

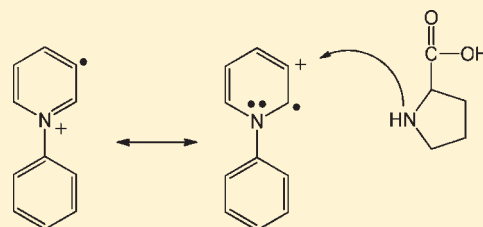
Reactivity and Selectivity of Charged Phenyl Radicals toward Amino Acids in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

George O. Pates, Leonard Guler, John J. Nash, and Hilkkka I. Kenttämaa*

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

S Supporting Information

ABSTRACT: The reactivity of 10 charged phenyl radicals toward several amino acids was examined in the gas phase in a dual-cell Fourier transform ion cyclotron resonance mass spectrometer. All radicals abstract a hydrogen atom from the amino acids, as expected. The most electrophilic radicals (with the greatest calculated vertical electron affinities (EA) at the radical site) also react with these amino acids via NH₂ abstraction (a nonradical nucleophilic addition–elimination reaction). Both the radical (hydrogen atom abstraction) and nonradical (NH₂ abstraction) reaction efficiencies were found to increase with the electrophilicity (EA) of the radical. However, NH₂ abstraction is more strongly influenced by EA. In contrast to an earlier report, the ionization energies of the amino acids do not appear to play a general reactivity-controlling role. Studies using several partially deuterium-labeled amino acids revealed that abstraction of a hydrogen atom from the α-carbon is only preferred for glycine; for the other amino acids, a hydrogen atom is preferentially abstracted from the side chain. The electrophilicity of the radicals does not appear to have a major influence on the site from which the hydrogen atom is abstracted. Hence, the regioselectivity of hydrogen atom abstraction appears to be independent of the structure of the radical but dependent on the structure of the amino acid. Surprisingly, abstraction of two hydrogen atoms was observed for the *N*-(3-nitro-5-dehydrophenyl)pyridinium radical, indicating that substituents on the radical not only influence the EA of the radical but also can be involved in the reaction. In disagreement with an earlier report, proline was found to display several unprecedented reaction pathways that likely do not proceed via a radical mechanism but rather by a nucleophilic addition–elimination mechanism. Both NH₂ and ¹⁵NH₂ groups were abstracted from lysine labeled with ¹⁵N on the side chain, indicating that NH₂ abstraction occurs both from the amino terminus and from the side chain. Quantum chemical calculations were employed to obtain insights into some of the reaction mechanisms.



INTRODUCTION

Attack of radicals on proteins is known to cause proteins to fragment, oxidize, denature, and lose enzymatic activity.^{1,2} The size and complexity of proteins make it difficult to obtain knowledge on these processes at the molecular level. Hence, many solution studies aimed at improving the understanding of the reactivity of radicals toward proteins have been carried out by using small peptides or individual amino acids as the substrates.^{1,2}

One of the most thoroughly studied radicals is the OH radical. In aqueous solution, the OH radical reacts with most amino acids predominantly via hydrogen atom abstraction and with aromatic amino acids via addition to the aromatic ring.^{3,4} The hydrogen atom abstraction occurs from the α-carbon or from the side chain of the amino acid (abstraction of a hydrogen atom from the side chain is preferred over abstraction from the α-carbon for amino acids with side chains), and it corresponds to the major reaction pathway for amino acids with aliphatic non-sulfur-containing side chains.⁵ For the sulfur-containing amino acids methionine and cysteine, the OH radical attacks the sulfur atom. Abstraction of a hydrogen atom from the SH group in cysteine by the OH radical has also been observed.^{3,4}

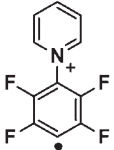
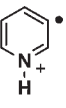
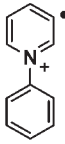
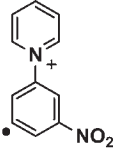
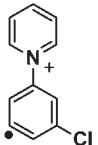
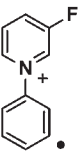
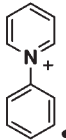
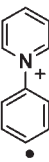
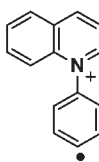
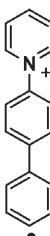
While the reactions of the OH radical and other oxygen-centered radicals in solution have been extensively investigated,³ the reactivity of carbon-centered σ-type organic radicals, such as the phenyl radical, toward amino acids and proteins is nearly entirely unexplored.^{6a} To the best of our knowledge, only one such study has appeared in the literature—and in this study, only glycine was examined.^{6a} This lack of studies is due to the difficulty in cleanly generating carbon-centered organic radicals in solution.^{7–9} However, these reactions are of great interest since organic radicals and biradicals released by some drugs and antitumor antibiotics, such as the enediynes, are known to attack proteins, in addition to other biopolymers.¹⁰

Our laboratory has advanced the “dystonic ion approach” for the investigation of the reactivity of phenyl radicals in the gas phase,^{11,12} that is, via the addition of a chemically inert charged group (to form a dystonic radical ion) that allows the manipulation of the radical in mass spectrometers, such as a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.^{11,12}

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Chart 2. Structures and Calculated Vertical EAs²⁰ of the Positively Charged Phenyl Radicals Studied

					
	a	b	c	d	e
EA (eV)	6.18	6.11	5.78	5.40	5.11
					
	f	g	h	i	j
EA (eV)	5.05	4.87	4.38	4.05	3.31

cell was about 1×10^{-5} Torr. The ions were collisionally activated with argon for 0.5–1 s at a frequency 1000 Hz above the cyclotron frequency of the ions. All other radicals were generated using a similar procedure except that different precursors were used: 3-iodopyridine for **b**, 3-iodopyridine and bromobenzene for **c**, pyridine and 3,5-dinitroiodobenzene for **d**, pyridine and 3,5-dichloroiodobenzene for **e**, 3-fluoropyridine and 1,3-diiodobenzene for **f**, pyridine and 1,3-diiodobenzene for **g**, pyridine and 1,4-diiodobenzene for **h**, quinoline and 1,4-diiodobenzene for **i**, and pyridine and 4,4'-diiodobiphenyl for **j**.

