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Phenyl Radical-Induced Damage to Dipeptides

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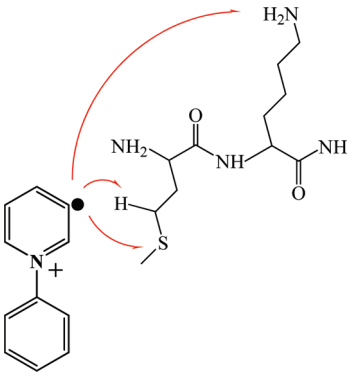


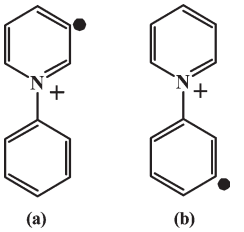
Laser-induced acoustic desorption (LIAD) incorporated with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR) has been utilized to investigate phenyl radical-induced damage to dipeptides in the gas phase. On the basis of the product branching ratios measured for the reactions of two different positively charged phenyl radicals with 17 different dipeptides, the overall order of susceptibility to attack of the different sites in the dipeptides was determined to be heteroaromatic side chain ≈ S atom in SCH_3 group > H atom in SH group > H atom in CH group > aromatic side chain > S atom in SH group > NH_2 in side chain > N-terminal NH_2 > COOH in side chain ≈ C-terminal COOH. The amino acid sequence also influences the selectivity of these reactions. As expected, the ability of a phenyl radical to damage dipeptides increases as the electrophilicity of the phenyl radical increases.

Introduction

Radicals can induce damage to proteins, which is considered to be the cause of various diseases.[1] Numerous condensed-phase studies have been carried out on reactions of simple oxygen-containing radicals (especially hydroxyl radical) with proteins.[2] For example, the hydroxyl radical has been reported to abstract a H atom from Cα, from an alkyl group, and from an amino group from aliphatic amino acids.[2] Further, the hydroxyl radical has been also reported to add to the aromatic ring in aromatic amino acids and to add to the sulfur atoms in methionine and cysteine.[2]

In sharp contrast to the hydroxyl radical, only limited efforts have been directed toward understanding the

CHART 1. Phenyl Radicals Studied



(a) (b)

(1) (a) Garrison, M. W. *Radiat. Res. Rev.* **1972**, *3*, 305. (b) Easton, J. C. *Chem. Rev.* **1997**, *97*, 53. (c) Davies, J. M.; Fu, S.; Wang, H.; Dean, T. R. *Free Radical Biol. Med.* **1999**, *27*, 513. (d) Garrison, W. M. *Chem. Rev.* **1987**, *87*, 381. (e) Davies, M. J. Dea, R. T. *Radical-Mediated Protein Oxidation: From Chemistry to Medicine*; Oxford University Press: Oxford, 1997. (f) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746. (g) Griffiths, J.; Murphy, J. A. *J. Chem. Soc., Chem. Commun.* **1992**, *1*, 24. (h) Davies, J. M.; Fu, S.; Wang, H.; Dean, R. T. *Free Radical Biol. Med.* **1999**, *27*, 1151. (i) Dean, T. R.; Fu, S.; Stocker, R.; Davis, J. M. *Biochem. J.* **1997**, *324*, 1. (j) Hawkins, C. L.; Davies, M. J. *Biochim. Biophys. Acta* **2001**, *1504*, 196. (k) Hawkins, C. L.; Davies, M. *J. Chem. Soc., Perkins Trans. 2* **1998**, 1937.

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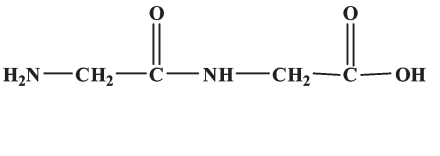
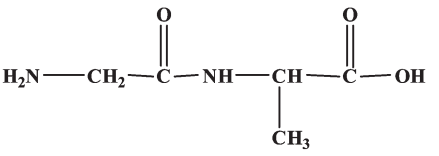
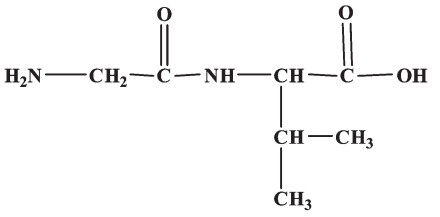
CHART 2. Structures of Dipeptides Containing Aliphatic Alkyl Side Chains



