyl disulfide (acting as sulfur radical probes) with the glutathione radical cation, which was formed in the ion trap by means of CID of protonated S-nitrosoglutathione. The kinetic profiles for these two experiments are shown in Figure 2. Figure 2a shows the profile for the reaction with allyl iodide. It shows that the reaction is observed for the first 1000 ms or so and then nearly stops. A similar observation was made for dimethyl disulfide (Figure 2b), in which the formation of products is observed for the first 500 ms before leveling off. The differences in the kinetics of dimethyl disulfide reactions versus those of allyl iodide are currently under investigation. To confirm that the loss of reactivity was a result of the radical migration to the \(\alpha\)-carbon atom, IRMPD spectra
were obtained and compared to theoretical IR spectra generated from DFT calculations.

**FELIX IRMPD of the glutathione radical cation**

The experimental IRMPD spectrum of in-source-formed $\gamma$-EC(S)G$^+$ is shown in Figure 3 (red trace). The theoretical spectra for the four lowest-energy isomers are shown as the black trace in Figure 3b–e. The structures for each of these isomers are shown in Scheme 2. The spectral comparison shows that the calculated spectra for the sulfur radical (Scheme 2a) and the cysteine $\alpha$-carbon radical (Scheme 2b) are the best match to the experimental spectrum, although the cysteine $\alpha$-carbon radical can be excluded because it has a higher energy than the sulfur radical. At the same time, because of the considerable line broadening of the experimental spectrum, the glycine (Scheme 2c) and glutamic acid $\alpha$-carbon radicals (Scheme 2d) cannot be excluded. None of the matches is perfect, but major bands in the experimental spectrum correspond to the C–O carboxyl stretches (1130–1170 cm$^{-1}$), CH$_2$ bends or C–C stretches at around 1400 cm$^{-1}$, C–N stretches at 1490–1530 cm$^{-1}$, and carboxyl stretches in the 1650–1770 cm$^{-1}$ range. The latter were previously used by us as the main diagnostic bands for distinguishing between the distonic and captodative radical ions of cysteine derivatives and dipeptides.$^{[61–63]}$ In glutathione, however, there are four carboxyl stretches making unambiguous assignment more difficult. In addition, the calculated structures suggest considerable hydrogen-bonding interactions for most carboxyls, which is consistent with the broadening and redshifting of these features.

**University of Florida IRMPD**

We also investigated IR absorption properties of the glutathione radical cation by performing IRMPD in the N–H and O–H stretching regions (2800–3700 cm$^{-1}$). The experimental IRMPD spectrum (Figure 4, red trace) reveals two sharp peaks at 3560 and 3580 cm$^{-1}$ plus several less intense, broader features, most of them in the 3200–3450 cm$^{-1}$ region. Comparison with the theoretical vibrational spectrum of $\gamma$-EC(S)G$^+$ (the starting species) is shown in Figure 4b, black trace (Scheme 2a). The sharp features at 3560 and 3580 cm$^{-1}$ can be attributed to the carboxyl O–H stretches of the glutamic acid and the C-terminal glycine, respectively, and they match the experimental spectrum very well, both in position and intensity. The theoretical spectrum also predicts the two amide stretches at 3310 and 3380 cm$^{-1}$ and two stretching modes of the protonated N-terminal amine at 3230 and 3380 cm$^{-1}$, respectively, the latter coinciding with one of the amide N–H stretches. The fact that there are no distinct features corresponding to the N–H stretches in the experimental IRMPD spectrum can be explained by hydrogen bonding. Both amide hydrogen atoms, and especially the N-terminal ammonium hydro-
The calculated IR spectrum for $\gamma$ECG$^+$ (Scheme 2b and Figure 4c) in principle shows a close match to the experimental data, in that all of the calculated bands are compatible with the experimental spectrum. However, the theoretical calculations show that the lowest-energy structure for this isomer is 8.9 kJ mol$^{-1}$ higher in energy than the sulfur radical, which suggests that this radical migration to the $\alpha$-carbon atom of cysteine is unfavorable. It has also been shown with other cysteine-containing systems that this radical migration has a very high energy barrier owing to a four-membered ring intermediate. Thus we propose that this isomer is not present in the ion population. As for the DFT-calculated glyoxyl radical cation ($\gamma$ECG$^+$) (Scheme 2c) shown in Figure 4d, there is a large peak at about 3075 cm$^{-1}$ in the calculated IR spectrum that is absent in the experimental spectrum. This peak is associated with an N–H stretch of the N-terminal amine group. Since this peak does not match up with any in the experimental IR spectrum, it is improbable that this species is present. It should be mentioned that our calculated glyoxyl radical cation (Figures 3d and S4 in the Supporting Information) has a different conformation to that calculated by Zhao et al.$^{[58]}$ We were able to locate a conformationally more compact structure for this isomer that was lower in energy than the more open structure reported in reference $^{[58]}$. A comparison of these two structures and their calculated IR spectra is shown in Figure S6. Besides the glyoxyl-based radical, the other structures shown in Scheme 2 were fairly close to those calculated by Zhao et al.$^{[58]}$

