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PAPER

Glutathione radical cation in the gas phase; generation, structure and fragmentation†

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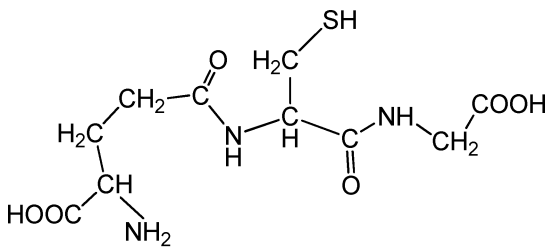
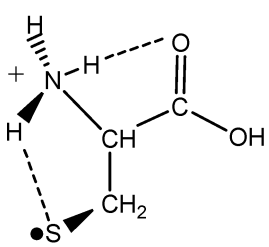
Two different chemical methods have been used to form glutathione radical cations: (1) collision-induced dissociations (CIDs) of the ternary complex $[Cu^{II}(tpy)(M)]^{\bullet 2+}$ (M = GSH, tpy = 2,2′:6′,2″-terpyridine) and (2) homolysis of the S–NO bond in protonated S-nitrosoglutathione. The radical cations, $M^{\bullet +}$, were trapped and additional CIDs were performed. They gave virtually identical CID spectra, suggesting a facile interconversion between initial structures prior to fragmentation. DFT calculations at the B3LYP/6–31++G(d,p) level of theory have been used to study interconversion between different isomers of the glutathione radical cation and to examine mechanisms by which these ions fragment. The N-terminal α-carbon-centred radical cation, strongly stabilized by the captodative effect, is at the global minimum, which is 8.5 kcal mol^{-1} lower in enthalpy than the lowest energy conformer of the S-centred radical cation. The barrier against interconversion is 18.1 kcal mol^{-1} above the S-centred radical.

Introduction

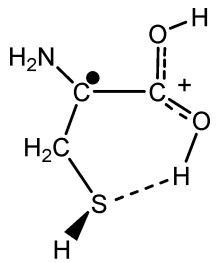
Thiols are commonly used to scavenge radicals by hydrogen atom transfer thereby creating stable thiyl radicals.[1] Cysteine is the only naturally occurring amino acid that has a thiol group. Glutathione (GSH, γ-glutamylcysteinylglycine, **I**) is a tripeptide that has a cysteine residue as its second residue; it plays an important role as an antioxidant in biological systems by scavenging radicals while being converted into a thiyl radical.[2] The cysteine radical cation has been examined both computationally[3–5] and subsequently by mass spectrometry.[6] There are a number of low-lying structures on the potential energy surface (PES).[5,6] The sulfur-centred radical, Structure **II**, is a distonic ion with the charge formally located on the protonated amino group but delocalized by hydrogen bonding to both the carbonyl group and the sulfur atom. Structure **II** is the second lowest energy structure on the PES. The isomer at the global minimum **III** (6.1 kcal mol^{-1} below **II**) has a captodative structure with the radical at the α-carbon and the charge on the protonated carboxy group. This latter structure has the structural features that heavily stabilize a radical: a powerful electron donor, NH_2, and a powerful electron acceptor, $COOH_2^+$, flanking the radical centre.[5,7,8] Conversion of **II** to **III** is a multistep process with an overall barrier of 37.1 kcal mol^{-1}; consequently, the S-centred radical, if formed, is expected to be stable and experimentally detectable.[5,6] Recently, O'Hair *et al.*[6,9] have shown that it is possible to form thiyl radicals by the loss of NO from nitrosylated cysteine derivatives; IRMPD (infrared multiphoton dissociation) spectroscopy of the radical cation of cysteine[10] and of its methyl ester[11] thus created show that the ions have predominately the structure of **II**.



I



II



III

The relatively high barrier against conversion of the S-centred radical into the α-radical for cysteine is primarily due to steric strain experienced in the transition state for the required 1,3-hydrogen shift.[5] In the case of GSH, the analogous process involves a 1,8-hydrogen atom migration and does not suffer from the severe

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† Electronic supplementary information (ESI) available: CID of protonated GSNO, GSNO-$(OCH_3)_2$ and GSNO-OCH_2CH_3. Tables of total energies and Cartesian coordinates of the structures shown in Fig. 4–7 determined at the B3LYP/6–31++G(d,p) level. See DOI: 10.1039/c1ob05968h

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geometric constraints that make the barrier against the 1,3-H-shift in cysteine radical cation so high. For the neutral glutathione radical the S-centred radical has been calculated to be 10.6 kcal mol^{-1} higher in energy than the N-terminal α-radical, and the barrier against interconversion is 7.4 kcal mol^{-1} above the S-centred radical.[12] The glutathione radical cation ($GSH^{\bullet+}$) carries an extra proton which should further enhance stabilization of the captodative structures,[5,8,13] with the N-terminal α-radical being favoured over the other two α-carbon radicals (*vide infra*).[14]

Experimentally, $GSH^{\bullet+}$ can be generated by means of two different mass spectrometry (MS) strategies. Generation from the S-nitrosylated glutathione should initially create an isomer containing the S-centred radical ion, while that from a redox reaction *via* the dissociation of $[Cu^{II}(tpy)(GSH)]^{\bullet 2+}$ (tpy = 2,2′:6′,2″-terpyridine) will probably create initially a different isomer, possibly a carboxy or canonical radical ion.[15–24] Subjecting the $GSH^{\bullet+}$ formed by the two methods to collision-induced dissociation (CID) may result in different dissociation products that are indicative of the precursor structures. Conversely, if the dissociation products are the same, then the two initially formed ions must either have had the same structure or have undergone interconversion prior to dissociation.