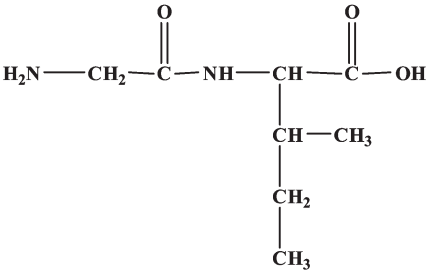
Gly-Gly



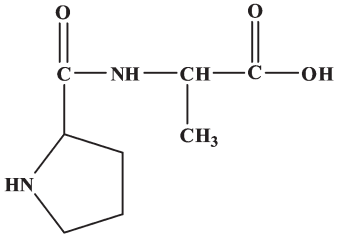
Gly-Ala



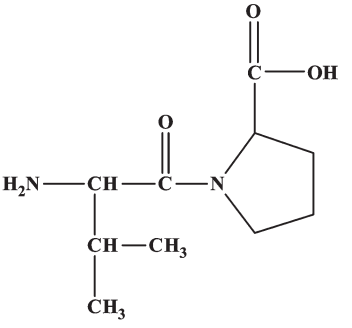
Gly-Val



Gly-Ile



Pro-Ala



Val-Pro

reactions of carbon-centered organic radicals (e.g., phenyl radicals) with proteins due to the complexity of the biological environment and the difficulty in generating pure radicals in solution.[3a] The few studies published include an examination of the *para*-benzoic acid radical (4-dehydrobenzoic acid) that was reported to abstract a D atom from the α-position of α, α-dideuterioglycine in solution.[3a]

Gas-phase experiments provide a way to examine reactions of phenyl radicals with organic molecules in a solvent-free environment. For example, 5-dehydro-2-(2-hydroxymethyl-18-crown-6)naphthoate radical was reported to abstract a H atom from the α-position and abstract a H atom from an alkyl side chain from peptides while noncovalently attached to the peptides in the gas phase.[3b] Recently, the "distonic ion approach", in which a chemically inert, charged group is added to the radical of interest for mass spectrometric manipulation, has been used to study reactions of various radicals in mass spectrometers.[4] This approach has been used, for example, to examine the gas-phase reactivity of different positively charged phenyl radicals toward thermally evaporated neutral amino acids in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR).[5] These studies have demonstrated that the reactions of positively charged phenyl radicals occurring under the gas-phase conditions are similar to those observed for neutral phenyl radicals in solution. For example, both a condensed-phase neutral phenyl radical (the 4-dehydrobenzoic acid) and gas-phase positively charged phenyl radicals (e.g., *N*-(3-dehydrophenyl)pyridinium) rapidly react with

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SCHEME 1

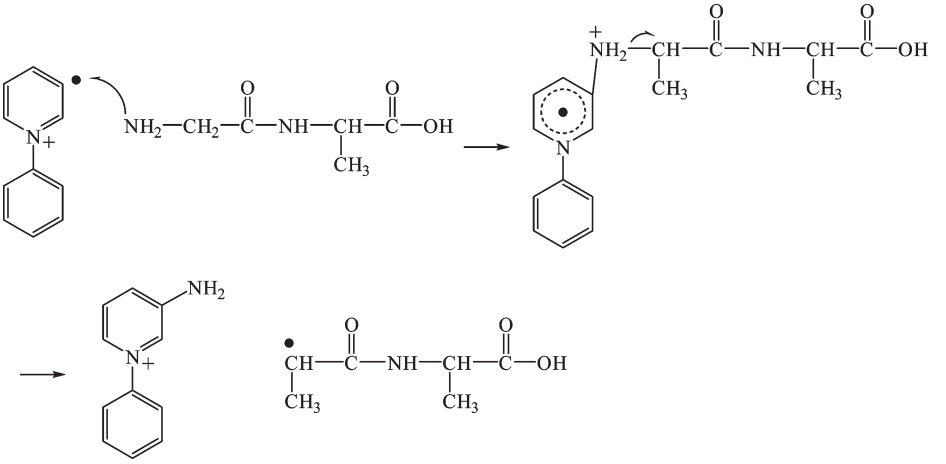

TABLE 1. Branching Ratios[a] for Product Ions Formed in Reactions of Radical a and Radical b with Dipeptides Containing Alkyl Side Chains

dipeptide[b]	radical **a** (EA = 5.78 eV)	radical **b** (EA = 4.87 eV)
Gly-Gly (132)	H-abs: 0.84 NH_2-abs: 0.16	H-abs: 1.00
Gly-Ala (146)	H-abs: 0.87 NH_2-abs: 0.13	H-abs: 1.00
Gly-Val (174)	H-abs: 0.90 NH_2-abs: 0.10	H-abs: 1.00
Gly-Ile (188)	H-abs: 0.92 NH_2-abs: 0.08	H-abs: 1.00
Pro-Ala (186)	H-abs: 0.67 $NHCH{=}CH_2$-abs: 0.12 $NHCH_2CH_3$-abs: 0.21	H-abs: 1.00
Val-Pro (214)	H-abs: 0.79 NH_2-abs: 0.21	H-abs: 1.00

[a]Average values from several experiments. [b]Molar masses given in parentheses.

TABLE 2. Branching Ratios[a] for Product Ions Formed in Reactions of Radical a and Radical b with Dipeptides Containing Sulfur

dipeptide[b]	radical **a** (EA = 5.78 eV)	radical **b** (EA = 4.87 eV)
Cys-Gly (178)	H-abs: 0.65 SH-abs: 0.35	H-abs: 1.00
Glu-Cys (250)	H-abs: 0.56 NH_2-abs: 0.10 SH-abs: 0.34	H-abs: 1.00
Met-Gly (206)	H-abs: 0.17 SCH_3-abs: 0.77 addition-CH_3: 0.06	H-abs: 0.82 SCH_3-abs: 0.18
Val-Met (248)	H-abs: 0.17 SCH_3-abs: 0.76 addition-CH_3: 0.07	H-abs: 0.79 SCH_3-abs: 0.21
Met-Lys (277)	H-abs: 0.14 NH_2-abs: 0.06 SCH_3-abs: 0.71 addition-CH_3: 0.06 addition: 0.03	H-abs: 0.90 SCH_3-abs: 0.08 addition-CH_3: 0.02

[a]Average values from several experiments. [b]Molar masses given in parentheses.

