Glycyl Radical Is a Stable Species in the Gas Phase
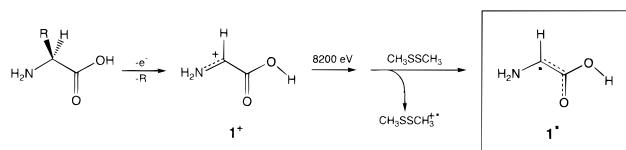
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Radicals derived from amino acids, peptides, and proteins have been of long-standing interest in radiation chemistry and biology.[3] The recent discovery of enzymes such as ribonuclease reductases[4] and pyruvate formate lyase,[5] which catalyze radical reactions under anaerobic conditions and involve reactive protein intermediates, attracted considerable attention to the properties of amino acid radicals.[6] Although radicals derived from glycine have been studied by electron-spin resonance spectroscopy[4b,7] and ab initio calculations,[8] the *intrinsic properties* of the prototypical 2-glycyl radical, $H_2N-CH^\bullet-COOH$ (**1**), have not been elucidated experimentally.[9] In this Communication, we report the first unambiguous preparation of radical **1** in the gas phase and study the thermochemistry of its unimolecular dissociations.

We used collisional neutralization of the stable 2-glycyl cation (**1**$^+$) to generate radical **1** in the rarefied gas phase and to study its properties by neutralization–reionization mass spectrometry (NRMS).[10] NRMS relies on femtosecond electron transfer from a thermal electron donor, such as trimethylamine or dimethyl disulfide,[10d] to a fast precursor ion of kiloelectronvolt kinetic energy. The structure of the precursor ion is thus important, because it determines the structure of the nascent radical. The internal energy of the radical is composed of the internal energy of the precursor ion and the Franck–Condon energy acquired upon vertical electron transfer.[11] The precursor ion (**1**$^+$) was generated by dissociative ionization of several amino acids, e.g., valine, serine, leucine, isoleucine, and phenylalanine (Scheme 1). An α-cleavage of the $R-CH(NH_2)COOH$ bond favored the formation of **1**$^+$ + R$^\bullet$ against the complementary products **1** +

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(3) See, for example: Bonifacic, M.; Stefanic, I.; Hug, G. L.; Armstrong, D. A.; Asmus, K.-D. *J. Am. Chem. Soc.* **1998**, *120*, 9930 and references therein.
(4) (a) Sun, X.; Harder, J.; Krook, M.; Sjoberg, B.-M.; Reichard, P. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 577. (b) Mulliez, E.; Fontecave, M.; Gaillard, J.; Reichard, P. *J. Biol. Chem.* **1993**, *268*, 2296. (c) Sun, X.; Eliasson, R.; Pontis, J.; Andersson, J.; Buist, G.; Sjoberg, B.-M.; Reichard, P. *J. Biol. Chem.* **1995**, *270*, 2443.
(5) (a) Knappe, J.; Neugebauer, F. A.; Blaschkowski, H. P.; Ganzler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1332. (b) Parast, C. V.; Wong, K. K.; Lewisch, S. A.; Kozarich, J. W. *Biochemistry* **1995**, *34*, 2393.
(6) For a recent comprehensive review, see: Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705.
(7) Volker, V.; Wagner, A. F.; Frey, M.; Neugebauer, F. A.; Schafer, W.; Knappe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 996.
(8) (a) Yu, D.; Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 1789. (b) Barone, V.; Adamo, C.; Grand, A.; Subra, R. *Chem. Phys. Lett.* **1995**, *242*, 351. (c) Barone, V.; Adamo, C.; Grand, A.; Jlibois, F.; Brunel, Y.; Subra, R. *J. Am. Chem. Soc.* **1995**, *117*, 12618. (d) Himo, F.; Eriksson, L. A. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 305.
(9) A recent paper reported on collisional neutralization of (M − H)$^+$ ions from glycine. (O'Hair, R. A. J.; Blanksby, S.; Styles, M.; Bowie, J. H. *Int. J. Mass Spectrom.* **1999**, *182/183*, 203.) Spurious peaks in the published spectrum indicate that those ions probably consisted of a mixture of isomers.
(10) For recent reviews of the technique, see: (a) Turecek, F. *J. Mass Spectrom.* **1998**, *33*, 779. (b) Schalley, C. A.; Hornung, G.; Schroder, D.; Schwarz, H. *Chem. Soc. Rev.* **1998**, *27*, 91. (c) Goldberg, N.; Schwarz, H. *Acc. Chem. Res.* **1994**, *27*, 347. (d) Turecek, F. *Org. Mass Spectrom.* **1992**, *27*, 1087.
(11) Nguyen, V. Q.; Turecek, F. *J. Mass Spectrom.* **1996**, *31*, 843.
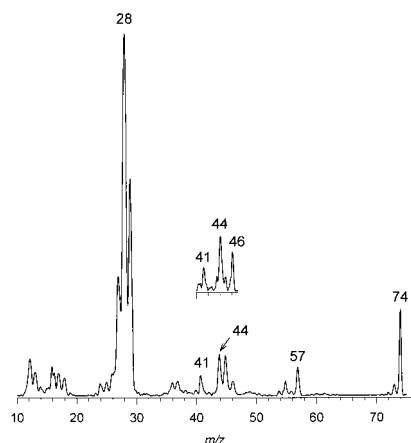
Scheme 1

