Radicals Hot Paper

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A Mass Spectrometric Approach for Probing the Stability of Bioorganic Radicals**

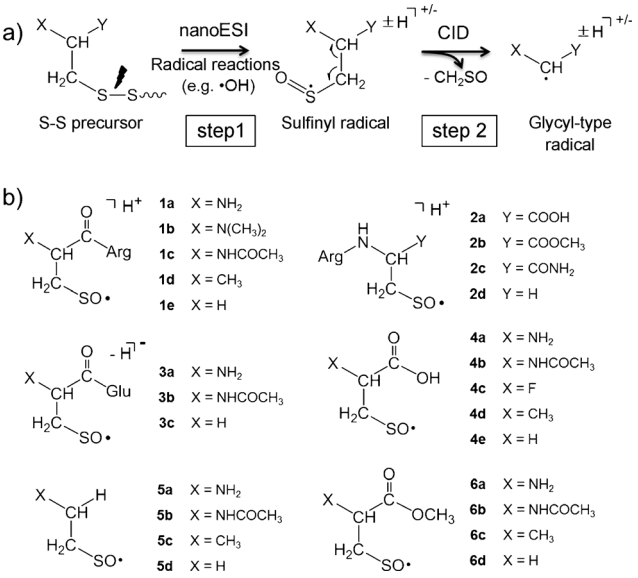
Lei Tan, Hanfeng Hu, Joseph S. Francisco, and Yu Xia**

Abstract: *Glycyl radicals are important bioorganic radical species involved in enzymatic catalysis. Herein, we demonstrate that the stability of glycyl-type radicals (X-·CH-Y) can be tuned on a molecular level by varying the X and Y substituents and experimentally probed by mass spectrometry. This approach is based on the gas-phase dissociation of cysteine sulfinyl radical (X-Cys$^{SO\cdot}$-Y) ions through homolysis of a C_α–C_β bond. This fragmentation produces a glycyl-type radical upon losing CH_2SO, and the degree of this loss is closely tied to the stability of the as-formed radical. Theoretical calculations indicate that the energy of the C_α–C_β bond homolysis is predominantly affected by the stability of the glycyl radical product through the captodative effect, rather than that of the parent sulfinyl radical. This finding suggests a novel experimental method to probe the stability of bioorganic radicals, which can potentially broaden our understanding of these important reactive intermediates.*

Bioorganic radicals have been implicated as important intermediates in a wide variety of biochemical processes. At the molecular level, they are associated with enzymatic digestion[1] and oxidative damage of proteins.[2] Among them, the glycyl radical that bears the -NH-·CH-C(O)- prototype has been of particular interest because of its outstanding stability[3] and its involvement in the catalytic function of many enzymes.[4] Moreover, selective formation of the glycyl radical is also implicated in the oxidative side-chain cleavage of other amino acid residues.[5] In several theoretical studies, the intrinsic thermochemical properties of relevant model systems have been investigated.[3] The synergistic effect known as captodative effect, in which the radical center is located between an electron donor and acceptor, has been postulated to greatly stabilize the radical.[6]

Experiments have been conducted to determine the stability of radical species from the electron spin resonance (ESR) coupling constant,[7] or free radical reactions toward *N*-bromosuccinimide.[8] Mass spectrometry has been demonstrated as an effective experimental methodology to interrogate the intrinsic property of many radical species in the gas phase.[9] Glycyl radicals have been successfully generated and characterized in the gas phase by neutralization–reionization mass spectrometry[10] and side-chain loss from collision-induced dissociation (CID) of hydrogen-deficient peptide radicals through β cleavage.[11] Chu et al. have studied the interconversion of the three isomeric α-carbon-centered radical ions of triglycine, and suggested that the stability of radicals can affect radical migration and thus the CID pattern of the species.[11a] In this study, we demonstrate a new experimental approach to probe the stability of the glycyl radical.

In previous studies, we have utilized radical reactions within the nanoelectrospray ionization (nanoESI) plume to generate gas-phase site-specific sulfinyl radical ions from the interchain disulfide-linked peptides (Scheme 1 a, step 1).[12] Unimolecular dissociation of protonated cysteine sulfinyl radical (Cys$^{SO\cdot}$) ions proceeds predominantly through a radical-driven fragmentation channel.[12b] Upon the loss of CH_2SO (sulfine) by homolysis of the C_α–C_β bond, glycyl radicals are formed (Scheme 1 a, step 2). In this work, we ask two fundamental questions: 1) Can the as-formed glycyl radical be stabilized by functional-group substitution, and



Scheme 1. a) Generation of gas-phase sulfinyl radical ions through radical reactions in the nanoESI plume (step 1) and fragmentation pathway to form the glycyl-type radical (step 2) upon CID. b) Sulfinyl radicals studied experimentally (**1**–**3**) and theoretically (**4**–**6**).