α,α-dideuterioglycine by abstraction of a D atom from the α-position.[3a,5] Further, more electrophilic (and hence more reactive) positively charged radicals (e.g., *N*-phenyl-3-dehydropyridinium) were also found to undergo NH_2, SH, and SCH_3 group abstractions, as well as aromatic side-chain abstraction and adduct formation (only for aromatic amino acids).[5] These differences in reactivity were proposed to arise from the different electrophilicities of the radicals, which were evaluated by comparing their calculated vertical electron affinities (EA).[6] The more electrophilic radicals (with greater EA) can better stabilize (via polarization) the transition states of their reactions than the less electrophilic radicals (lower EA), which results in observation of new reactions.[4c−e] Indeed, oxidation of the amino group, H atom abstraction from the amino group, and addition at sulfur atoms and aromatic rings in amino acids have been also reported for the electrophilic hydroxyl radical in solution, as mentioned above.[2]

Until recently, these gas-phase studies were limited to amino acids because peptides and proteins are thermally fragile and hence cannot be readily introduced into mass spectrometers as neutral molecules. However, the laser-induced acoustic desorption (LIAD) technique now allows the introduction of neutral, intact peptides with low internal and kinetic energies into an FT-ICR.[7] The examination of radical reactions of peptides may provide information useful for the understanding of radical reactions of proteins. We report here the results of gas-phase studies on the reactions of two phenyl radicals (Chart 1) with different EAs with 17 dipeptides, which were introduced into an FT-ICR by using LIAD. These results permit an assessment of the susceptibility of the different sites in neutral dipeptides toward attack by electrophilic phenyl radicals in the gas phase.

Results and Discussion

The two phenyl radicals (**a** and **b**; Chart 1) show very different reactivity toward the dipeptides studied. This is likely due to their different electrophilicities, which are evaluated here by comparing their calculated ((U)B3LYP/6-31+G(d)//(U)B3-LYP/6-31+G(d)) vertical electron affinities (EA).[9] The more

(6) Note that, for these calculations, the vertical electron affinity of the radical site, not the vertical electron affinity of the molecule, is computed.

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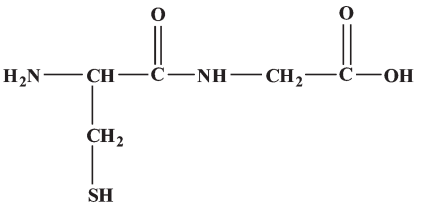
CHART 3. Structures of Dipeptides Containing Sulfur



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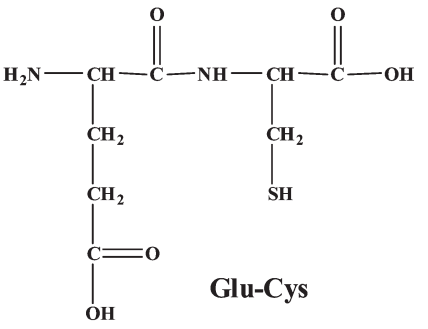
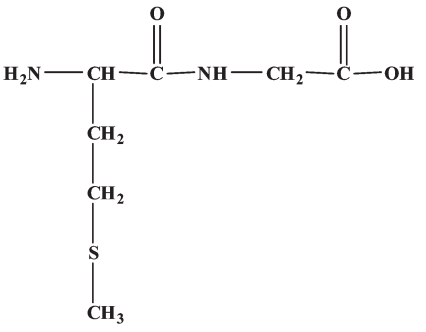
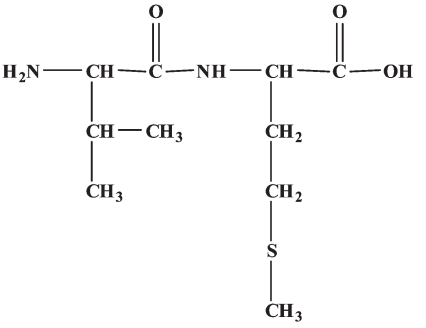