The results from IRMPD spectroscopy in the amide-stretching region rule out the glyoxyl $\alpha$-carbon radical and the cysteine $\alpha$-carbon radical can be ruled out based on our DFT energy calculations. It is, however, consistent with the initial distonic sulfur radical species rearranging into the glutamic $\alpha$-carbon radical.

$\gamma$-Glutamylcysteine and cysteinylglycine radical cations

Ion–molecule reactions

As stated above, IMR points to radical migration from the sulfur to an $\alpha$ carbon. However, there are three $\alpha$-carbon sites in glutathione. To narrow down these rearrangement possibilities, the dipeptide radical cations $\gamma$EC(S)$^+$ and C(S)$^+$, derived from the glutathione sequence $\gamma$EC(S)$^+$ by truncation at either the C or N terminus, were investigated. The kinetic profiles for IMR reactions between the radical-cation species and allyl iodide are shown in Figure 5.

These three peptides were analyzed because of their amino acid sequences. It can be seen that $\gamma$EC(S)$^+$ (diamonds) and $\gamma$EC(S)$^+$ (triangles) have very similar profiles, in which they react with the neutral reagent rapidly at first and then the reaction seems to stop. Conversely, C(S)$^+$ (circles) reacts completely with the neutral reagent over time as has been reported in our recent study.$^{[63]}$ Since the kinetic profiles of $\gamma$EC(S)$^+$ and $\gamma$EC(S)$^+$ are very similar, this suggests that if radical rearrangement is occurring in $\gamma$EC(S)$^+$, it is likely that the radical is migrating from the sulfur to the unreactive.
The α-carbon atom of the γ-glutamic acid. In contrast, C(SC)G reacts completely with allyl iodide, which suggests that the radical remains at the reactive sulfur position rather than rearranging to the unreactive α-carbon atom of glycine or cysteine. To further test this hypothesis, the IRMPD spectra of both γ-EC⁺ and CG⁺ were obtained.

**FELIX IRMPD of the γ-glutamylcysteine radical cation**

The experimental IRMPD spectrum of in-source-formed γ-EC(SC)G⁺ (Scheme 3a and Figure 6b, black trace) matches the experimental one very poorly. Most notably, the C-terminal carbonyl stretch calculated to be at 1725 cm⁻¹ is absent in the experimental spectrum, as is the NH₂ scissoring band at 1615 cm⁻¹. The calculated spectrum for γ-EC⁺ is shown in Figure 6c (black trace). As with the sulfur-based radical, the match with the experimental spectrum is not satisfactory, as the C-terminal carbonyl predicted to appear at 1680 cm⁻¹ is not present in the experimental IRMPD (red trace). The theoretical spectrum for γ-EC⁺ (Scheme 3c) shown in Figure 6d (black trace) represents the best match of the three isomers. The Gaussian structures and calculated Cartesian coordinates for each of these isomers are shown in Figures S7–S9.

These results fully support our ion–molecule reaction experiments as well as theoretical calculations, which predict the γ-EC⁺ isomer to be the most stable one and the barrier for its formation from γ-EC(SC)G⁺ is only 73.4 kJ mol⁻¹ (Figure S10). This is in agreement with calculations performed by Zhao et al. who showed that for the glutathione radical cation the energy barrier for the rearrangement of the sulfur radical to the α-carbon of glutamic acid is 76 kJ mol⁻¹.[58]

We have previously used IRMPD spectroscopy to examine whether HAT occurs in C(SC)G, the N-terminally truncated dipeptide of glutathione. It was found that the radical cation of C(SC)G retains its initial radical position at the sulfur atom.[63] This was consistent with the relatively high DFT-calculated barrier for the S-to-α-carbon (of glycine) radical migration of 134 kJ mol⁻¹. Thus, the ion spectroscopy data also suggest rearrangement of the sulfur-based radical to the α-carbon atom of glutamic acid but not glycine or cysteine.