The radicals of interest were isolated by ejecting all other ions via the application of a series of SWIFT excitation pulses to the plates of the cell. The isolated charged phenyl radicals were allowed to react with each amino acid for a variable period of time (typically 0.5–1000 s). Detection was performed by using “chirp” excitation of 124 V amplitude, 2.7 MHz bandwidth, and 3.2 kHz/ms sweep rate. All of the spectra are the average of 10 transients, which were recorded as 64k data points and subjected to one zero fill prior to Fourier transformation. Each reaction spectrum was background corrected by using a procedure described previously.¹⁸

In the FT-ICR mass spectrometer, the concentration of ions (in this case, charged monoradicals) inside the cell is much smaller than that of neutral molecules (amino acid molecules). Hence, the concentration of the amino acid can be assumed to be constant. Indeed, all the reactions studied followed pseudo-first-order kinetics, which allowed for the derivation of the second-order reaction rate constant (k_{exp}) from a semilogarithmic plot of the relative abundance of the reactant ion versus reaction time and the concentration of the amino acid. The pressure readings inside the cell were measured by two ionization gauges located on each side of the dual cell. The ion gauge pressure readings were corrected for the sensitivity of the ion gauges toward each amino acid and for the pressure gradient between the ion gauge and the cell. The correction factors were obtained by measuring the reaction rate of an exothermic proton transfer reaction from protonated acetone or protonated methanol to the given amino acid.¹⁹ Such reactions can be expected to occur at the collision rate.²⁰ The accuracy of the measured rate constants is estimated to be about 50%, with the precision estimated to be better than 20%. The theoretical collision rate constants (k_{coll})

were calculated using a parametrized trajectory theory.²¹ The efficiency of each reaction (the fraction of collisions that leads to reaction) is given by $k_{\text{exp}}/k_{\text{coll}}$. The primary products' relative abundances are reported as branching ratios, which are given as the ratio of a primary product ion's abundance to the sum of all primary product ions' abundances.

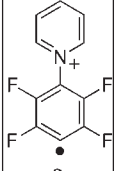
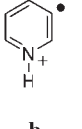
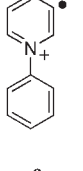
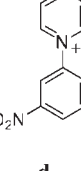
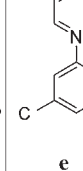
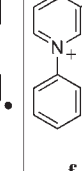
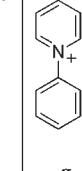
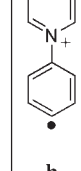
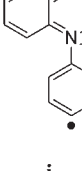
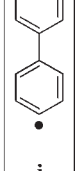
All amino acids (purity $\geq 98.5\%$), except for DL-lysine- ϵ -¹⁵N and DL-glycine-2,2- d_2 , were obtained from Fluka Biochemika. DL-Lysine- ϵ -¹⁵N (purity $\geq 98.5\%$, isotopic purity (¹⁵N) 98+%) was obtained from Cambridge Isotope Laboratories, Inc. and DL-glycine-2,2- d_2 (purity $\geq 98.5\%$, isotopic purity (D) 98%) from Sigma-Aldrich. DL-Proline-1- d_1 was synthesized from L-proline by using racemization via a Schiff base procedure.²² This procedure involves selective exchange of the α -carbon hydrogen atom of L-proline with deuterium by racemization of this stereogenic center (α -carbon) in monodeuterated acetic acid (CH₃CO₂D). A vial with 500 mg of L-proline was refluxed with 0.04 mL of salicylaldehyde and 9.4 mL of monodeuterated acetic acid at 100 °C for 1 h. The deuterated amino acid was recrystallized. The product's structure was verified by ¹H NMR and mass spectrometry (isotopic purity 95 atom % D).

Quantum chemical calculations were performed with the Gaussian 98²³ electronic structure program suite. Molecular geometries for the radicals were calculated as previously described.^{12c} Charge and spin densities for the radicals were calculated at the UBPW91/cc-pVDZ level of theory. Relative transition-state energies for addition of ammonia to different positions in the 3-dehydropyridinium ion were calculated at the UHF/6-31G(d,p)//UHF/6-31G(d,p) level of theory.

RESULTS AND DISCUSSION

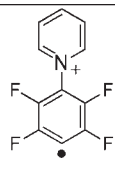
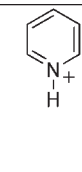
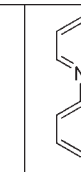
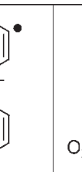
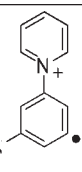
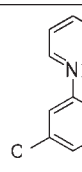
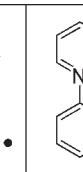
The 10 positively charged phenyl radicals used in this study (a–j) are shown in Chart 2. These radicals were generated by using literature methods.^{11,12} The isolated charged phenyl radicals were allowed to react with L-glycine, DL-glycine-2,2- d_2 , L-lysine, ¹⁵N-labeled DL-lysine, L-isoleucine, L-leucine, DL-leucine-1- d_1 , L-proline, and DL-proline-1- d_1 (Chart 1) for variable periods of time to determine the reaction efficiencies and product branching ratios (Tables 1–5).

Table 1. Reaction Efficiencies (Eff.) and Product Branching Ratios for Reactions of Monoradicals a–j with Glycine and Glycine-2,2-d₂

											
	EA ^a (eV)	6.18	6.11	5.78	5.40	5.11	5.05	4.87	4.50	4.05	3.31
L-glycine MW = 75	NH ₂ abs. ^b (%)	96 ^c	79 ^c	57 ^c	0	0	0	0	0	0	0
	H abs. (%)	4 ^c	21 ^c	43 ^c	100	100	100	100	100	100	0
	Eff. (%)	50 ^c	22 ^c	8.5 ^c	0.9	0.5	0.2	0.1	0.1	0	0
DL-glycine-(2,2-d ₂) MW = 77	NH ₂ abs. (%)	98 ^c	87 ^c	63.5 ^c	0	0	0	0	0	0	0
	H abs. (%)	2 ^c	6 ^c	18.5 ^c	72	83	54	71	59	100	0
	D abs. (%)	0.5 ^c	7 ^c	17.5 ^c	28	17	46	29	41	0	0
	Eff. (%)	53 ^c	25 ^c	10 ^c	0.9	0.4	0.2	0.1	0.1	0 ^d	0

^a Calculated vertical EAs from ref 12c. ^b abs. = abstraction. ^c Data taken from refs 12b and 12c. ^d The efficiency (%) is actually 0.00003.