[*] L. Tan, H. Hu, Prof. J. S. Francisco, Prof. Y. Xia
Department of Chemistry, Purdue University
West Lafayette, IN 47907 (USA)
E-mail: francisc@purdue.edu
yxia@purdue.edu
Prof. J. S. Francisco
Department of Earth, Atmospheric, and Planetary Sciences
Purdue University
West Lafayette, IN 47907 (USA)

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2) can the degree of stabilization be experimentally probed for the prototype radical (X-·CH-Y) through gas-phase dissociation of the sulfinyl radical? To answer these questions, we surveyed a series of sulfinyl radical ions (X-Cys$^{SO\cdot}$-Y) functionalized with various electron-donating (X) or electron-withdrawing (Y) substituents (Scheme 1b, **1**–**3**). The impact of these substituents on the stability of thus formed glycyl radicals is evaluated by a combined experimental and theoretical approach.

The formation of sulfinyl radical ions was achieved through on-line radical reactions at the sampling interface of a mass spectrometer; the details of these experiments have been described previously and can be found in the Supporting Information. Briefly, oxidative radicals (presumably OH radicals), produced by discharges in the air, react with the disulfide precursors entrained in the nanoESI plume and cleave the disulfide bond through dissociative addition, leading to the formation of sulfinyl radicals (SO$^{\bullet}$).[12] The purity of the sulfinyl radicals (structures shown in Scheme 1, **1**–**3**) is high, the reaction yields are moderate ($\approx$40%). The sulfinyl radical ions are subjected to on-resonance CID in a linear ion trap mass spectrometer. The degree of CH_2SO loss (CH_2SO%: the percentage of CH_2SO loss among all product ions) is compared at a parent survival yield of 50% to keep a constant decomposition rate across different sulfinyl radical ions studied herein. The CH_2SO% from all experimentally studied sulfinyl radical ions is summarized in Table 1. The sulfinyl radical ions **1** were designed to test the effect of the X group for a generic structure of X-Cys$^{SO\cdot}$-C(O)-Arg. Arginine is included in the structure to reduce proton mobility in order to limit the proton-driven amide bond cleavages in the protonated peptide system.[12a] There is a significant drop in CH_2SO% (from 98% to 3%, experimental data in Table 1) as the electron-donating capability of the X group decreases among **1**. In case of strong electron-donating groups, such as X = NH_2 (**1a**) and X = $N(CH_3)_2$ (**1b**), the C_α–C_β bond cleavage is the only predominant fragmentation channel from CID (i.e., Figure 1a), corresponding to 98% of CH_2SO%. For X = $NHCOCH_3$ (**1c**), the acetylamino group is less effective in donating electrons as compared to NH_2. As a result, CH_2SO% drops to 66% and other fragmentation channels become more competitive (i.e., loss of SOH, Figure 1b). The loss of CH_2SO further decreased to 57% upon CID of **1d** (X = CH_3), likely because of the lack of lone pair electrons to donate from the methyl group. The most dramatic change in fragmentation behavior is observed when X = H (**1e**, Figure 1c), where the loss of SOH (−49 Da) is prevalent while the loss of CH_2SO is almost negligible (3%). The effect of the Y group was tested using sulfinyl radical ions **2** (Arg-NH-Cys$^{SO\cdot}$-Y). When the Y group is a carbonyl substituent (-COOH, -$COOCH_3$, or -$CONH_2$, all of which are considered to be moderately electron-withdrawing), the loss of CH_2SO is always the predominant channel upon CID (CH_2SO% = 90–85%). Not surprisingly, for Y = H (**2d**), CH_2SO% is reduced significantly to 24%. The above data clearly demonstrate the critical role of the electron-donating and electron-withdrawing nature of the substituents as well as their synergistic effect in the formation of glycyl-type radicals upon CID of sulfinyl radical ions.

Although the Arg side-chain can efficiently sequester a proton to its guanidine group, we cannot exclude the possibility that the charge is in close proximity to the radical site through ionic hydrogen bonding,[13] and thus alternates the behavior of the radical. Studies have shown that hydrogen bonds are likely to form between the protonated guanidine and amide carbonyl group, which can assist in magnifying the electron-withdrawing capability of the carbonyl group.[11b] In order to evaluate the effect of the charge, sulfinyl radical

Table 1: CH_2SO% observed in experiments and BDEs calculated theoretically.