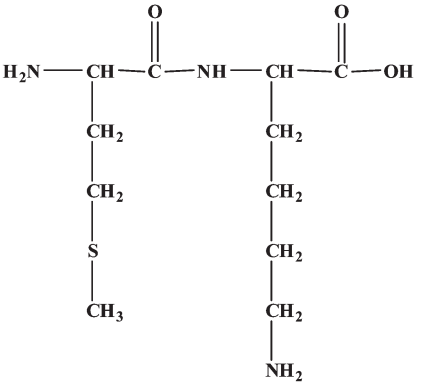
Glu-Cys



Met-Gly



Val-Met



Met-Lys

reactive phenyl radical **a** (EA = 5.78 eV[9]) is more electrophilic. Indeed, in addition to fast H atom abstraction, this radical reacts with all dipeptides that contain an aliphatic alkyl side chain (Chart 2) also by a slower NH_2 group abstraction (for a proposed[5] reaction mechanism, see Scheme 1), while the less electrophilic radical **b** (EA = 4.87 eV[9]) only undergoes H atom abstraction (Table 1). It is not a surprise that no NH_2 group abstraction was observed for the less electrophilic radical since NH_2 abstraction may be best viewed as nucleophilic addition−elimination reaction, and the more electrophilic the radical, the faster the reaction is expected to be.[5a]

The branching ratios for H atom abstraction from the dipeptides Gly-Gly (0.84), Gly-Ala (0.87), Gly-Val (0.90), and Gly-Ile (0.92) by radical **a** are substantially greater than those observed for the individual amino acids (Gly, 0.43; Ala, 0.53; Val, 0.67; Ile, 0.72),[5a] and they appear to increase as the size of the alkyl side chain and the size of the dipeptide increase (Table 1). The experimental results reported below suggest that the size of the alkyl side chain, rather than the size of the dipeptide, is the main factor influencing the H atom abstraction branching ratio. The rates of H atom abstraction reactions of charged phenyl radicals in these experiments have been earlier demonstrated to be independent of the homolytic C−H bond dissociation energies of the H atom donor bonds (and rather depend on the ionization energy of the substrate and EA of the radical).[10] Hence, differences in the C−H bond dissociation energies of the possible donor sites can be ignored. Recognizing this, the experimental findings reported here suggest that, in spite of the thermodynamic preference for H atom abstraction from

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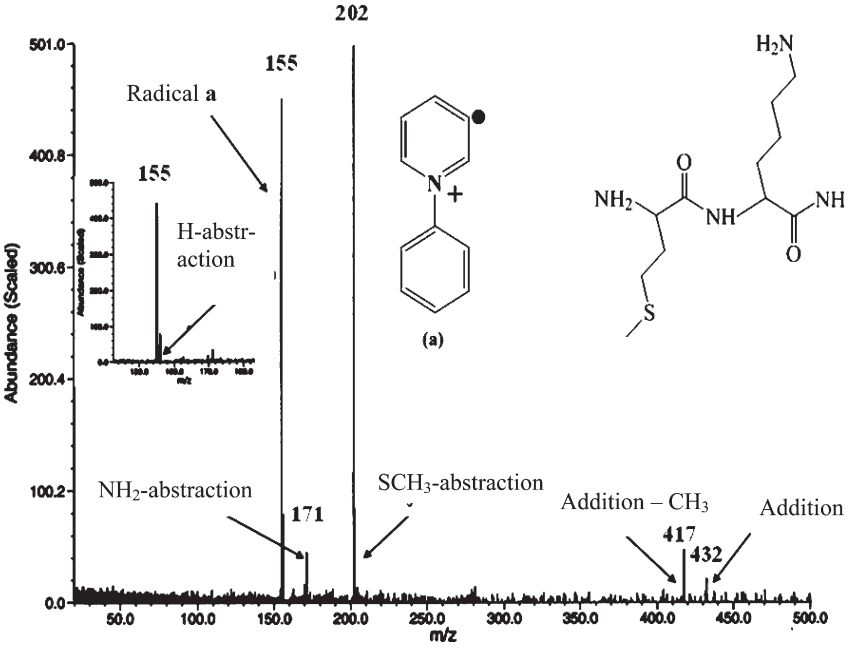


FIGURE 1. Mass spectrum measured after reaction of radical **a** with Met-Lys.

TABLE 3. Branching Ratios[a] for Product Ions Formed in Reactions of Radical a and Radical b with Dipeptides Containing Aromatic Side Chains

dipeptide[b]	radical **a** (EA = 5.78 eV)	radical **b** (EA = 4.87 eV)
Phe-Val (264)	H-abs: 0.61 NH_2-abs: 0.07 $C_6H_4CH_2$-abs: 0.30 addition-H: 0.02	H-abs: 0.54 $C_6H_4CH_2$-abs: 0.37 addition-H: 0.09
Ala-Phe (236)	H-abs: 0.52 NH_2-abs: 0.07 C_6H_5-abs: 0.09 $C_6H_4CH_2$-abs: 0.25 addition-H: 0.07	H-abs: 0.64 $C_6H_4CH_2$-abs: 0.36
Tyr-Leu (294)	H-abs: 0.56 NH_2-abs: 0.06 HOC_6H_5-abs: 0.06 $HOC_6H_4CH_2$-abs: 0.08 addition-OH: 0.16 addition-H: 0.08	H-abs: 0.82 HOC_6H_5-abs: 0.03 addition-OH: 0.08 Addition-H: 0.07
Leu-Tyr (294)	H-abs: 0.55 NH_2-abs: 0.05 HOC_6H_5-abs: 0.11 $HOC_6H_4CH_2$-abs: 0.12 addition-OH: 0.10 addition-H: 0.07	H-abs: 0.90 addition-OH: 0.07 addition-H: 0.03
Trp-Gly (261)	H-abs: 0.18 $C_6H_4C_2H_2N$-abs: 0.13 $C_6H_4C_3H_4N$-abs: 0.60 addition-OH: 0.03 addition-H: 0.04 addition: 0.02	H-abs: 0.22 $C_6H_4C_2H_2N$-abs: 0.19 $C_6H_4C_3H_4N$-abs: 0.52 addition-H: 0.07
Leu-Trp (317)	H-abs: 0.20 NH_2-abs: 0.03 $C_6H_4C_2H_2N$-abs: 0.15 $C_6H_4C_3H_4N$-abs: 0.59 addition: 0.03	H-abs: 0.26 $C_6H_4C_2H_2N$-abs: 0.13 $C_6H_4C_3H_4N$-abs: 0.57 addition-H: 0.04