Herein we report generating $GSH^{\bullet+}$ by means of both methods and compare their fragmentation products. Additionally, we compare these products with those from the radical cations of S-methylglutathione and glutathione ethyl ester, ions for which the number of potential fragmentation channels are more limited. Both ions of these derivatives were generated from the dissociation of $[Cu^{II}(tpy)(M)]^{\bullet 2+}$ (where M is the glutathione derivative). We examine the dissociation products from the radical cation of glutathione dimethyl ester, which was generated from S-nitrosoglutathione dimethyl ester. Furthermore, we also use density functional theory (DFT) calculations at the B3LYP/6–31++G(d,p) level to optimize structures for several isomers of $GSH^{\bullet+}$, to examine the pathways to interconversion, and to calculate barriers to fragmentation.

Experimental section

1. Chemicals and syntheses

Glutathione and its derivates: S-methylglutathione (denoted S-methyl-GS), glutathione ethyl ester and S-nitrosoglutathione, copper(II) perchlorate hexahydrate, 2,2′:6′,2″-terpyridine (tpy), and solvents were available from Sigma–Aldrich (St. Louis, MO). Copper(II)-containing ternary complexes were prepared in 1 : 1 water/methanol solutions by mixing copper(II) perchlorate, tpy and glutathione in equimolar amounts to a final concentration of 200 μM $[Cu^{II}(tpy)(GSH)]^{\bullet 2+}$. Methyl esterification and *trans*-nitrosylation reactions of glutathione were performed according to Lam *et al.* [9] The synthesized S-nitrosoglutathione dimethyl ester and S-nitrosoglutathione ethyl ester were used directly for MS experimentation (as MS isolation afforded concurrent purification).

2. Mass spectrometry

The mass spectrometers used were prototype versions of the API 2000 linear ion-trap instrument and the API 3000 triple-quadrupole mass spectrometer (both MDS SCIEX). The collision gas used for both instruments was nitrogen. Samples were introduced by means of pneumatically assisted electrospray at flow rates of 50–60 μL h^{-1}. For the ion-trap mass spectrometer, MS^2 and MS^3 spectra were recorded typically with a fill time of 50–100 ms and a scan speed of 1000 Th s^{-1}. We adopt herein the convention of labeling the mass-selected precursor ion in the spectra with an asterisk (*). Throughout this report, unspecified Cu isotopes are always ^{63}Cu; the dissociation chemistries of the ^{65}Cu-containing complexes were used for verification.

3. Computational methods

All calculations were performed using the Gaussian03 quantum chemical program.[25] The total energies of glutathione radical cations were calculated by the unrestricted open-shell formalism within the framework of Becke's three-parameter DFT hybrid functional, B3LYP, which is based on a mixture of Hartree–Fock exchange and the Becke and Lee–Yang–Parr exchange–correlation functional.[26,27] The standard Pople Gaussian-type basis set, 6–31++G(d,p), was employed.[28] Local minima and transition structures were optimized and characterized by means of harmonic frequency analyses. Zero-point vibration energies were evaluated directly using normal-mode frequencies without anharmonic scaling. The local minima associated with all transition states were identified using the intrinsic reaction coordinates method.[29] Atomic charges and spin densities were evaluated using natural population analysis.[30]

Results and discussions

1. Formation of glutathione radical cations

(a) CID of $[Cu^{II}(tpy)(GSH)]^{\bullet 2+}$. The CID spectrum of $[^{63}Cu^{II}(tpy)(GSH)]^{\bullet 2+}$ (*m/z* 301.7, Fig. 1a) exhibits a rich gas-phase chemistry. In addition to the dissociative electron transfer reaction that gave rise to $GSH^{\bullet+}$ (*m/z* 307.3) in low abundance and its complementary ion $[Cu^{I}(tpy)]^{+}$ (*m/z* 296.3), varying degrees of competing neutral molecule losses from the copper complex were observed, including dipositive fragments: $[Cu^{II}(tpy)(GSH) - NH_3]^{\bullet 2+}$ (*m/z* 293.4), $[Cu^{II}(tpy)(CysGly)]^{\bullet 2+}$ (*m/z* 237.2), and $[Cu^{II}(tpy)(CysGly - NH_3)]^{\bullet 2+}$ (*m/z* 228.7). The other doubly charged, relatively low abundant ions at *m/z* 270.3, 264.0 and 261.2, corresponded to losses of ($NH_3 + H_2O + CO$), glycine, and ($NH_3 + CO + 2H_2O$). In addition, even electron product ions: y_2 (*m/z* 192.9), b_2 (247.1), b_1 (*m/z* 130) and their corresponding complementary ions were also evident. Binding of glutathione in the complex can, in principle, be canonical (giving a charge-solvated structure) or zwitterionic in which the carboxylate anion (on either the γ-glutamic acid or glycine residue) is bound to copper(II) and the amino group is protonated (thereby resulting in a salt-bridge structure). The abundant loss of NH_3 from the complex strongly suggests that the peptide probably adopted the zwitterionic coordination mode. Furthermore, formation of all the other product ions can also be most easily rationalized in terms of fragmentation of a complex in which the peptide was zwitterionic. We also note that binding through the C-terminal carboxylate group (of the glycine residue) appears to have been prevalent, resulting in highly abundant $[Cu^{II}(tpy)(CysGly)]^{\bullet 2+}$ and $[Cu^{II}(tpy)(CysGly - NH_3)]^{\bullet 2+}$ ions (Fig. 1a).