Table 2. Reaction Efficiencies (Eff.) and Product Branching Ratios for Reactions of Monoradicals a–g with L-Leucine, DL-Leucine-1-d₁, and L-Isoleucine

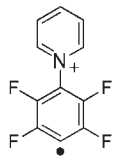

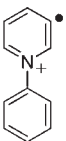
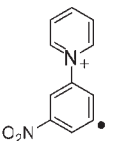
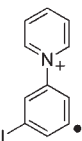
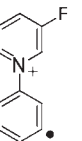
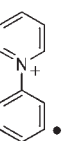
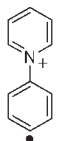
								
	EA ^a (eV)	6.18	6.11	5.87	5.40	5.11	5.05	4.87
L-leucine MW = 131	NH ₂ abs. ^b (%)	73	proton transfer only	28	0	0	0	0
	H abs. (%)	27		72	100	100	100	100
	Eff. (%)	55		45	33	16	16	15
DL-leucine-(1-d ₁) MW = 132	NH ₂ abs. (%)	60	proton transfer only	27	0	0	0	0
	H abs. (%)	31		70	100	100	100	100
	D abs. (%)	0		3	0	0	0	0
	unknown (%) (abs. 17 Da)	9		0	0	0	0	0
	Eff. (%)	55		48	36	17	15	10
L-isoleucine MW = 131	NH ₂ abs. (%)	64	proton transfer only	25	0	0	0	0
	H abs. (%)	36		75	100	100	100	100
	Eff. (%)	57		43	36	16	13	19

^a Calculated vertical EAs from ref 12c. ^b abs. = abstraction.

The ionic avoided curve crossing model^{14b,c} predicts a strong relationship between the vertical EA (the energy released upon attachment of an electron to the radical site with no geometry change) of an electrophilic radical and its gas-phase radical reactivity.²⁴ The higher the EA of the radical, the faster the

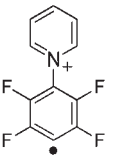

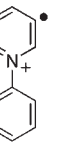
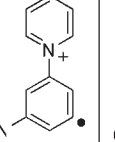
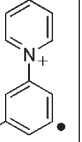
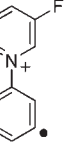
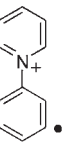
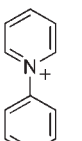
reactions are predicted to be. This has been found to be true for many reactions. Hence, favorable polarization of the transition state appears to be a crucial controlling factor in these reactions.²⁴ Consequently, radicals with widely different EA values, ranging from 3.31 to 6.18 eV (Chart 2), were chosen

Table 3. Reaction Efficiencies (Eff.) and Product Branching Ratios for Reactions of Monoradicals a–h with L-Lysine and DL-Lysine- ϵ - ^{15}N

									
	EA ^a (eV)	a 6.18	b 6.11	c 5.87	d 5.4	e 5.11	f 5.05	g 4.87	h 4.50
L-lysine MW = 146	NH ₂ abs. ^b (%)	62	proton transfer only	24	0	0	0	0	0
	H abs. (%)	38		44	45	100	100	100	100
	2 H abs. (%)	0		0	55	0	0	0	0
	addition (%)	0		32	0	0	0	0	0
	Eff. (%)	46		49	40	21	25	22	8
DL-lysine- ϵ - ^{15}N MW = 147	NH ₂ abs. (%)	53	proton transfer only	21	0	0	0	0	0
	$^{15}\text{NH}_2$ abs. (%)	18		9	0	0	0	0	0
	H abs. (%)	29		47	66	100	100	100	100
	2 H abs. (%)	0		0	34	0	0	0	0
	addition (%)	0		23	0	0	0	0	0
		Eff. (%)	42		39	38	19	24	24

^a Calculated vertical EAs from ref 12c. ^b abs. = abstraction.

Table 4. Reaction Efficiencies (Eff.) and Product Branching Ratios for Reactions of Monoradicals a–h with L-Proline and DL-Proline-1- d_1

									
	EA ^a (eV)	a 6.18	b 6.11	c 5.87	d 5.4	e 5.11	f 5.05	g 4.87	h 4.50
L-proline MW = 115	NH ₂ abs. ^b (%)	0	proton transfer only	see table	0	0	0	0	0
	H abs. (%)	100		5	64	100	100	100	100
	2 H abs. (%)	0			36	0	0	0	0
		Eff. (%)	92			44	22	21	19
DL-proline-(1- d_1) MW = 116	NH ₂ abs. (%)	0	proton transfer only	see table	0	0	0	0	0
	H abs. (%)	100		5	58	100	100	98	93
	2 H abs. (%)	0			42	0	0	0	0
	D abs. (%)	0			0 ^c	0	0	2	8
		Eff. (%)	92			46	24	22	18

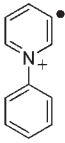
^a Calculated vertical EAs from ref 12c. ^b abs. = abstraction. ^c Distinguishing between D atom abstraction (2.014102 amu) and 2 H atom abstraction (2.01588 amu) is difficult due to the closeness of their masses and the detection limits of the mass spectrometer.

for this study. The vertical EAs of the radicals were calculated earlier at the (U)B3LYP/6-31+G(d) level of theory.^{12c}

The amino acids L-glycine, L-lysine, L-isoleucine, and L-leucine react with the radicals predominantly by hydrogen atom transfer and addition–elimination pathways (e.g., NH₂ transfer). The less electrophilic radicals only exhibit hydrogen atom abstraction,

while the more electrophilic radicals also show NH₂ abstraction. The relative reactivity of the radicals was found to depend on their EA, and was independent of the types of reactions observed. This is intriguing as these reactions occur via drastically different mechanisms. While hydrogen atom transfer is a radical reaction, the mechanism for NH₂ transfer is thought to be a nucleophilic

Table 5. Reaction Efficiencies (Eff.) and Product Branching Ratios for Reactions of Monoradical *c* with L-Proline and DL-Proline-1-*d*₁

		 c
	EA ^a (eV)	5.87
L-proline MW = 115	NH ₂ abs. ^b (%)	0
	H abs. (%)	73
	HO abs. (%)	4
	C ₂ H ₄ N abs. (%)	12
	addition-OH (C ₃ H ₇ NO abs.) (%)	6
	addition (%)	5
	Eff. (%)	42
DL-proline-(1- <i>d</i> ₁) MW = 116	NH ₂ abs. ^b (%)	0
	H abs. (%)	56
	D abs. (%)	6
	HO abs. (%)	12
	C ₂ H ₄ N abs. (%)	12
	addition-OH (C ₃ H ₇ NO abs.) (%)	7
	addition (%)	7
	Eff. (%)	46