R$^+$ [12] and gave rise to an abundant ion **1**$^+$. The glycyl ion from phenylalanine ($C_2H_4NO_2$, measd 74.0236, calcd 74.0242) was accompanied by a minor (1.5%) $C_6H_2^{+\bullet}$ ion (measd 74.0155, calcd 74.0156).[14] Ions **1**$^+$ were characterized by collision-induced dissociation spectra[15] and theoretical calculations,[16,17] as discussed below.

Collisional neutralization of **1**$^+$ (Scheme 1), followed by reionization and mass spectrometric analysis,[18] afforded the spectrum shown in Figure 1. The spectrum showed a substantial peak at *m/z* 74, corresponding to undissociated **1**$^+$. This implied that a fraction of intermediate radicals **1** survived the time (4.1 μs) between the neutralizing and ionizing collisions. Hence, isolated radical **1** was a stable species that had a microsecond lifetime. The dissociations upon NR of **1**, which can originate from the neutral radical **1** (Scheme 2) and/or reionized **1** and its fragments, resulted in the formation of $H_2NCH=C=O$ (*m/z* 57, loss of OH), CH_4NO (*m/z* 46, loss of CO), COOH (*m/z* 45, loss of H_2N-C-H), CO_2 (*m/z* 44, loss of H_2NCH_2), H_2N-C-H (*m/z* 29), HC=NH (*m/z* 28), and HCN (*m/z* 27). In addition, small peaks at *m/z* 49, 37, and 36 appeared in the NR mass spectrum of **1**$^+$ from phenylalanine that were assigned to the minor $C_6H_2^{+\bullet}$ contaminant. From the reference NR mass spectrum of $C_6H_2^{+\bullet}$,[19] the $C_6H_2^{+\bullet}$ ion coinciding with **1**$^+$ contributed <20% to the signal intensity at *m/z* 74.

The structure and dissociation energetics of **1** were addressed by combined density functional theory[16] and ab initio calculations up to the G2(MP2) level of theory (Table 1).[17] Four rotamers of **1** have been identified as local energy minima by B3LYP/6-31+G(d,p) geometry optimizations.[20] The optimized geometry of the most stable rotamer, anti-syn-**1**,[21] differed only little from