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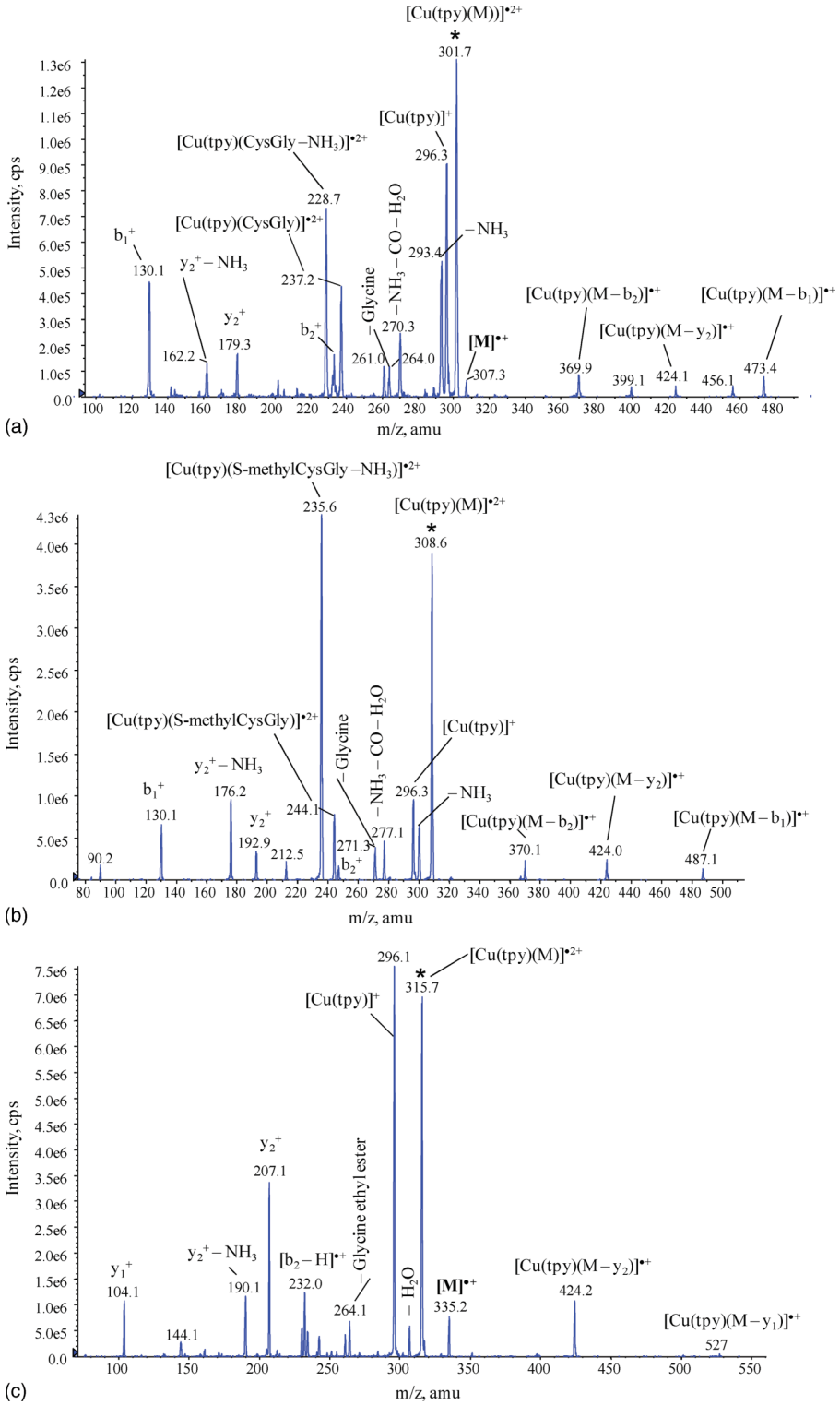

Fig. 1 CID spectra of (a) $[^{63}Cu^{II}(tpy)(GSH)]^{\bullet 2+}$; (b) $[^{63}Cu^{II}(tpy)(S\text{-}CH_3\text{-}GS)]^{\bullet 2+}$; (c) $[^{63}Cu^{II}(tpy)(GSH\text{-}OCH_2CH_3)]^{\bullet 2+}$: the precursor ion in each spectrum is labeled with an asterisk (*).

Our analysis is further supported by CIDs of the copper(II) complexes of two related GSH derivatives: S-methylglutathione (S-methyl-GS) and glutathione ethyl ester ($GSH\text{-}OCH_2CH_3$), the spectra of which are shown in Fig. 1b and 1c, respectively. Every dissociation reaction for $[Cu^{II}(tpy)(S\text{-methyl-GS})]^{\bullet 2+}$ appears to have an analogue for $[Cu^{II}(tpy)(GSH)]^{\bullet 2+}$; albeit that the (S-methyl-GS)$^{\bullet +}$ ion (*m*/*z* 321) is barely noticeable. By contrast, the CID of $[Cu^{II}(tpy)(GSH\text{-}OCH_2CH_3)]^{\bullet 2+}$ is much simpler, showing only y_1 and y_2 ions, and their complementary ions, plus the radical cation $(GSH\text{-}OCH_2CH_3)^{\bullet +}$ (*m*/*z* 335.2) (in relatively high abundance) and

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its complementary ion $[Cu^{I}(tpy)]^{+}$. Previous studies have shown that homolytic cleavage of the Cu^{II}-carboxylate binding results in a carboxy radical, which readily loses CO_2.[13] All spectra in Fig. 1 show no evidence of this loss. We attribute this to a facile rearrangement of the nascent carboxy radical to give the more stable sulfur-centered radical upon collisional activation (*vide infra*).

(b) CID of protonated S-nitrosoglutathione. CID of protonated S-nitrosoglutathione gives abundant $GSH^{\bullet+}$ *via* loss of $NO^{\bullet}$ (Fig. S1 in the ESI†). This is consistent with the results of previous studies on protonated nitrosylated derivatives of cysteine, cysteine methyl ester and cysteine-containing peptides[6,9–11] that showed loss of $NO^{\bullet}$ being the most energetically favorable dissociation pathway. Furthermore, very recent IRMPD studies on both the cysteine radical cation[10] and its methyl ester[11] have established that gas-phase homolysis of the S–NO bond gives radical cations in which the radical resides on the sulfur atom.