^a Calculated vertical electron affinity from ref 12c. ^b abs. = abstraction.

addition–elimination reaction rather than a radical reaction (illustrated for lysine in Scheme 1 and discussed in detail below).^{12b} In general, for the radicals and amino acids studied here, more NH₂ abstraction and less hydrogen atom abstraction were observed as the EA of the radical increased. This finding is in agreement with a literature report that, as the EA of a radical increases, its addition reactions become faster than hydrogen atom abstraction.¹¹ While NH₂ abstraction is the most common group abstraction observed, other group abstraction products were observed for reactions of L-proline and DL-proline-1-*d*₁ with radical *c*. The reactivities observed for the different amino acids are discussed in detail below.

On the basis of studies on polar effects in radical reactions,^{12c} amino acids with lower ionization energies (IEs) are expected to react faster via hydrogen atom transfer than those with higher IE values. The preliminary study on a smaller group of amino acids supported this expectation.^{12b} However, in the present study, only some of the radicals were found to follow this trend. For example, although radical *e* abstracts a hydrogen atom fastest from proline (IE = 8.3 eV) and slowest from glycine (IE = 8.9 eV), radical *a* reacts at similar efficiencies with glycine and lysine (IE = 8.6 eV), in spite of their quite different IE values.

L-Glycine and DL-Glycine-2,2-*d*₂. The more electrophilic radicals *a*–*c* display two pathways upon reaction with glycine, namely, hydrogen atom abstraction and NH₂ abstraction (Table 1). The greater the EA of the radical, the more the NH₂ abstraction is favored and the greater the total reaction efficiency. The less electrophilic radicals *d*–*i* react with L-glycine solely by hydrogen atom abstraction (Table 1). This finding is in agreement with previous studies^{12b} that have shown that radicals *d* and *e* only react by hydrogen atom abstraction with glycine.

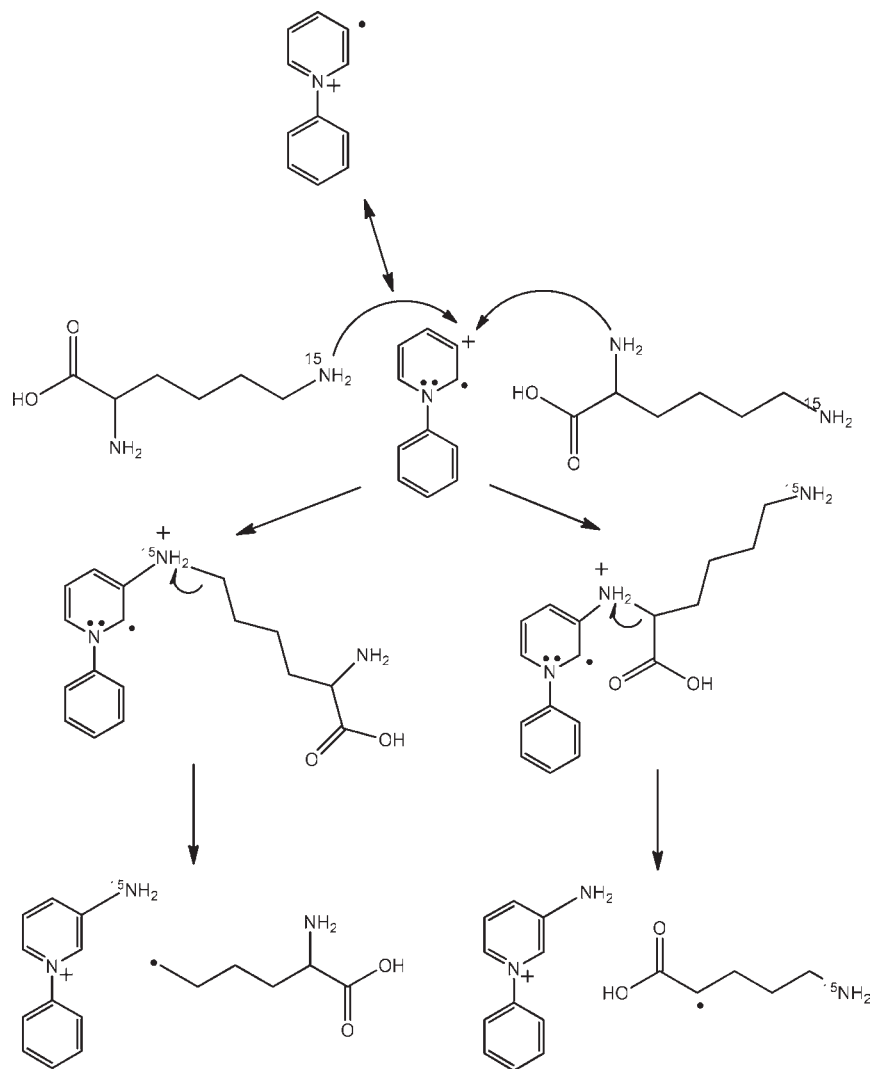
The hydrogen atom abstraction efficiency of radicals *d*–*i* increases with increasing EA of the radical.

No reactions were observed for radical *j*. This lack of reactivity is rationalized by its very low EA of 3.31 eV (the other radicals have EAs ranging from 4.50 to 6.18 eV). In agreement with the present findings, previous studies^{12c} have shown that radical *j* abstracts a hydrogen atom from cyclohexane (IE = 10.32 eV) and isopropyl alcohol (IE = 10.44 eV) only at very low reaction efficiencies of 0.0034 and 0.0029, respectively.