[a]Average values from several experiments. [b]Molar masses given in parentheses.

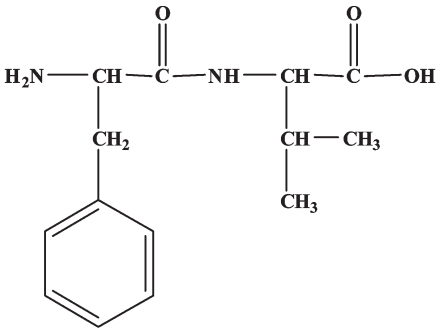
the αC−H bond (for example, the homolytic αC−H bond dissociation energy in glycine (79.2 kcal/mol) is substantially lower than that of the N−H (102.6 kcal/mol) and O−H bonds (112.9 kcal/mol),[5a] most H atoms are abstracted from the alkyl side chains, and H atom abstraction from the side chains is hence favored kinetically. As a result, the H atom branching ratio increases as the number of H atoms in the side chain increases. Steric hindrance caused by larger side chains may further hinder H atom abstraction from the αC−H group.

An examination of dipeptides containing Pro provides information about the origin of the NH_2 group that is abstracted by radical **a** from most of the dipeptides studied. For example, radical **a** abstracts an NH_2 group from Val-Pro but $NHCH{=}CH_2$ and $NHCH_2CH_3$ groups from Pro-Ala (Table 1). This observation suggests not only that the position of Pro in the dipeptide has a strong influence on its reactivity but also that the N-terminal amino group is the preferentially attacked amino group in dipeptides (additional support for this hypothesis is provided below). Earlier studies have shown that $NHCH{=}CH_2$ and $NHCH_2CH_3$ abstractions do not occur for the free amino acid Pro (only H atom abstraction).[5a] Hence, the reactivity of a phenyl radical toward a free amino acid can be drastically different from the reactivity of the radical toward the same amino acid in a dipeptide.

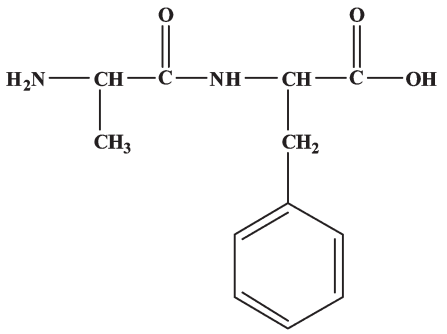
In addition to H atom and NH_2 group abstraction, radical **a** also abstracts other groups from dipeptides that contain side chains other than aliphatic alkyl groups. For example, radical **a** also abstracts an SH group from Glu-Cys, and this reaction is faster than NH_2 group abstraction from this dipeptide (Table 2 and Chart 3). SH group abstraction from the N-terminal Cys in Cys-Gly is so favorable that NH_2 group abstraction is not observed for this dipeptide (for comparison, the free amino acid Cys reacts[5a] with radical **a** by H atom abstraction (0.60) and SH group abstraction (0.40)). Hence, the position of Cys in the dipeptide has a strong influence on its reactivity. Furthermore, these results indicate that the N-terminal amino acid is more vulnerable to radical attack than the C-terminal amino acid.

The presence of the carboxylic acid group in the side chain of Glu-Cys does not appear to have a significant influence on

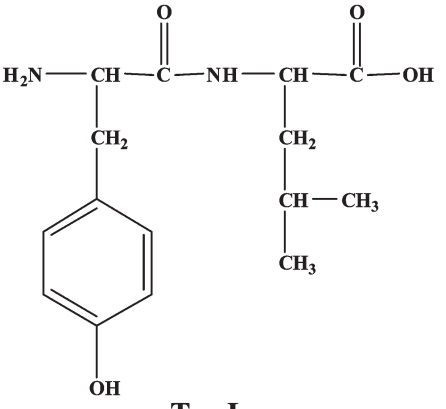
CHART 4. Structures of Dipeptides Containing Aromatic Side Chains