2. Dissociations of the radical cations

The GSH radical cations generated by the two aforesaid methods were mass-selected and subjected to a third stage of MS (see Fig. 2a–b). It is apparent that the CID spectra are almost identical, strongly suggesting that these two radical ions either readily interconverted prior to the fragmentation or that they had the same initial structure. The most abundant fragment ion is $[b_2 - H]^{\bullet+}$ at *m/z* 232.1, formed by loss of the glycine residue. This is substantiated by CID of the glutathione ethyl ester radical cation where glycine ethyl ester is lost (Fig. 3a). In addition, there is an almost equally abundant product ion at *m/z* 289 in the CID of $GSH^{\bullet+}$ (Fig. 2); this product was formed by eliminating H_2O. It is of note that prominent water loss is also observed in the CID of $[GSH\text{-}OCH_2CH_3]^{\bullet+}$, suggesting that the water lost may originate either from the N-terminal carboxylic group or from a backbone amide oxygen, as was found in a previous study on protonated peptides containing a cysteine residue.[31] Loss of water from the backbone is herein verified by the CID of glutathione dimethyl ester radical cation that was produced *via* CID of the protonated nitrosylated dimethyl ester (Fig. 3b), in which losses of both CH_3OH and H_2O were observed, with CH_3OH loss being predominant. It may also be of note that a minor second water loss giving ions at *m/z* 271 and *m/z* 299 was observed in the CIDs of glutathione (Fig. 2) and glutathione derivative radical cations (Fig. 3), respectively.

Other fragment ions of note in CID of the glutathione radical cation include the y_2 ion at *m/z* 179 (Fig. 2, shifted to *m/z* 207 for the ethyl ester in Fig. 3) and an ion at *m/z* 202 (Fig. 2, shifted to *m/z* 230 and *m/z* 216 for the ethyl ester and the dimethyl ester, respectively, in Fig. 3); the latter probably produced from the dehydrated $GSH^{\bullet+}$ ion, *m/z* 289 in Fig. 2, by eliminating 2-amino-2-propenoic acid, $CH_2{=}C(NH_2)COOH$, from the γ-glutamic acid residue. This product is most easily rationalized in terms of an intermediate in which water having been lost from the N-terminal (first) peptide bond of captodative $GSH^{\bullet+}$ (Scheme 1).

3. Computational investigations

To gain further insights into the experimental results, we used DFT calculations to examine profiles to interconversion between isomers of the glutathione radical cation and to establish fragmentation pathways.

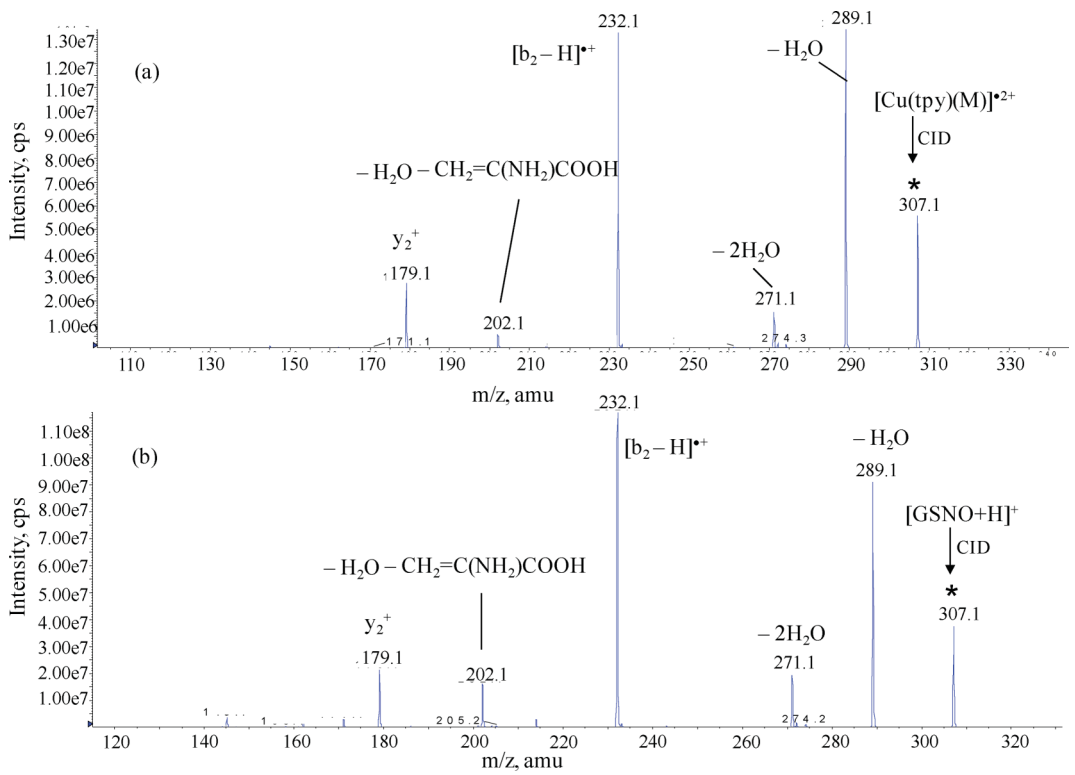

Fig. 2 CID spectra of glutathione radical cations: (a) generated from CID of $[Cu^{II}(tpy)(GSH)]^{\bullet2+}$; (b) generated from CID of protonated S-nitrosoglutathione. * signifies the precursor ion.