Experiment[a]		Theoretical Calculation					
	CH_2SO%		-X	-Y	BDE[b]	$RSE_{CH\cdot}$[b]	$RSE_{SO\cdot}$[b]
1a	*98*	**4a**	NH_2	COOH	35.2	21.3	3.4
1b	98						
1c	*66*	**4b**	acetyl-amino	COOH	41.0	18.3	2.5
1d	*57*	**4d**	CH_3	COOH	45.4	10.3	3.5
1e	*3*	**4e**	H	COOH	49.6	4.7	3.6
2a	90						
2b	*90*	**6b**	acetyl-amino	$COOCH_3$	42.2	18.0	2.6
2c	85						
2d	*24*	**5a**	NH_2	H	45.9	10.4	3.3
3a	50	**4a**	NH_2	COOH	35.2	21.3	3.4
3b	15	**4b**	acetyl-amino	COOH	41.0	18.3	2.5
3c	1	**4e**	H	COOH	49.6	4.7	3.6

[a] Corresponding spectra provided in Figure 1 and Figures 2 and 3 in the Supporting Information. [b] In kcal mol^{-1}.

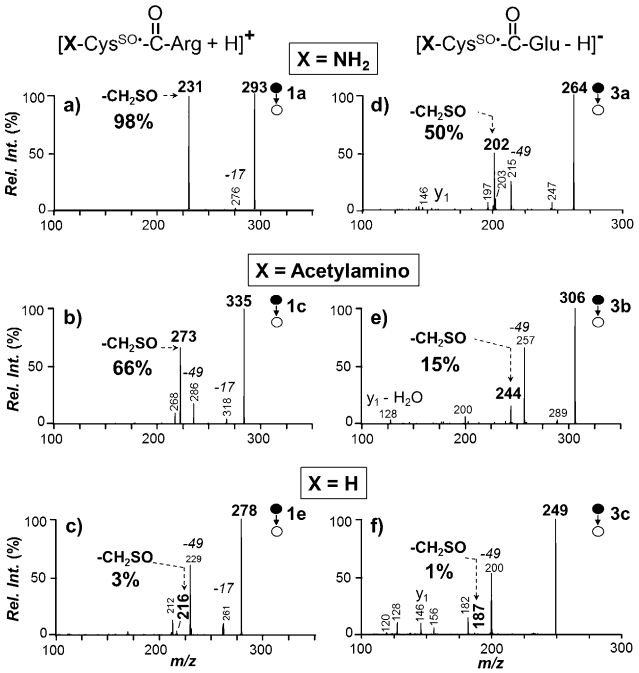
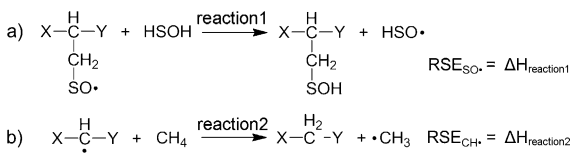


Figure 1. MS^2 CID of protonated sulfinyl radical cations X-Cys$^{SO\cdot}$-C(O)-Arg: a) **1a**; b) **1c**; and c) **1e**. MS^2 CID of deprotonated sulfinyl radical anions X-Cys$^{SO\cdot}$-C(O)-Glu: d) **3a**; e) **3b**; f) **3c**.

anions are also investigated (**3**, X-Cys$^{SO\cdot}$-C(O)-Glu). For these anionic species, loss of 62 Da is also observed to various degrees (Figure 1 d–f). Accurate mass measurement confirmed the elemental composition of CH_2SO and thus it is not the sequential loss of H_2O and CO_2. Similar to the radical cations, the CH_2SO% also drops as the electron-donating capability of the X group decreases (Table 1, **3a**–**c**). Note that the sulfine loss of radical anions is not as favorable as in radical cations, probably because of the absence of proton-induced enhancement of the electron-withdrawing nature of the carbonyl group as in the protonated species. Nevertheless, the data from both sulfinyl radical cations and anions indicate that the CH_2SO% is not significantly affected by the nature of the charge.

Since the loss of CH_2SO is a single-bond fission process upon the unimolecular dissociation of the cysteine sulfinyl radical, the fragmentation energy barrier is directly affected by the bond dissociation energy (BDE) of the C_α–C_β bond. We therefore calculated the BDE of the C_α–C_β bond in sulfinyl radical systems, as defined by the enthalpy change in step 2 shown in Scheme 1 a.[12b] Given that the BDE is affected by the stability of both parent and product radicals, we further characterized the relative stability of these radical species based on the radical stabilization energy (RSE).[14] Isodesmic reactions shown in Scheme 2 were used to calculate the RSE for sulfinyl ($RSE_{SO\cdot}$) and glycyl ($RSE_{CH\cdot}$) radicals, respectively.



Scheme 2. Isodesmic reactions for the calculation of the RSE.