Previous examination of the reactions of OH radicals with glycine in solution has shown that hydrogen atoms can be abstracted from either the NH₂ or OH group in glycine, in addition to the α -carbon, which is thermodynamically favored.²⁵ This occurs in spite of the fact that the homolytic C–H bond dissociation energy in glycine (79.2 kcal/mol) is lower than that of the N–H bond (102.6 kcal/mol), which is substantially lower than that of the O–H bond (112.9 kcal/mol).²⁶

Glycine-2,2-*d*₂ was allowed to react with radicals *a*–*j* to obtain a better understanding of which position is preferred for hydrogen atom abstraction from glycine. All these reactions, except those of radicals *a*–*c*, occur via both hydrogen and deuterium atom abstraction (NH₂ abstraction was also observed for radicals *a*–*c*, Table 1), and hydrogen atom abstraction dominates over deuterium atom abstraction in most cases. Hence, hydrogen atom abstraction also occurs from other sites besides the α -carbon. Since abstraction of a deuterium atom is likely to be slower than of a hydrogen atom from the same site due to the primary kinetic isotope effect,^{24b} abstraction from the α -carbon is seemingly less favored than it actually is.

For the more electrophilic monoradicals *a*–*c*, a trend was observed between the EA of the radical and hydrogen/deuterium

Scheme 1. Possible Mechanisms for the NH₂ Abstraction Reactions of Radical **c** with Lysine-ε-¹⁵N

atom vs NH₂ abstractions (as the EA of the radical decreases, the extent of both deuterium and hydrogen atom abstractions increases relative to that of NH₂ abstraction; however, the total reaction efficiency goes down). This competition between hydrogen/deuterium abstraction and NH₂ abstraction is not present for radicals **d–j**, where hydrogen and deuterium atom abstractions are the only observed reaction pathways. When NH₂ group abstraction is not observed, there is no correlation between EA and deuterium vs hydrogen atom abstraction. A similar finding was reported earlier for ethanol.^{24a} However, a correlation has been reported in the literature for 2-propanol (less electrophilic radicals are more selective for hydrogen atoms bound to the α -carbon).^{24b}

For a given radical, abstraction of a hydrogen atom from the α -carbon rather than from the NH₂ group is not only thermodynamically but also kinetically favored. Previously calculated transition-state energies for radicals **a–c**, **e**, and **g** show that the transition state for hydrogen atom abstraction from the α -carbon in glycine is 2–3 kcal/mol^{12b} lower than from the NH₂ group (calculations were not performed for the OH group). However, both the present and previous results show that the α -carbon in glycine is not the site from which a hydrogen atom is abstracted.^{12b}

The site at which the initial attack occurs in the NH₂ abstraction reaction pathway is likely the radical site since this readily leads to a homolytic C–N bond cleavage in the amino acid (Scheme 1), and charged aromatic compounds with no radical sites do not undergo this reaction.^{12b} This is in spite of the fact that, for different radicals, the radical site may or may not be the most electrophilic site. This was explored by calculating the charge densities for 2-, 3-, and 4-dehydropyridinium cations at the UBPW91/cc-pVDZ//UBPW91/cc-pVDZ level of theory (Figure 1). For 2-dehydropyridinium cation (radical site charge density 0.162) and 4-dehydropyridinium cation (radical site charge density 0.126), the radical sites are the most electrophilic sites. Thus, for these two isomers, the radical site is the preferred site for nucleophilic attack. For 3-dehydropyridinium cation, the radical site (charge density 0.033) is less electrophilic than the 2-position (charge density 0.076) or the 4-position (charge density 0.087; in spite of a smaller positive charge, the 2-position may be favored over the 4-position for nucleophiles that can form a stabilizing hydrogen bond with the NH⁺ group). Although the 2- and 4-positions are calculated to be the most electrophilic sites, addition also must occur at the 3-position since a homolytic C–N bond cleavage was observed to occur for the adduct.

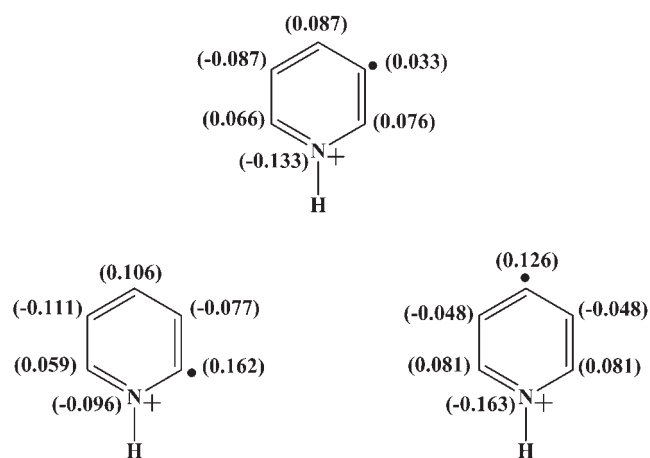


Figure 1. Calculated (UBPW91/cc-pVDZ//UBPW91/cc-pVDZ) charges for heavy atoms of 2-, 3-, and 4-dehydropyridinium cations.

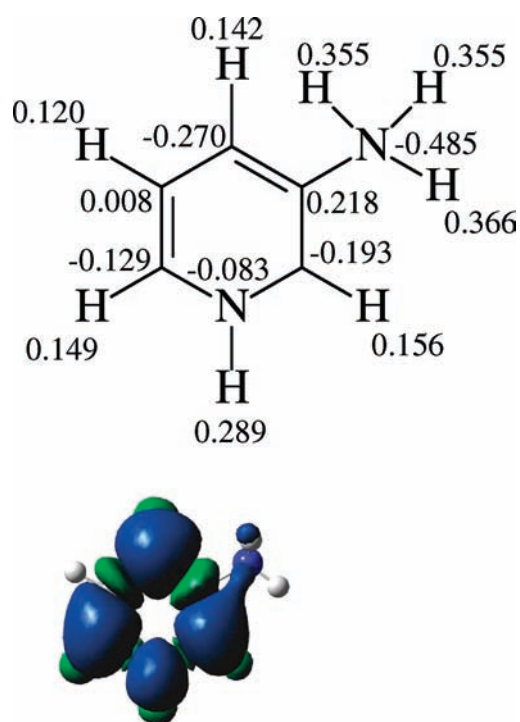


Figure 2. Calculated (UBPW91/cc-pVDZ//UBPW91/cc-pVDZ) charges (top) and spin density (bottom) for the NH_3 adduct of 3-dehydropyridinium cation.