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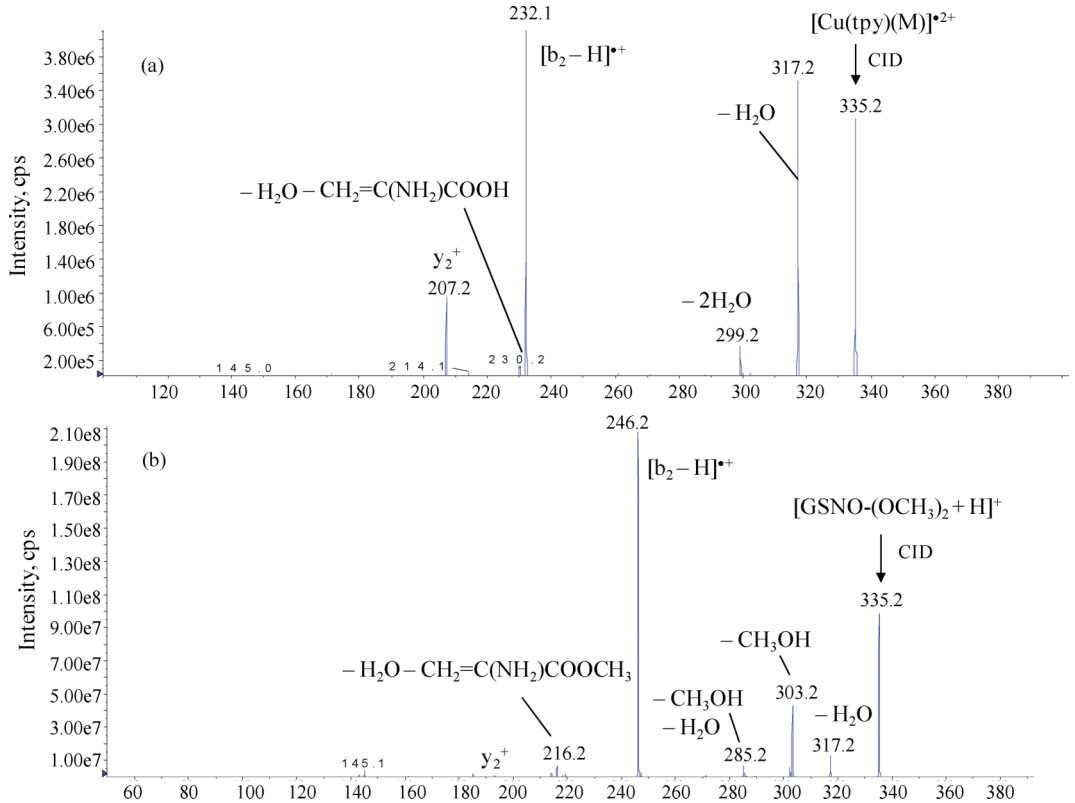
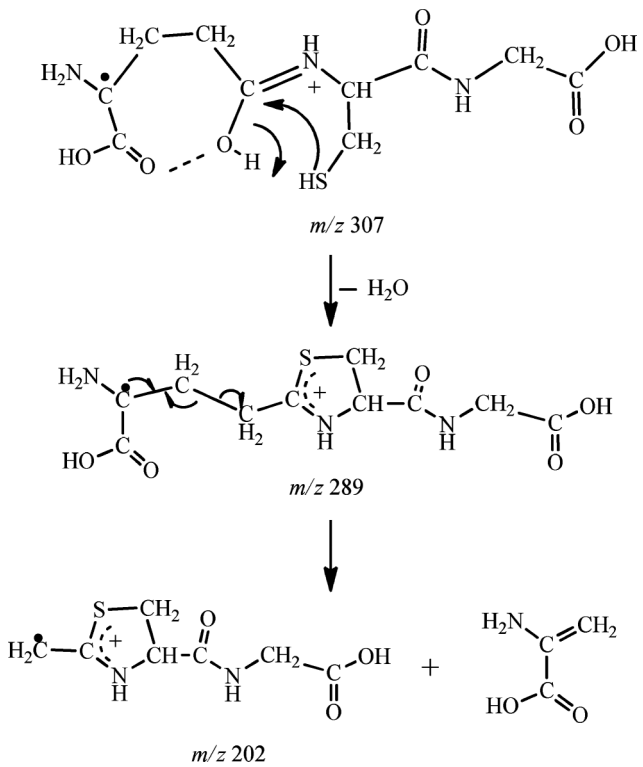


Fig. 3 CID spectra of (a) glutathione ethyl ester radical cation generated from CID of $[Cu^{II}(tpy)(GSH\text{-}OCH_2CH_3)]^{\bullet 2+}$; (b) glutathione dimethyl ester radical cation generated from CID of protonated nitrosylated glutathione dimethyl ester, * signifies the precursor ion.



Scheme 1

(a) Energies and structures of the glutathione radical cation. A survey of the potential energy surface of the glutathione radical cation revealed a number of low-lying classes of structures. The lowest energy structures for the four classes of radicals, the α-carbon-centred radical, the sulfur-centred radical, the carboxy radical and the canonical radical, are shown in Fig. 4. The N-terminal α-carbon-centred radical ion, **1**, is at the global minimum on the PES. In this structure, the radical centre is attached to a powerful π-donor, NH_2, and a π-acceptor, COOH. In addition, the proton located on the amide oxygen of the γ-linkage has a strong hydrogen bond to the carbonyl oxygen ($CO\text{–}H^+ \cdots O_{COOH}$ = 1.366 Å). This effectively transfers some of the positive charge onto the COOH group, thereby enriching the π-accepting ability of this group, which in turn enhances the stabilization due to the captodative effect. The other two isomeric α-carbon-centred radical ions, the middle α-carbon-centred radical ion, **2**, and the C-terminal α-carbon-centred radical ion, **3**, are higher in enthalpy than **1** by 8.8 and 7.4 kcal mol^{-1}, respectively. The second class of radicals, the sulfur-centred radical ion, **4**, in which the charge is formally localized on the NH_3 group and the spin on the sulfur atom, is higher in enthalpy than **1** by 8.5 kcal mol^{-1}. The remaining two classes, the canonical and the carboxy radicals, are all significantly higher in energy. The canonical ion, **5**, having the majority of the charge and spin on the sulfur atom, is 34.5 kcal mol^{-1} above **1** in enthalpy. Two possible carboxy radicals, denoted **6** and **7**, have the charge on the protonated amino group. Ion **6** is lower in enthalpy than **7** by 5.0 kcal mol^{-1}, but is higher than **1** by 31.5 kcal mol^{-1}. These two carboxy radicals could potentially be formed by dissociation of $[Cu^{II}(tpy)(GSH)]^{\bullet 2+}$ in which the carboxylate anion (either from the γ-glutamic acid or the glycine residue) is bound to copper(II). However, as noted