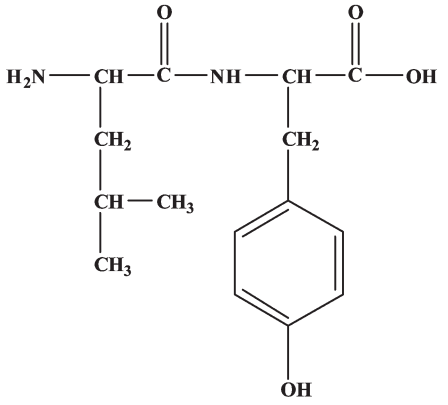
Phe-Val



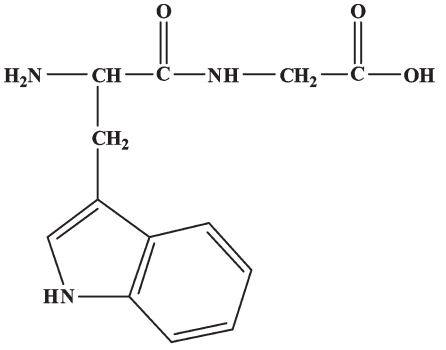
Ala-Phe



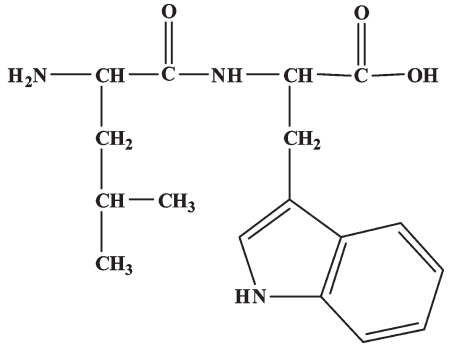
Tyr-Leu



Leu-Tyr



Trp-Gly

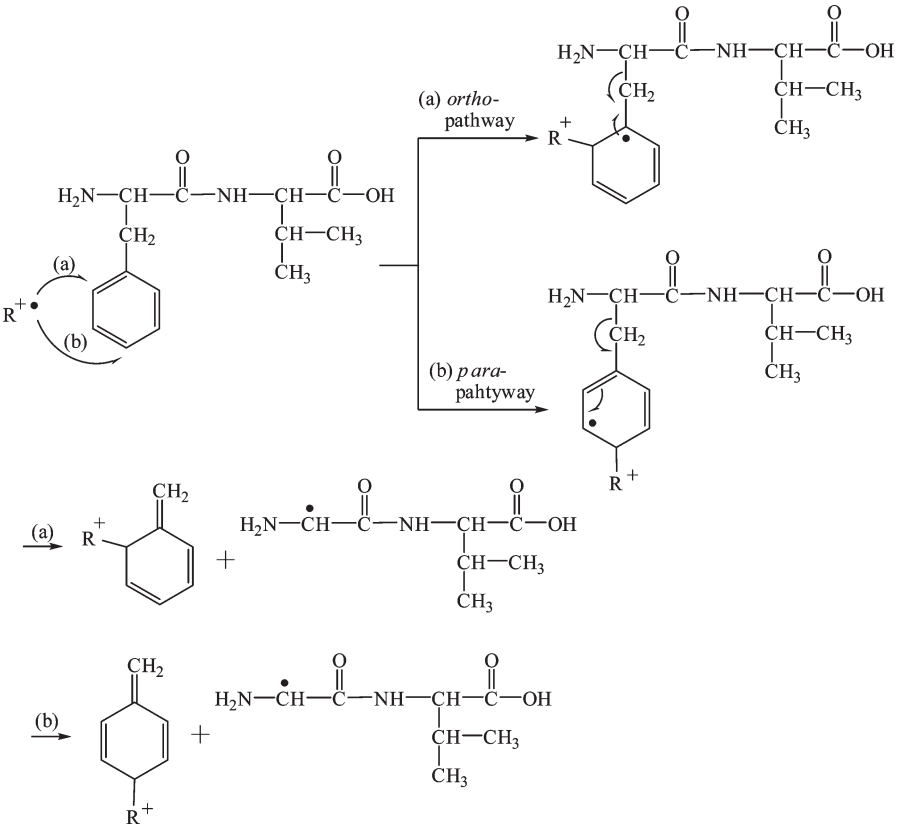


Leu-Trp

its reactivity (i.e., the reactivity of this dipeptide is similar to that observed for Cys-Gly, which does not contain a carboxylic acid group in the side chain; Table 2). In sharp contrast, the presence of a SCH_3 group in the side chain (i.e., those containing Met) has a significant influence on the reactivity of these dipeptides, and SCH_3 group abstraction, rather than H atom abstraction, is the principal pathway for reactions of these dipeptides with radical **a** (Table 2). Interestingly, even the less electrophilic radical **b** is able to abstract SCH_3 from Met-containing dipeptides. In addition to SCH_3 group abstraction, radical **a** reacts with Met-Gly by H atom abstraction and addition to SCH_3 followed by the loss of CH_3 (similar behavior has been reported[5a] previously for the reaction of the free amino acid Met with radical **a**; H atom abstraction = 0.10; SCH_3 group abstraction = 0.86; addition to SCH_3 followed by the loss of CH_3 = 0.04). The reactivities of both radicals toward Val-Met are similar to those for Met-Gly. Hence, radical attack at the S atom in Met, located either at the N- or the C-terminus, appears to be favored over attack at any other heteroatom.