The relative transition-state energies calculated at the UHF/6-31G(d,p)//UHF/6-31G(d,p) level of theory for addition of ammonia (instead of an amino acid to simplify the calculations) to 3-dehydropyridinium cation are 0.5 kcal/mol (C-2), 0.0 kcal/mol (C-3), 1.4 kcal/mol (C-4), 19.5 kcal/mol (C-5), and 2.5 kcal/mol (C-6). Thus, nucleophilic attack at the 3-position has the lowest transition-state energy, making addition of ammonia to the 3-position kinetically favored for radical **b**, although additions to the other sites may also occur. Figure 2 shows the calculated charge and spin densities for the ammonia adduct of 3-dehydropyridinium cation. On the basis of these results, it seems likely that most of the positive charge resides on

the hydrogen atoms bound to the nitrogen atom, and the odd spin is delocalized over the pyridinium ring, in agreement with the Lewis structure presented for an analogous adduct in Scheme 1. These findings suggest that the nucleophilic attack of amino acids to the charged phenyl radicals, leading to NH_2 group abstraction, occurs at the radical site and yields an adduct with the odd spin delocalized over the pyridine ring.

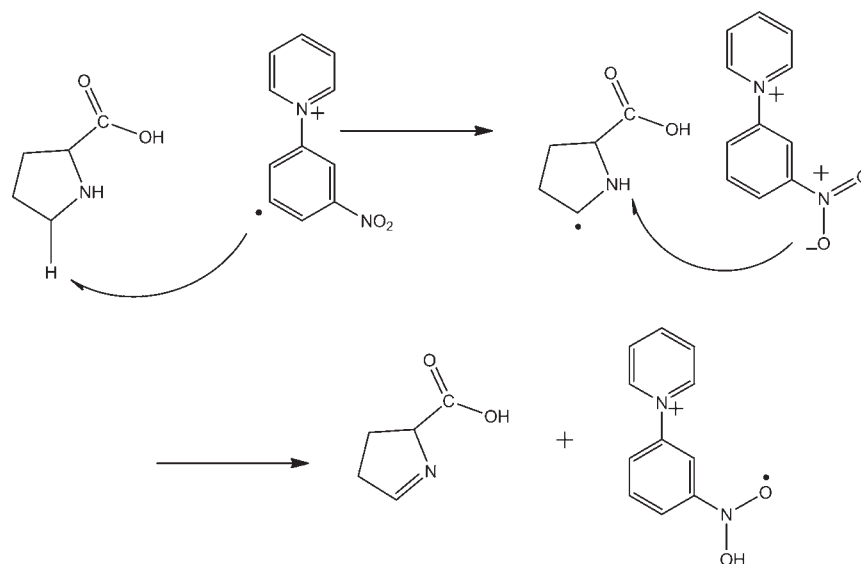
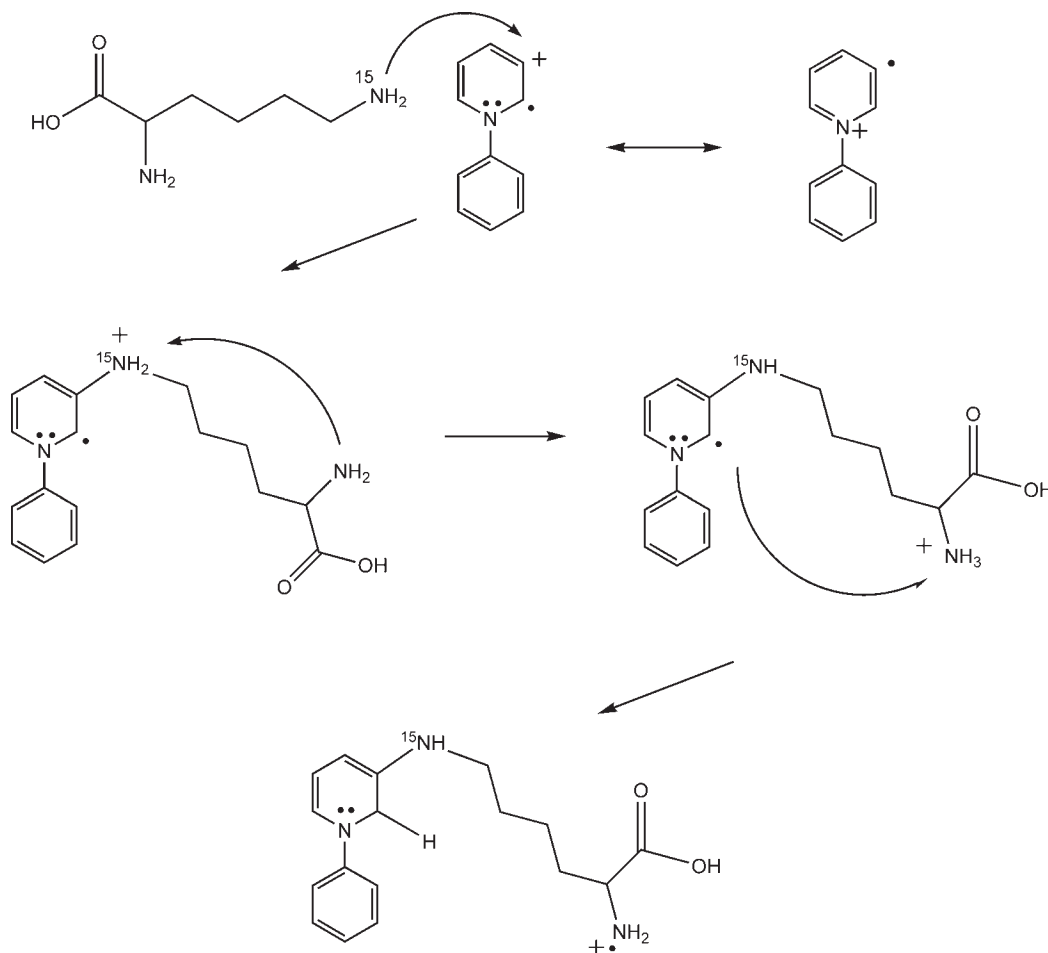
L-Leucine, DL-Leucine-1- d_1 , and L-Isoleucine. The difference in the alkyl side chain for isomeric L-leucine and L-isoleucine does not substantially influence their reactivity (Table 2). Just as for glycine, both NH_2 abstraction and hydrogen atom abstraction reactions were observed when this group of amino acids was allowed to react with the highly electrophilic charged radicals **a** and **c** (Table 2). Radical **a** shows more NH_2 abstraction than radical **c**. Radical **b** is an exception to the above behavior. This radical reacts by proton transfer with these amino acids (L-leucine, PA = 218.6 kcal/mol;¹⁵ L-isoleucine, PA = 219.3 kcal/mol¹⁵) because the amino acids are more basic than the conjugate base of radical **b**.^{12b} The proton transfer is so facile that it suppresses all radical reactions. Radicals **d–g**, with relatively low EA, react exclusively by hydrogen atom abstraction (Table 2). The lower the EA of the radical, the slower the hydrogen atom abstraction.