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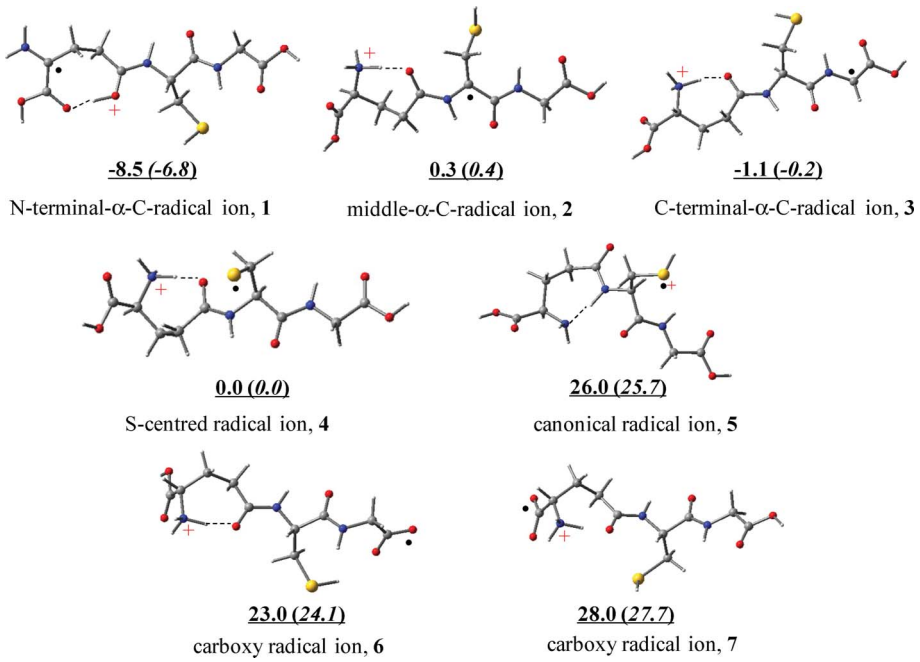

Fig. 4 B3LYP-optimized structures of the glutathione radical cation: enthalpies (kcal mol^{-1}) are shown in normal font; free energies (kcal mol^{-1}) are in italics.

above, the absence of any $(GSH - CO_2)^{\bullet+}$ ions suggests that ions **6** and **7** are kinetically unstable, probably due to hydrogen migration within the nascent ions as $GSH^{\bullet+}$ cleaves from the copper.

(b) Interconversion between isomers. As discussed above, the glutathione radical cations generated *via* the two methods gave virtually identical CID spectra, suggesting facile interconversion prior to fragmentation. We consider in this section the barriers against rearrangement between the four classes of radicals, and then compare these barriers against those for dissociation reactions. The energies of all structures are reported relative to the most stable S-centred radical ion, **4**.

The reaction profiles for interconversions between the isomers of the glutathione radical cation are given in Fig. 5a–c. The canonical radical ion, **5**, can undergo a 1,6-H^+ shift *via* **TS-1** to form a high-energy conformer of the sulfur-centred radical cation, **8**, in which the proton is located on the carbonyl oxygen of the N-terminal amide. This conversion is barrierless (Fig. 5a), which means that the canonical structure of $GSH^{\bullet+}$ is too fragile to be isolated experimentally. Conversion of ion **5** into a conformer of the captodative N-terminal α-carbon-centred radical cation, **1**, can occur by a mechanism in which the hydrogen of the α-carbon undergoes a 1,8-H atom shift to the sulfur, followed by a 1,6-H^+ shift to the carbonyl oxygen of the N-terminal amide. In this *one* step process, the sulfur atom acts as a catalyst, abstracting a hydrogen atom and then donating a proton. The barrier, 12.1 kcal mol^{-1}, against this reaction is clearly not competitive relative to rearrangement to the sulfur-centred radical cation, **8**.

Fig. 5b shows the reaction profiles for conversions of the carboxy radical cations, **6** and **7**, to the sulfur-centred radical cation, **4**. The calculated barriers against the 1,8-H shift *via* **TS-3**, and the 1,10-H shift *via* **TS-4**, to give **4** are only 5.1 and 0.5 kcal mol^{-1}, respectively. These low barriers are in accordance with the experimental observation that $GSH^{\bullet+}$ generated from $[Cu(tpy)(GSH)]^{\bullet2+}$ and that from protonated S-nitrosoglutathione give identical CID spectra. Presumably, ro-vibrationally hot carboxy radical cations **6** and **7** cleaved from $[Cu(tpy)(GSH)]^{\bullet2+}$ rapidly isomerize to the S-centred radical cation **4** due to the low barriers.

The energy profiles for isomerization of ion **4** to the three α-carbon radicals are shown in Fig. 5c. Only the highest barrier on each profile is displayed for the sake of tractability. Starting with the lowest-energy sulfur-centred radical, **4**, migration of the α-hydrogen from the γ-glutamic acid residue in **4** to the sulfur atom creates an N-terminal α-carbon radical ion, **9**, that is 3.5 kcal mol^{-1} higher in enthalpy than the structure at the global minimum, ion **1**. In a low energy first step, the proton migrates from NH_3^+ to the amide oxygen of the first peptide bond; this is followed by a higher energy 1,8-H atom shift from the α-carbon to the sulfur atom ***via* TS-5**, 18.1 kcal mol^{-1} above **4** in enthalpy. By comparison, migration of the α-hydrogen atoms of the second (middle) residue and the C-terminal residue to the sulfur radical *via* **TS-6** and **TS-7** requires 28.2 and 24.6 kcal mol^{-1}, respectively. This means these two latter pathways are energetically less favorable.

In summary, the profiles in Fig. 5 show that the canonical radical and the carboxy radical ions all collapse readily into the sulfur-centred radical ion **4**. This radical should be experimentally observable as it is sitting in a relatively deep well with the lowest barrier against rearrangement to the structure at the global minimum, the N-terminal α-carbon radical, being 18.1 kcal mol^{-1}.