(12) The relevant ionization energies for CH$_3$$^\bullet$, 2-C$_3H_7$$^\bullet$, 2-C$_4H_9$$^\bullet$, CH$_2OH^\bullet$, and C$_6H_5CH_2$$^\bullet$, 9.84, 7.37, 7.25, 7.56, and 7.249 eV, respectively,[13] favor the positive charge on **1**$^+$, IE$_a$(**1**) = 6.90 eV by G2(MP2) calculations (this work).
(13) Mallard, W. G., Linstrom, P. J. Eds. *NIST Chemistry Webbook*; NIST Standard Reference Database No. 69; National Institute of Standards and Technology: Gaithersburg, MD, March 1998; http://webbook.nist.gov/chemistry.
(14) From accurate mass measurements under high-resolution conditions, *M*/Δ*M* >10 000.
(15) The CID spectra were obtained on a JEOL HX-110 instrument as B/E linked scans with air as collision gas at 50% ion beam transmittance. **1**$^+$: *m/z* (rel intensity, %ΣI$_{CID}$) 57(4.4), 56(3.4), 55(1), 54(0.7), 46 (16.7), 45(12.3), 44(3.4), 41(2.9), 29(12.6), 28(36.6), 27(6.1). [**1**-D$_3$]$^+$: *m/z* (rel intensity, %ΣI$_{CID}$) 59(4.8), 57(2.1), 49(11.8), 46(6.6), 44(1.2), 41(2.1), 31(15.7), 30(8.8), 29(22.4), 28(5.4), 27(1.2).
(16) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372, 5648. (b) Stephens, P. J.; Devlin, F. J.; Chablowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623.
(17) (a) Curtiss, L. A.,; Raghavachari, K.; Pople, J. A. *J. Chem. Phys.* **1993**, *98*, 1293. (b) Curtiss, L. A.; Raghavachari, K.; Redfern, P. C.; Pople, J. A. *J. Chem. Phys.* **1997**, *106*, 1063. (c) Raghavachari, K.; Stefanov, B. B.; Curtiss, L. A. *J. Chem. Phys.* **1997**, *106*, 6764.
(18) For experimental details of NRMS measurements, see: (a) Sadilek, M.; Turecek, F. *J. Phys. Chem.* **1996**, *100*, 224. (b) Polce, M. J.; Cordero, M. M.; Wesdemiotis, C.; Bott, P. A. *Int. J. Mass Spectrom. Ion Processes* **1992**, *113*, 35.
(19) The NR mass spectrum of C$_6$H$_2$$^{+\bullet}$ from 1,2,4,5-tetrachlorobenzene gave the following ion relative intensities: *m/z* (%ΣI$_{NR}$) 74(26.3), 73(8.1), 72(3), 61(2), 49(6.4), 48(1.7), 38(3.4), 37(11.5), 36(11.5), 25(3), 24(11), 13(2.1), 12(8).
(20) Previous calculations of Yu et al.[8a] found only the anti-syn and syn-syn conformers of **1**.
(21) The syn, anti, and gauche assignments refer to 0°, 180°, and twisted dihedral angles of the H$_2$N−C−C−OH, and H−O−C=O systems in this order. Detailed optimized structures are available from the corresponding author upon request and will be published in a full paper.

Figure 1. Neutralization (CH_3SSCH_3, 70%T)−reionization (O_2, 70%T) mass spectrum of 1^+ from phenylalanine. Inset shows a region in the NR mass spectrum of $[1\text{-}D_3]^+$.

Scheme 2

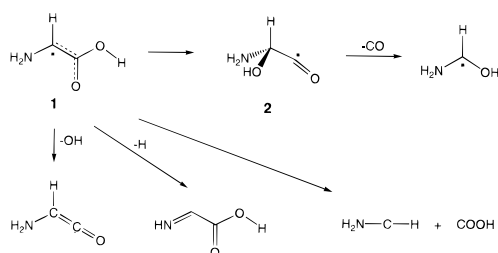**Table 1.** Relative and Dissociation Energies of Glycyl Radicals and Cations

radicals	relative energy^{a,b}		cations	relative energy^{a,b}	
	B3 LYP^c	G2 (MP2)		B3 LYP^c	G2 (MP2)
anti-syn-**1**	0	0	anti-syn-**1**$^+$	0	0
anti-syn-**1** (VN)^d	39		anti-syn-**1**$^+$(VI)^e	50	
syn-syn-**1**	6	6	syn-syn-**1**$^+$	8	9
anti-anti-**1**	23	21	anti-anti-**1**$^+$	38	36
gauche-anti-**1**	44	40	gauche-anti-**1**$^+$	51	57
2	145	121	**3**$^+$	57	59
TS(1 → 2)	278	310	$H_2N-CH-OH^+$ + CO	−6	−13
$H_2N-CH-OH^\bullet$ + CO	155	129	$HC=N-H\cdots OH_2^+$ + CO	79	60
$HN=CH-C=O^\bullet$ + H_2O	182	165	$HC=NH^+$ + HCOOH	165	160
$HN=CH-COOH$ + $H^\bullet$	228	197	$HC=NH^+$ + CO + H_2O	204	175
TS(H$\cdots$NH=CH−COOH)	231	207	$H_2N-CH=C=O^{+\bullet}$ + $OH^\bullet$	366	404
$H_2N-CH=C=O$ + $OH^\bullet$	316	316	H_2N-C-H + $COOH^+$	419	424
H_2N-C-H + $COOH^\bullet$	328	324	$H_2N-C-H^{+\bullet}$ + $COOH^\bullet$	422	444