DL-Leucine-1- d_1 was allowed to react with radicals **a–g** to obtain a better understanding of the selectivity of hydrogen atom abstraction from leucine. The radicals (with the exception of radical **c**) did not abstract a deuterium atom from the α -carbon of the partially labeled leucine, as expected, but instead a hydrogen atom from somewhere else in the molecule. Although abstraction of the deuterium atom is slowed by the primary kinetic isotope effect, this finding nevertheless suggests that the α -carbon is not the kinetically preferred site for hydrogen atom abstraction from this amino acid. This finding is in sharp contrast to the behavior of partially labeled glycine, where extensive deuterium atom abstraction from the α -carbon was observed. Apparently, the presence of several hydrogen atoms in the side chain of leucine, as opposed to only one at the α -carbon, makes hydrogen atom abstraction from the side chain kinetically favored. Furthermore, the side chain may sterically hinder abstraction of the hydrogen atom from the α -carbon.

Reactions of Phenyl Radicals with L-Lysine and DL-Lysine- ϵ - ^{15}N . As observed for leucine and isoleucine, radical **b** (PA = 216 kcal/mol), the only Brønsted acid among the radicals studied, reacts exclusively by proton transfer with L-lysine (PA = 238 kcal/mol¹⁵). Furthermore, as reported for glycine, leucine, and isoleucine above, NH_2 and hydrogen atom abstractions were observed for the most electrophilic radicals **a** and **c** with lysine (Table 3), while the less reactive radicals **d–h** exhibit only hydrogen atom abstraction.

Radical **d** deviates from the other radicals in that it displays an additional reaction pathway—abstraction of two hydrogen atoms from L-lysine and DL-lysine- ϵ - ^{15}N (Table 3). This reaction is as fast as a single hydrogen atom abstraction. Apparently, after hydrogen atom abstraction by the radical site, the NO_2 group can abstract another hydrogen atom from the lysine radical. The same reaction was observed for radical **d** upon interaction with proline (as discussed below) but not for other amino acids. A possible mechanism for proline is shown in Scheme 2.

Radical **c** forms a stable adduct with L-lysine and DL-lysine- ϵ - ^{15}N , with branching ratios of 32% and 23%, respectively (Table 3). The formation of a stable adduct is most likely due to (1) the presence of two amino groups in lysine (since only this

Scheme 2. Possible Mechanism for the Abstraction of Two Hydrogen Atoms by Radical **d** from ProlineScheme 3. Possible Mechanism for the Formation of a Stable Adduct between Radical **c** and Lysine

amino acid yields an abundant stable adduct) and 2) the intrinsic properties of radical **c** (the only radical that forms a stable adduct). With the exception of radical **b**, which only undergoes

proton transfer reactions, radical **c** is the only radical studied here wherein the radical site is in the same ring as the charge site. Hence, lysine can interact with both these sites of the radical.

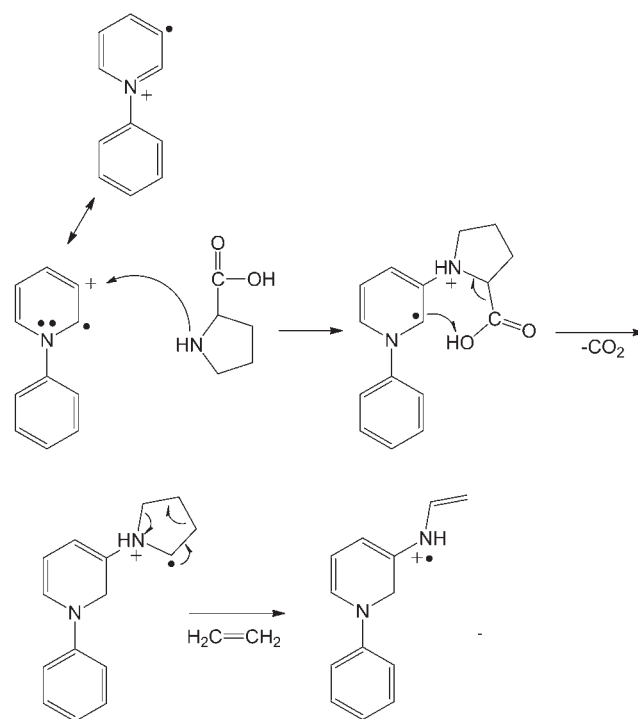
A possible mechanism for the formation of a stable adduct (m/z 301) for radical **c** with lysine only is shown in Scheme 3. The initial addition is proposed to occur as in Scheme 1 and involve the amino group in the lysine side chain since this group should be more nucleophilic than that in the amino acid moiety (the adjacent electron-withdrawing carbonyl group reduces its nucleophilicity). However, addition of either amino group should be followed by the same reaction sequence. After addition, the second amino group may abstract the proton from the protonated amino group attached to the aromatic ring. This reversible step would explain why this reaction was only observed for lysine. An irreversible hydrogen atom transfer to the radical site (now delocalized over the ring) would generate a resonance-stabilized radical cation. Some support for this mechanism was obtained when the stable adduct ion (m/z 301) was subjected to collision-activated dissociation. It does not fragment to yield back the reactant monoradical **c** via loss of intact lysine, which demonstrates that the adduct has not retained the initially formed structure (shown at the left in the middle row in Scheme 3). It also does not fragment via loss of NH_3 , which indicates that it does not contain a protonated amino group formed upon proton transfer to the second amino functionality (shown at the right in the middle row in Scheme 3). Instead, the stable adduct (m/z 301) fragments by loss of an NH_2 group (to yield an ion of m/z 285) and loss of CO_2 (to yield an ion of m/z 257), in support of the final structure proposed for the adduct in Scheme 3.