In addition to the above reactions, radical **a** also abstracts an NH_2 group from Met-Lys (Table 2 and Figure 1). NH_2 group

SCHEME 2



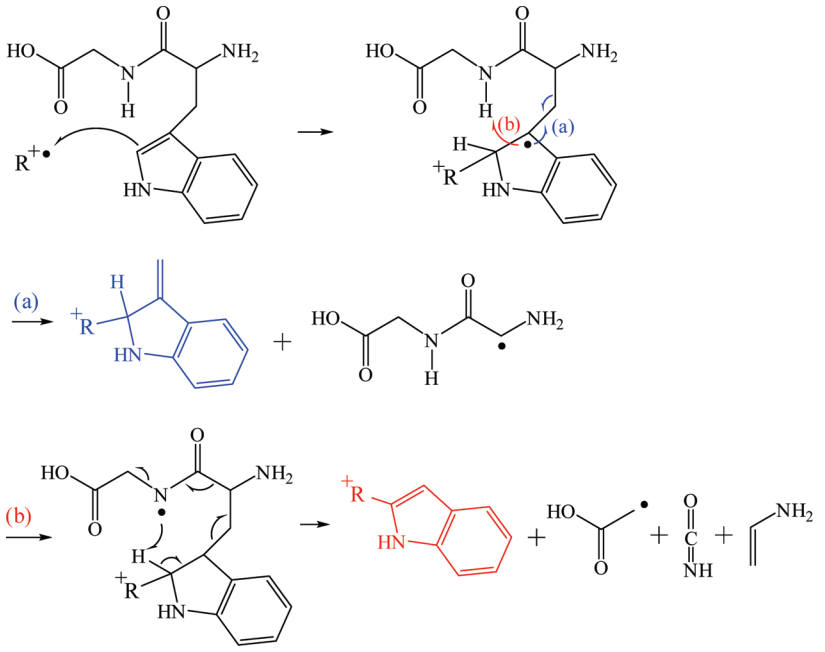
abstraction from Met-Lys but not from Met-Gly suggests that the NH_2 group is abstracted from the side chain of Lys instead of the N-terminus, and that the side-chain NH_2 group is more susceptible to radical attack than the N-terminal NH_2 group. Finally, like Met-Lys, the formation of a stable adduct with radical **a** was also observed for the free amino acid Lys. These findings suggest that the presence of a side-chain NH_2 group facilitates the formation of a stable adduct. The mechanism for adduct formation in these molecules is under investigation.

Both phenyl radicals **a** and **b** react with all of the dipeptides studied that contain an aromatic side chain (Chart 4) by H atom abstraction and addition to the aromatic ring (Table 3). For Phe-Val, H atom abstraction is the dominant reaction pathway for both radicals (branching ratios for the reaction of radical **a** with Val[5a] are H abstraction = 0.67; NH_2 group abstraction = 0.33). However, both radicals also add to the aromatic ring of Phe-Val followed either by the loss of a H atom (radical **a** only) or the homolytic Cα−Cβ bond cleavage resulting in the elimination of the side chain (radicals **a** and **b**) from the dipeptide (Table 3; the reaction mechanism for the aromatic side-chain abstraction from Phe-Val shown in Scheme 2 is essentially identical to that proposed[5b] earlier for aromatic side-chain abstraction from aromatic amino acids by the same phenyl radicals; computational support for the proposed mechanism was presented[5b]). Addition to the aromatic ring has been reported also to be the major reaction pathway for the hydroxyl radical with aromatic amino acids in solution.[2] However, no Cα−Cβ bond cleavage was reported.[2] This is probably due to stabilization of the addition product by collisions with solvent molecules in solution which is not possible for the gas-phase conditions used here.

That aromatic side-chain abstraction reactions were observed for both radicals indicates that the aromatic side chain of Phe-Val is more susceptible to radical attack than the side chains of aliphatic dipeptides containing no Met. The occurrence of NH_2 group abstraction for Phe-Val (but not for Cys-Gly or Met-Gly) upon reaction with radical **a** indicates that the side-chain aromatic ring is less susceptible to radical attack than either the SH or the SCH_3 group at the N-terminus because it does not hinder the competing NH_2 group abstraction. Radical **a** abstracts the $C_6H_4CH_2$ group almost equally efficiently from Ala-Phe (Table 3) with a side-chain aromatic ring in the C-terminus, as from Phe-Val with a side-chain aromatic ring in the N-terminus, which indicates that the side-chain aromatic ring is highly susceptible to radical attack.

Similar to Phe-Val, H atom abstraction is the predominant pathway for reactions of both radicals with Tyr-Leu and Leu-Tyr (Table 3). Tyr has been shown[5b] to be a relatively poor H atom donor (H atom abstraction branching ratios for radicals **a** and **b** are 0.16 and 0.31, respectively). However, Leu appears to be an excellent H atom donor (H atom abstraction branching ratios for radicals **a** and **b** are 0.73 and 1, respectively). Tyr-Leu and Leu-Tyr show nearly equal reactivity toward radicals **a** and **b**. For example, in addition to H atom abstraction, both radicals add to the aromatic side chain followed by elimination of either a H atom or an OH group. The more electrophilic radical **a** also slowly abstracts an NH_2 group from the N-terminus.