^{a} In kJ mol^{-1}. ^{b} At 0 K, including B3LYP/6-31+G(d,p) zero-point corrections. ^{c} From single-point energies with the 6-311+G(2df,p) basis set. ^{d} Vertical neutralization of the cation. ^{e} Vertical ionization of the radical.

that of the most stable ion conformer, anti-syn-1^+. In particular, the C−C bond was slightly longer in the ion (1.525 and 1.430 Å for 1^+ and **1**, respectively), while the N−C bond was shorter (1.278 and 1.361 Å for 1^+ and **1**, respectively). This most likely reflected the electrophilic nature of the C-2 carbocation, which

increased the bond order of the N−C bond by participation of the nitrogen lone electron pair. Conjugation with the carboxylate π-system of the semioccupied π-orbital at C-2 in **1** increased the order of the C−C bond and decreased that of the C−O bond, which resulted in changes of the corresponding bond lengths.[8a] The Franck−Condon energies upon vertical electron capture in 1^+ and vertical ionization of **1** were moderate, 39 and 50 kJ mol^{-1}, respectively.

Elimination of CO to give the $H_2N-CH^\bullet-OH$ radical was the lowest-energy dissociation of **1** (Table 1). The elimination was likely to proceed through an isomer (**2**, Scheme 2), which was 121 kJ mol^{-1} less stable than anti-syn-**1**. However, the isomers were separated by a 310 kJ mol^{-1} energy barrier (**TS(1 → 2)**, Table 1). Elimination of water to give $HN=CH-C^\bullet=O$ was another low-energy dissociation of **1** (Table 1). Loss of the syn-amine hydrogen atom was the most favorable simple-cleavage dissociation of **1** (Table 1). In addition to its intrinsic endothermicity, the loss of H showed a small activation barrier, 10 kJ mol^{-1} above the products. Dissociations of the C−OH and C−C bonds were both more endothermic, requiring 316 and 324 kJ mol^{-1}, respectively. The calculated dissociation energies confirmed that **1** was a bound species in the gas phase that required a substantial internal energy for unimolecular dissociation and isomerization. Note that dissociations of **1** cannot be driven solely by the Franck−Condon energy acquired by vertical neutralization but would require contribution from the internal energy of the precursor ion.

Four ion rotamers were found for 1^+, e.g., anti-syn-1^+, syn-syn-1^+, anti-anti-1^+, and gauche-anti-1^+ (Table 1). In contrast to the recent report by O'Hair et al.,[9] we found anti-syn-1^+ to be the most stable rotamer at all levels of theory. The lowest-energy dissociation of anti-syn-1^+ was elimination of CO to give the stable hemiaminal ion, H_2N-CH^+-OH, corresponding to protonated formamide. The latter reaction was slightly exothermic at the thermochemical threshold (Table 1). An attempted optimization of a presumed intermediate acylium ion, $H_2N-CH(OH)-C=O^+$ (2^+), resulted in a dissociation by loss of CO. Hence, structure 2^+ was not supported at the present level of theory. Because of the low threshold for the elimination of CO, the stability of 1^+ must be due to an energy barrier to OH group migration that precedes CO expulsion. Another isomer possibly formed by CO elimination from 1^+, $HC=N-H^+\cdots OH_2$, was 73 kJ mol^{-1} less stable than H_2N-CH^+-OH. Other dissociations of 1^+ to form $HC=NH^+$, $H_2N-CH=C=O^{+\bullet}$, $COOH^+$, and $H_2N-C-H^{+\bullet}$ were all substantially endothermic (Table 1).[22] Some of these dissociations may proceed through another isomer, $HN=CH-C(OH)_2^+$ (3^+), which was calculated to be 59 kJ mol^{-1} less stable than 1^+.

In conclusion, 2-glycyl radical (**1**) was generated by collisional neutralization of the stable 2-glycyl cation and shown to be stable on the microsecond time scale. Losses of CO, water, and an amine hydrogen atom were calculated to be the lowest-energy dissociations of **1**. However, even these lowest-energy unimolecular dissociations required substantial threshold energies and are, therefore, predicted to be very slow in thermalized **1** at room temperature. Depletion of glycyl radicals in biological systems most likely occurs via bimolecular reactions.[23]

(22) The ion energetics was also investigated with MP2/6-311+G(2df,p) calculations, which yielded bond dissociation energies which were of the same order as and within ± 25 kJ mol^{-1} of the B3LYP/6-311+G(2df,p) data.

(23) Himo, F.; Eriksson, L. A. *J. Am. Chem. Soc.* **1998**, *120*, 11449.