$\text{DL-Lysine-}\epsilon\text{-}^{15}\text{N}$ was studied to provide support for the mechanism shown in Scheme 3. Indeed, radical **c** was found to abstract either one of the two NH_2 groups in $\text{DL-lysine-}\epsilon\text{-}^{15}\text{N}$, with branching ratios for $^{15}\text{NH}_2$ and NH_2 group abstractions of 8% and 21%, respectively. Radical **a** also abstracts $^{15}\text{NH}_2$ and NH_2 groups, with branching ratios of 18% and 53%, respectively. On the basis of these four values, it can be concluded that the electrophilicity of the radical does not have a major influence on the radicals' preference toward side chain NH_2 abstraction versus NH_2 abstraction from the amino acid moiety. Scheme 1 shows possible mechanisms for NH_2 and $^{15}\text{NH}_2$ abstraction from $\text{DL-lysine-}\epsilon\text{-}^{15}\text{N}$. Abstraction of the NH_2 group from the amino acid moiety is more facile than the abstraction of the $^{15}\text{NH}_2$ group (Table 3), likely due to the different stabilities of the radicals produced in these reactions. The radical produced upon the abstraction of the NH_2 group of the amino acid moiety is resonance stabilized and hence more stable than the radical produced in the abstraction of the NH_2 group from the side chain.

L-Proline and DL-Proline-1- d_1 . Radical **b** again reacts only by proton transfer when allowed to react with proline (Table 4). In contrast, radicals **a** and **e–h** react with proline via exclusive hydrogen atom abstraction (Table 4). NH_2 abstraction from proline does not occur because the nitrogen atom is a part of a five-membered ring. $\text{DL-Proline-1-}d_1$ was allowed to react with radicals **a–h** to obtain a better understanding of the selectivity of hydrogen atom abstraction from proline. Only a minor amount of deuterium atom abstraction was observed (Tables 4 and 5). Hence, hydrogen atom abstraction from the unlabeled proline mostly occurs from positions other than the α -carbon.

Radicals **c** and **d** (and **b**, as discussed above) are the only radicals that undergo another reaction with proline besides hydrogen atom abstraction. Radical **d** abstracts two hydrogen atoms from L-proline . The abstraction of two hydrogen atoms by **d** was also observed for lysine. The mechanism for this reaction is

Scheme 4. Possible Mechanism of $\text{C}_2\text{H}_4\text{N}$ Abstraction upon the Reaction of Radical **d** with Proline



likely the same for proline and lysine (a possible mechanism is shown in Scheme 2). Steric hindrance due to the methyl branch may slow this reaction for leucine and isoleucine. As opposed to an earlier study,^{12b} wherein only hydrogen atom abstraction was reported for the reaction of radical **c** with proline, additional reaction pathways were observed in the present study. Although hydrogen atom abstraction was found to dominate, HO abstraction, $\text{C}_2\text{H}_4\text{N}$ abstraction, $\text{C}_3\text{H}_7\text{NO}$ abstraction, and stable adduct formation were also observed. This discrepancy between the two studies may arise from the greater sensitivity of the more recent measurements. The different reactivity of radical **c**, when compared to the other radicals studied, may again be explained by the fact that radical **c** is the only radical studied (with the exception of acidic radical **b**) that has the radical site and the positive charge in the same ring, hence allowing interactions of proline with both. For example, $\text{C}_2\text{H}_4\text{N}$ abstraction from proline by radical **c** may be initiated by nucleophilic addition of the proline nitrogen to the radical carbon, as shown in Scheme 1 for lysine. For lysine, N–C bond cleavage leads to the NH_2 abstraction product. For proline, this is not possible. Instead, the radical (now delocalized over the ring) may abstract a hydrogen atom from the carboxylic acid group of proline, leading to the elimination of CO_2 (Scheme 4). Finally, C_2H_4 may be eliminated from the ring in proline, resulting in a resonance-stabilized radical cation (the $\text{C}_2\text{H}_4\text{N}$ abstraction product).

CONCLUSIONS

Examination of the gas-phase reactions of 10 different phenyl radicals with several amino acids, many partially isotope labeled, revealed the commonly observed hydrogen atom abstraction, but also a number of other reactions. Results obtained for partially deuterium-labeled leucine and proline demonstrate that only a very small amount of hydrogen atoms, if any, are abstracted from

the α -carbon and a large amount from elsewhere (likely the alkyl side chain). This is in sharp contrast to glycine whose α -carbon is the predominant hydrogen atom donor site. The regioselectivity of hydrogen atom abstraction appears to be independent of the structure of the radical but dependent on the structure of the amino acid.

The mechanism of the previously reported NH_2 abstraction (proposed to occur via the nucleophilic addition–elimination mechanism^{12b}) was examined computationally. The relative transition-state energies for addition of ammonia to different positions in 3-dehydropyridinium cation revealed that addition to the radical carbon is kinetically favored and hence the most likely addition site for amino acids in charged phenyl radicals. The calculated charge and spin densities in the ammonia adduct of 3-dehydropyridinium cation support the Lewis structure chosen to illustrate the amino acid adducts of the radicals.

Several unprecedented reaction pathways were also observed. These include the abstraction of two hydrogen atoms from proline and lysine by the nitro-substituted radical **d**. Furthermore, abstractions of $\text{C}_2\text{H}_4\text{N}$, $\text{C}_5\text{H}_7\text{NO}$, and OH groups by radical **c** were observed for proline. These reactions are likely to occur by nucleophilic addition–elimination pathways, similar to that leading to NH_2 abstraction from all the other amino acids but proline, where the nitrogen atom is part of a ring structure. Finally, both NH_2 and $^{15}\text{NH}_2$ groups were abstracted from lysine labeled with ^{15}N on the side chain, indicating that NH_2 abstraction occurs both from the amino terminus and from the side chain of lysine.

The electron affinity of the radical appears to be the major factor in controlling the radical's reaction rates with the amino acids. Both the radical (hydrogen atom abstraction) and non-radical (NH_2 abstraction) reaction efficiencies were found to depend on the electrophilicity of the radical, although the nonradical reactions are influenced more strongly. However, in contrast to an earlier report,^{12b} the ionization energies of the amino acids do not appear to have a general reactivity-controlling role.

■ ASSOCIATED CONTENT

S Supporting Information. Absolute energies and coordinates of atoms for all optimized structures and complete ref 23. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author
hilkka@purdue.edu

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