SCHEME 3



Furthermore, this radical adds to the aromatic side chain, resulting in two side-chain abstraction products (i.e., HOC_6H_5 and $HOC_6H_4CH_2$ group abstractions). Neither of these side-chain abstraction products has been observed for the free amino acid Tyr.

Finally, both radicals react with the aromatic side chain of Trp-Gly and Leu-Trp (Table 3), predominantly leading to $C_6H_4C_3H_4N$ abstraction (possibly as shown in Scheme 3; only the shown addition reaction can lead to the cleavage of the bond needed for formation of the final ionic product of pathway a). H atom abstraction is substantially less favorable for Trp-Gly and Leu-Trp than, for example, for Gly-Gly (Table 1) and Leu-Tyr (Table 3), and NH_2 group abstraction was not observed for Trp-Gly. These results suggest that the heteroaromatic side chain is comparable to a SCH_3 group in its susceptibility to radical attack.

Conclusions

The gas-phase experiments discussed here demonstrate that the N-terminal amino acid of dipeptides is intrinsically more vulnerable to attack by electrophilic phenyl radicals than the C-terminal amino acid. H atom abstraction (predominantly from a side chain) by the phenyl radicals occurs for all dipeptides studied, and the amino acids Ile, Leu, Val, Ala, and Gly (in decreasing order) are especially good H atom donors. The overall order of susceptibility of different sites in dipeptides for attack by phenyl radicals in the gas phase is heteroaromatic side chain ≈ S atom in SCH_3 group > H atom in SH group > H atom in CH group > aromatic side chain > S atom in SH group > NH_2 in side chain > N-terminal NH_2 > COOH in side chain ≈ C-terminal COOH. In most cases, radical reactivity toward a given amino acid residue in a dipeptide is influenced by not only the location of the amino acid in the peptide but also the other amino acid present. Hence, radical reactivity toward free amino acids is not a good predictor for the reactivity toward the same amino acids in a dipeptide. As expected, a more electrophilic phenyl radical causes more substantial damage to a dipeptide.

The results presented here were obtained for neutral dipeptides in the gas phase. However, similar behavior is expected for solution conditions based on the discussions above. For example, the reactivity of a phenyl radical toward glycine in solution has been demonstrated to be identical to that of a positively charged analogue in the gas phase.[3a] Further, the gas-phase reactivity of highly electrophilic positively charged phenyl radicals resembles that of the (electrophilic) hydroxyl radical in solution.[2] We are currently examining reactions of positively charged phenyl radicals with amino acids and dipeptides in solution in order to further elucidate the differences between reactions occurring in the gas phase and in solution.

Experimental Section

Most of the experiments were performed in a Nicolet model FTMS 2000 Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with a 3 T superconducting magnet. The nominal base pressure was maintained at less than 10^{-9} Torr by two Edwards Diffstak diffusion pumps. The instrument contains a dual cell, which allows ion generation in one side and their examination in the other (clean) side. The positively charged phenyl radicals were synthesized in the mass spectrometer as described previously.[8] For example, radical **a** was generated as follows. Its precursor, the chlorobenzene radical cation (generated via electron ionization of chlorobenzene), was allowed to react with 3-iodopyridine to form the *N*-phenyl-3-iodopyridinium ion. The *N*-phenyl-3-iodopyridinium ion was isolated and subjected to sustained off-resonance irradiated collision-activated dissociation (SORI-CAD) to cause a homolytic carbon−chlorine bond cleavage, which resulted in radical **a**.[8]

The dipeptides were used as received from commercial suppliers. Their purity varied from 85 to 99%. Each dipeptide was dissolved in methanol (ca. 10 mM solutions) and deposited by

electrospray on the surface of thin (12.7 μm) titanium foils with a diameter of 0.695 in. A high intensity laser pulse from a Nd:YAG laser (3 ns pulse width, 532 nm wavelength) was used to generate acoustic waves, as described earlier, by focusing it at the back side of the Ti foil.[7] The laser (10 Hz repetition rate) was fired 100 times (100 ms interval between each shot) while continuously rotating the LIAD probe so that each laser pulse irradiated a fresh spot on the Ti foil. The surface area affected by a single laser pulse was about 10^{-3} cm^2, and approximately 1 pmol of the dipeptide was introduced into the mass spectrometer per pulse. The neutral, intact dipeptide molecules were then allowed to react with the positively charged phenyl radicals, which were generated prior to the laser pulses and trapped in that side of the dual cell into which the peptides were evaporated. The reported (average) product branching ratios were determined from several experiments (each experiment involved 100 laser pulses to evaporate dipeptide molecules for interaction with the same ion population). Their reproducibility is better than $\pm 10\%$. Background spectra were obtained by trapping the phenyl radicals in the cell for the same period of time as needed for 100 laser shots, but without firing the laser. Each background spectrum was subtracted from the corresponding reaction spectrum.

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