Simplified structures (Scheme 1, **4**–**6**) were used as model systems in the theoretical calculations. The theoretical results based on each experimentally tested group are summarized in Table 1, and the rest of the calculated data are shown in Table 1 in the Supporting Information. The $RSE_{CH\cdot}$ varies from 0 to 21.3 kcal mol^{-1} as the substituent changes toward a more significant captodative effect (in the increasing order of X = H, CH_3, acetylamino, NH_2, and Y = H, $COOCH_3$, COOH). The tendency is consistent with previous theoretical studies on the stability of radicals and agrees with the prediction of the captodative effect.[3] On the other hand, the value of the $RSE_{SO\cdot}$ is relatively small and varies little with the identity of the X or Y groups (2.5–3.8 kcal mol^{-1}). This result suggests that the stability of the sulfinyl radical is not significantly affected by the substituents on C_α. Our previous study has shown that the spin is localized on the sulfinyl group (SO•; with almost equal probability on the sulfur and oxygen atom).[12b] The isolation of the spin density on sulfinyl radical and its further separation from the substituents may lead to the inconspicuous relationship between the $RSE_{SO\cdot}$ and the substituents on C_α.

Figure 2 a shows the plot of the $RSE_{SO\cdot}$ and the $RSE_{CH\cdot}$ versus the BDE with the identities of the X and Y groups

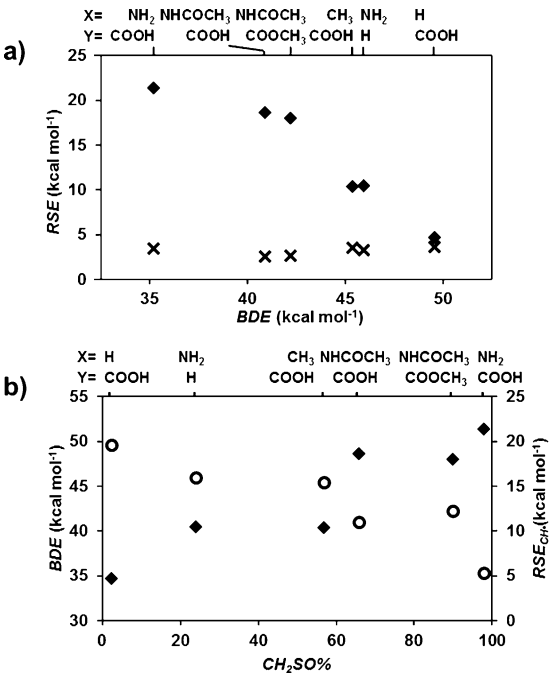


Figure 2. a) Plot of $RSE_{CH\cdot}$ (diamond) and $RSE_{SO\cdot}$ (cross) against BDE, and b) anti-correlation of BDE (open circle) and $RSE_{CH\cdot}$ (diamond) versus experimental CH_2SO%.

indicated correspondingly. Clearly, as the BDE increases, the $RSE_{CH\cdot}$ decreases, while there is no obvious correlation between the BDE and the $RSE_{SO\cdot}$. These data support the argument that the change in the BDE is largely a result of the RSE gained by forming the glycyl radical. Since CH_2SO% can be directly linked to the value of the BDE in the case of single-bond fission, this finding thus suggests that the stability of the as-formed glycyl radical can be directly probed experimentally through the C_α–C_β bond homolysis in the sulfinyl radical without considering the substituent effect of the parent radical. Such a relationship is depicted in Figure 2 b with an anti-correlation between the $RSE_{CH\cdot}$ and the BDE with CH_2SO%. Note that the experimental CH_2SO% data points in Figure 2 b are chosen from radical cations that have been theoretically evaluated (italicized in Table 1, column 2). An increased CH_2SO% corresponds to a lower BDE and a higher $RSE_{CH\cdot}$. It is this relationship that allows the stability of the product radical to be evaluated experimentally by monitoring the degree of CH_2SO loss upon CID.

In summary, a new approach based on gas-phase unimolecular dissociation of sulfinyl radicals (X-Cys$^{SO\cdot}$-Y) to probe the stability of glycyl-type radicals (X-$^{\cdot}$CH-Y) was demonstrated. The degree of sulfine loss increases correspondingly as the stability of the as-formed radical species increases, which can be tuned on a molecular level through the electronic effects of X or Y groups. Meanwhile, the stability of the parent sulfinyl radical is not significantly affected. This intrinsic property and the unique fragmentation pathway of the sulfinyl radical offer a direct way to explore the stability of radicals experimentally. It also allows us to investigate the effect introduced by different substituents in more detail than merely using the RSE to study the stability of radicals, and

also sheds light on the influence of the electronic nature of the connecting groups on the captodative effect in bioorganic radicals.

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