(c) Fragmentation of the glutathione radical cation. It is apparent from the above discussion that the experimentally observed $GSH^{\bullet+}$ ion is predominantly the sulfur-centred radical ion **4**. As shown in Fig. 2, CID of $GSH^{\bullet+}$ gives the $[b_2 - H]^{\bullet+}$ ion as a major product. A plausible fragmentation mechanism is given in Fig. 6. The initial step involves isomerization of ion **4** to the N-terminal-α-carbon radical ion **9** (as shown in Fig. 5). This is followed by transfer of the proton to the amide nitrogen of the

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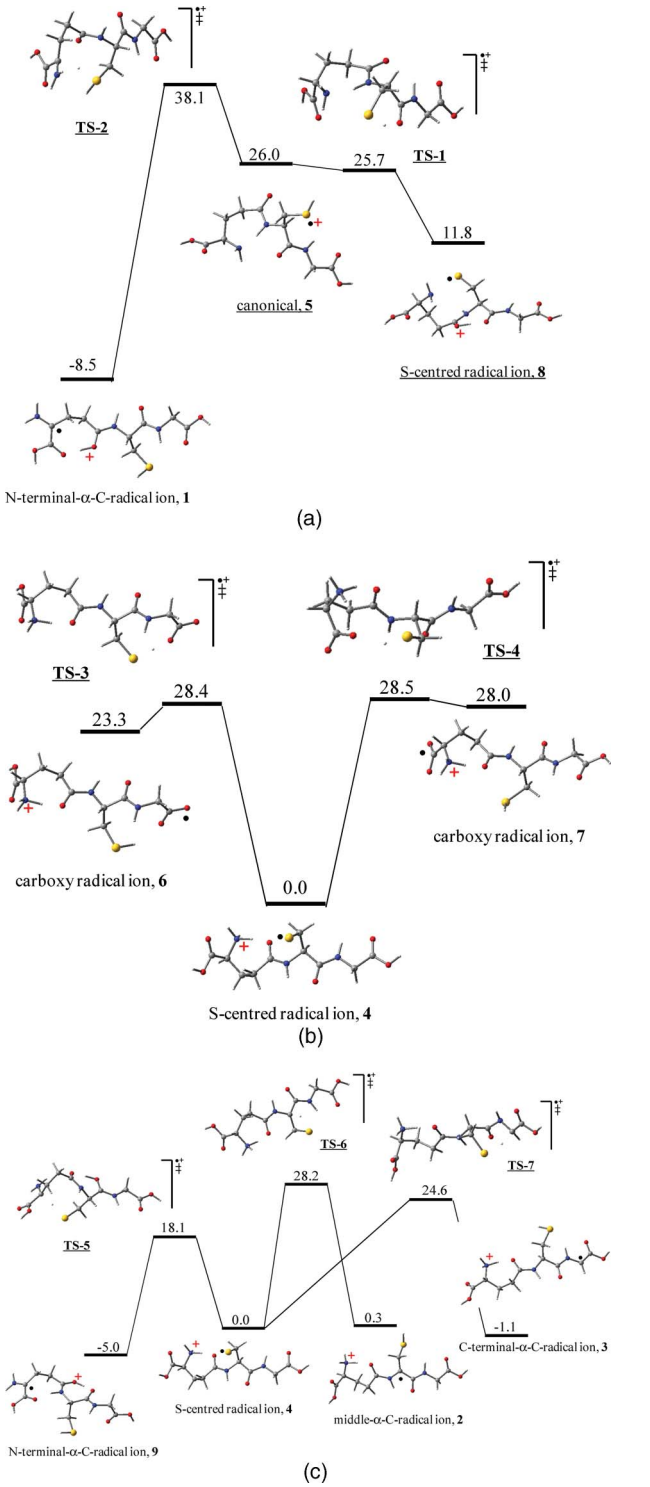

Fig. 5 Energy profiles for the interconversions (a) between the canonical radical ion and the N-terminal-α-carbon-centred radical ion; (b) between carboxy-centred radical ions and the sulphur-centred radical ion; (c) between the sulphur-centred radical ion and α-carbon-centred radical ions. (Enthalpies in kcal mol^{-1}).

C-terminal peptide bond *via* a 1,6-H^+ shift. Nucleophilic attack by the carbonyl oxygen of the N-terminal amide on the carbon of the C-terminal amide then ensues. Displacement of glycine gives a complex whose structure is best described as the nascent

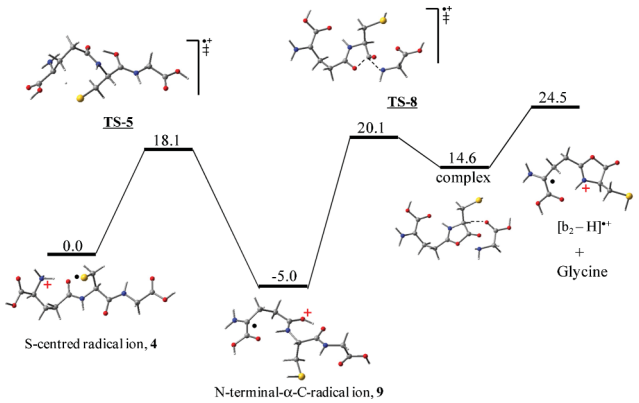

Fig. 6 Reaction profile for the loss of glycine from the glutathione radical cation. (Enthalpies in kcal mol^{-1}).

$[b_2 - H]^{\bullet+}$ ion being solvated by the departing glycine. Dissociation of this complex yields $[b_2 - H]^{\bullet+}$ and glycine with an overall endothermicity of 24.5 kcal mol^{-1} with respect to **4**. The $[b_2 - H]^{\bullet+}$ ion has a protonated oxazolone ring that carries the positive charge and a side-chain C_α radical that is stabilized by a captodative interaction created by the electron-donating amino group and the electron-accepting carboxy group, the latter of which is enhanced by a strong hydrogen bond with the positively charged protonated oxazolone ring. Starting from the radical cation at the global minimum, **1**, the endothermicity of the fragmentation reaction to give $[b_2 - H]^{\bullet+}$ is 33.0 kcal mol^{-1}, which is almost identical to the analogous barrier of 32.9 kcal mol^{-1} in the dissociation of $Gly^{\bullet}GlyGly^+$.[14]