**Comparison with previous gas-phase and solution results**

There have been several studies performed in solution and in the gas phase on γ-ECG radicals in the past. The majority of the solution studies have been performed using pulsed radiolysis.[6, 16–23] The results show that in solution the thyl radical of glutathione either equilibrates with RSSR⁻, as shown in Equation (5), or undergo intramolecular radical migration to an α-carbon position.[6, 17, 22, 23]

\[
\text{RS}^- + \text{RS}^- \rightarrow \text{RSSR}^-(5)
\]

In the latter case, the radical was shown to transfer from the sulfur to the α-carbon atom of the glutamic acid resi-
This intramolecular HAT only occurs in solutions with a pH greater than 7 (from 7.5 to 10.5). This pH dependence of radical migration is a result of bond strengths. Generally, C–H bonds are stronger than S–H bonds, which would make radical migration from the sulfur to an α-carbon atom thermodynamically unfavorable. However, when the ammonium group of an amino acid is converted into an amino group by deprotonation at high pH, the αC–H bonds become substantially weaker. Computational studies performed by Rauk and Armstrong support the solution studies, showing that the lowest-energy pathway for the intramolecular HAT would be from the sulfur to the α-carbon atom of glutamate, with an energy barrier of only about 40 kJ mol⁻¹. Conversely, the energy barriers for HAT from the α-carbon atom of glycine or cysteine were found to be 134 and 110 kJ mol⁻¹, respectively. More recent solution studies have shown that the radical can also migrate to the β- or γ-carbon atom of glutamate or to the α-carbon atom of glycine. However, it is believed that these pathways are likely solvent-assisted.

The combination of IRMPD spectroscopy and ion–molecule reactions presented here reveal that the radical cation of glutathione undergoes a related radical migration from the sulfur of cysteine to the α-carbon atom of glutamic acid in the gas phase. Our theoretical calculations and those reported by Zhao et al. highlight that the α-carbon radical of glutamic acid in the radical cation is stabilized by charge-enhanced captodative resonance, making the rearrangement thermodynamically favorable. Although these findings are consistent with the results obtained in solution, the factors that influence this migration are different: in solution the weakening of the C–H bonds at high pH is key, whereas in the gas phase captodative stabilization is important.

Conclusion

Ion–molecule reactions of the glutathione radical cation produced by S–NO bond homolysis of protonated S-nitrosoglutathione indicate that the radical migrates from the sulfur to an α-carbon position. Gas-phase reactivity studies on dipeptide radicals derived from the glutathione structure, γ-EC(S)⁺ and γ-C(S)G⁺, point to the glutamic acid α-carbon atom as the site of radical migration. This is consistent with findings of Zhao et al. who calculated it to be the most stable isomer of the glutathione radical cation.

IRMPD spectroscopy was employed in two regions—the approximately 1000–1800 cm⁻¹ region and in the 2800–3700 cm⁻¹ region. The IRMPD of the glutathione radical cation in the 2800–3700 cm⁻¹ region is consistent with a mixture of both the original sulfur-based radical and the resulting glutamic acid α-carbon-based species. The IRMPD spectrum of the radical cation of γ-EC in the approximately 1000–1800 cm⁻¹ region was consistent with the radical migrating from the sulfur to the α-carbon atom of glutamic acid.

Our recent investigation of two isomers of the tryptophan radical cation indicated that there can be a strong conformational dependence of the IRMPD signals in the fingerprint region. As the number of low-energy conformers is expected to increase with peptide size, we expect this issue to become more prevalent. Another issue that we encountered in this study was extensive hydrogen bonding that can broaden IRMPD signals in both the fingerprint and the N–H stretching region.

Experimental Section

Chemical reagents

All chemicals and reagents were used as received without any further purification. S-Nitrosylated glutathione, Cys-Gly, γ-Glu-Cys, methanol (HPLC grade), tert-butyl nitrite, dimethyl disulfide, and allyl iodide were all purchased from Sigma–Aldrich (Milwaukee, WI).

Ion–molecule reactions

Mass spectra were obtained by using a Bruker Esquire 3000 quadrupole ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany) that had been modified to conduct ion–molecule reactions (described previously). Cys-Gly and γ-Glu-Cys were nitrosylated by treating a 1:5:1 mixture of tert-butyl nitrite with a 1 μM solution of Cys-Gly or γ-Glu-Cys (in 50:50 methanol/water with 1% acetic acid) for 10 min at room temperature. The reaction mixture was diluted 100-fold using 50:50 methanol/water with 1% acetic acid and introduced into the electrospray ionization (ESI) source of the mass spectrometer at a flow rate of 5 μL min⁻¹. The sheath gas, capillary voltage, and temperature were adjusted to optimize the ion-peak intensity (10 arbitrary units, 3.0 kV, and 250 °C, respectively). Radical cations of glutathione, Cys-Gly, and γ-Glu-Cys were produced by CID. The protonated S-nitrosylated precursor ion was first isolated using mass selection with a peak width of 4.0, and then fragmented using a collision energy sufficient to dissociate the majority of the precursor ions. The radical cation was then isolated and treated with the neutral reagent introduced into the trap via a leak valve. To produce the kinetic profiles of the reactions, the scan delay time was varied from 0 to 5000 ms allowing the acquisition of mass spectra at different reaction time points.