As shown by our CID experiments (*vide supra*), elimination of H_2O from the glutathione radical cation to give the other major product ion at *m/z* 289 could occur from the N-terminal or C-terminal carboxyl group, or from the peptide backbone. Simplified energy profiles for these three possible routes, showing only the critical transition states, are given in Fig. 7a–b. Starting from the structure at the global minimum, **1**, rotation about the C–COOH bond in the γ-glutamic acid residue followed by migration of the proton from the carbonyl oxygen of the first amide bond to the OH group of the carboxylic group resulted in nucleophilic attack by the carbonyl oxygen of the γ-linkage with concomitant elimination of H_2O. The overall reaction has an activation enthalpy of 35.9 kcal mol^{-1} *via* **TS-9**. This is slightly higher than the endothermicity of 33.0 kcal mol^{-1} calculated for the formation of the $[b_2 - H]^{\bullet+}$ ion, starting from the structure at the global minimum, **1**. This small disagreement between the observed almost equal abundances for the two losses and the difference in calculated barriers is a little troublesome; the discrepancy is probably attributable to the inaccuracy of the DFT calculations. The critical barrier against elimination of H_2O from the C-terminal carboxylic group (to form an oxazolone-ring-containing product) *via* **TS-10** is 45.8 kcal mol^{-1}, thereby making this pathway energetically uncompetitive relative to the pathway *via* **TS-9**.

Fig. 7b shows the reaction profile for the elimination of water from a backbone peptide bond. Starting from the structure at the global minimum, **1**, transfer of the proton from the thiol to the carbonyl oxygen of the γ-glutamic acid residue with a concomitant attack on the carbonyl carbon by the sulfur forms the tetrahedral intermediate **10**. Proton migration from the carbonyl oxygen of the

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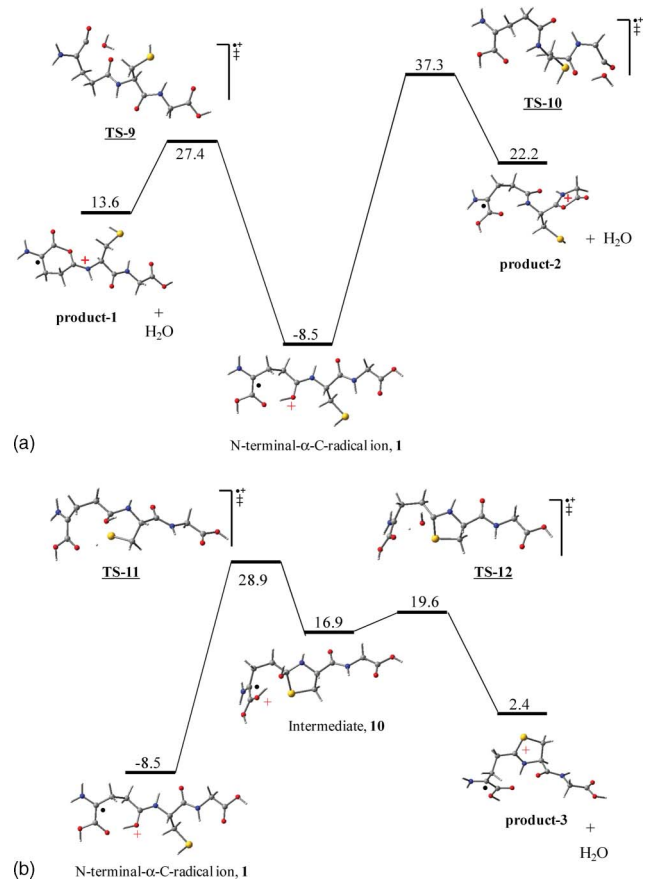

Fig. 7 Reaction profiles for the loss of H_2O from the glutathione radical cation. (Enthalpies in kcal mol^{-1}).

γ-glutamic residue to the hydroxyl oxygen, followed by cleavage of the C–OH_2^+, yields **product-3** and H_2O. The barrier of the rate-determining step against this multistep process is 37.4 kcal mol^{-1} relative to **1**, only 1.5 kcal mol^{-1} higher than the barrier against loss of water from the N-terminal carboxyl group (35.9 kcal mol^{-1} *via* **TS-9**). As typical errors in DFT calculations at the level of theory employed in this study are 2–3 kcal mol^{-1},[32] we conclude that the ion at *m*/*z* 289 is probably a mixture of products of water loss from the γ-glutamic acid residue and from the peptide backbone. The lowest-energy pathway against the loss of water has a calculated barrier of 35.9 kcal mol^{-1}, 2.9 kcal mol^{-1} higher than that against the formation of $[b_2 - H]^{\bullet+}$. Thus the relative barrier is consistent with experimental results shown in Fig. 3.

Conclusions

Glutathione radical cations have been produced by means of two different chemical methods: (1) collision-induced dissociation of $[Cu^{II}(tpy)(M)]^{\bullet 2+}$ and (2) gas-phase homolysis of the S–NO bond of protonated S-nitrosoglutathione. The radical cations generated *via* the two methods gave identical CID spectra, suggesting that either identical ions are formed or there is facile interconversion prior to fragmentation. DFT calculations show that isomerization of the canonical structure to the sulfur-centred radical is barrierless. Furthermore, the barriers against formation of the sulfur-centred radical from two possible carboxy radicals are very low (5.1 kcal mol^{-1} from the C-terminal radical and 0.5 kcal mol^{-1} from the γ-glutamic acid residue), thereby strongly suggesting that these carboxy radical ions are probably at best transient species in the mass spectrometer. The sulfur-centred radical is in a sufficiently deep potential well that it could be the ion observed experimentally. DFT calculations also show that the barrier against rearrangement from the sulfur-centred radical to isomerize to the structure at the global minimum, the α-carbon-centred radical, is 18.1 kcal mol^{-1}. The eliminations of H_2O from the N-terminal carboxylic group and the γ-linkage amide peptide bond are energetically competitive, but both are more favorable than that from the C-terminus. Both are slightly higher than the barrier against formation of the $[b_2 - H]^{\bullet+}$ ion. These predicted results are consistent with the experimental observations.

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