Infrared multiple-photon dissociation spectroscopy

The FELIX Facility in the Netherlands

IRMPD spectroscopy studies in the 1000–1800 cm⁻¹ region were carried out at the Free Electron Laser for Infrared eXperiments (FELIX) facility located at the FOM-Institute for Plasma Physics Rijnhuizen in Nieuwegein, The Netherlands (now relocated to the Radboud University in Nijmegen). The nitrosylated precursors were generated with a final concentration of 1 mM. The nitrosylated precursor was introduced into a custom-built FT-ICR mass spectrometer by means of an orthogonal Z-spray ESI source. Operating parameters for ESI were optimized to maximize the formation and transfer of ions into the ICR cell. The ions were trapped in the cell by applying a dc potential switch to the octopole ion guide without the use of a gas pulse. By removing the gas, collisional heating of the ions was eliminated. The radical cation was produced from the S-nitrosylated precursor by CID in the ion source. This was done by raising the cone voltage to cause in-source fragmentation. The radical cation was then isolated by using a stored waveform inverse Fourier transform (SWIFT) pulse before being irradiated with the FELIX infrared laser. Spectra were collected by monitor-
The Gaussian 09 suite of programs was used to calculate all geometrical and spectroscopic (described previously). This instrument is composed of a modified ESI source (Analytica, Branford, CT, USA), fitted with a laboratory-built stainless-steel capillary and ion funnel, for abundant generation of precursor ions; ions of interest are mass isolated with a quadrupole mass filter (Ardara Technologies LP, Ardara, PA, USA), followed by focused irradiation in a reduced-pressure (∼10⁻¹⁰ mbar) quadrupole ion trap (QIT), and a mass analysis in a time-of-flight (TOF) drift tube (Jordan TOF Products, Grass Valley, CA, USA). The focused beam in the trap is equivalent to approximately 300 W cm⁻², following focusing of an approximately 25 mW cw idler beam to a beam waist < 100 μm.

The glutathione radical cation was generated by in-source CID of its nitrosylated precursor ion by adjusting the voltage drop between the exit voltage of the ion funnel and the skimmer. The radical cation was isolated and stored in the quadrupole ion trap for laser irradiation (1 s) with the tunable idler wavelength output of the bench top OPO. The abundance of precursor versus photofragment ions were obtained by integrating the TOF mass spectral peaks with in-house LabView (National Instruments) software. The IRMPD yield was plotted as a function of OPO frequency to obtain the IRMPD spectrum. The IRMPD yield was also normalized linearly with laser power.

**Density functional theory calculations**

The Gaussian 09 suite of programs was used to calculate all geometry optimizations and harmonic vibrational frequencies. The unrestricted hybrid B3LYP functional and the 6-311+ +G(d,p) basis set were used in the optimization of all minima. For each isomer multiple conformations were explored and only the lowest-energy conformers were reported here. All transition-state calculations were performed using the QST2 function with Gaussian, and intrinsic reaction coordinate (IRC) calculations were used to confirm that the transition states linked to the correct minima. Vibrational frequency calculations were performed on the optimized structures and were used to determine whether the optimized structures were true minima (no imaginary frequencies) or transition states (one imaginary frequency), for zero-point energy corrections to electronic energies (used unscaled), and for prediction of infrared spectra for comparison to experimental IRMPD spectra. For comparison of DFT spectra to IRMPD spectra, the computed frequencies were scaled by a factor of 0.975, which is known to be adequate at the current level of theory. The intensities in the theoretical and experimental spectra were normalized (% relative intensity).

**Acknowledgements**

V.R. and S.O. acknowledge support from NIU and the Center for Biochemical and Biophysical Studies. R.A.J.O. thanks the ARC Centre of Excellence in Free Radical Chemistry and Biotechnology for financial support. J.O. and G.B. are supported by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO). N.P. and K.G. thank the National Science Foundation under CHE-084545 for financial support. This study is dedicated to the memory of Detlef Schröder who will be greatly missed.

**Keywords:** glutathione · ion–molecule reactions · IR spectroscopy · radical ions · rearrangement
