## Theoretical examination of competitive β-radical-induced cleavages of N-Cα and Cα-C bonds of Peptides

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Theoretical examination of competitive β-radical-induced cleavages of N-Cα and Cα-C bonds of peptides

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Abstract

Selective cleavages of N-Cα and Cα-C bonds of β-radical tautomers of amino acid residues in radical peptides have been examined theoretically by means of density functional theory at the M06-2X/6-311++G(d,p) level. Majority of the bond cleavages is homolytic via β-scission. Their energy barriers depend largely on the ability of the radical being stabilized in the transition structures and the availability of a mobile proton in the vicinity of the β-radical center. The N-Cα bond is less favorably cleaved than the Cα-C bond (except Ser and Thr) for systems without a mobile proton. It is because, firstly, the homolytic cleavage is less favorable for the more polar N-Cα bond than for the less polar Cα-C bond. Secondly, a less stable σ-radical localized on the amide nitrogen atom of the incipient N-terminal fragment is formed for the former, while a more stable radical delocalized in a π*(CO)k-like orbital of the incipient C-terminal fragment is formed for the latter. In the presence of a mobile proton N-terminal to the β-radical center, some degrees of heterolytic cleavage character, as preferred by the polar N-Cα bond, are observed. Consequently, its barrier is reduced. If the mobile proton is located at the C-terminal amide oxygen of the β-radical center, the Cα-C bond cleavage will be significantly suppressed. It is because the radical in the incipient C-terminal fragment becomes more localized as a σ-radical on the carbon atom of its protonated amide group. With basic amino-acid residues, the Cα-C bond cleavage can be re-activated. Heterolytic cleavage of the polar N-Cα bond can be largely facilitated if a mobile proton N-terminal to the β-radical center is available and the radical in the incipient C-terminal fragment is sufficiently stabilized, for instance, by the aromatic side chain of Trp and Tyr. Therefore, cleavages of the N-Cα bond induced by the β-radical tautomer of Trp and Tyr are often preferred as compared with cleavages of the Cα-C bond in peptide radical cations containing mobile protons.
Graphical Abstract:

β-radical: “N–C$_\alpha$ or C$_\alpha$–C ?!”

Keywords:

Peptide dissociation
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β-scission
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Introduction

Gas-phase chemistry of odd electron peptide radical cations is of fundamental importance with respect to its analytical applications to mass spectrometry-based protein identification. Numerous techniques for generation of peptide radical cations in the gas phase have been developed. Electron ionization is a classical method to produce molecular radical cations ([M]⁺), but only limited for small and volatile molecules (M). Major interests of generation of large peptide/protein radical cations have been continuously growing since the success of electron capture dissociation (ECD)¹ and electron transfer dissociation (ETD),² which involve recombination of a multiply protonated peptide/protein ion ([M+nH]ⁿ⁺) with a low-energy electron, resulting in hydrogen-rich peptide radical cations ([M+nH]ⁿ⁺⁻¹). Other techniques, for examples, collision-induced oxidative dissociations of transition metal-peptide complexes,³⁻⁵ photo-induced dissociations of iodo-peptides,⁶,⁷ and free-radical-initiated peptide sequencing,⁸ have also been developed to generate peptide radical cations that are stoichiometrically equivalent to [M]⁺⁺.

Because of the high reactivity of unpaired electrons, the peptide radical cations are very susceptible to dissociations, yielding smaller fragments containing information of peptide sequences. For [M+nH]ⁿ⁻¹⁺, the unpaired electron can give rise to rapid radical-induced dissociations⁸⁻¹¹ usually at the N-Cα bonds along the peptide backbone, producing predominantly c/z-type fragments.¹²,¹³ For [M]⁺⁺, tautomerizations prior to backbone or side-chain dissociations are commonly observed.¹⁴ Captodative α-radical is a typical tautomer formed through a hydrogen-atom abstraction from the α-carbon of an amino acid residue.¹⁵,¹⁶ Dissociations of the α-radical tautomer of [M]⁺⁺ are normally charge-induced by the mobile proton, forming b/y-type fragments.¹⁷ Interestingly, hydrogen-atom abstraction from the β-carbon on the side chain of an amino acid can result in a β-radical tautomer.⁵,¹⁸⁻²¹ This radical can cleave either its adjacent N-Cα or Cα-C bond, forming c/z-type or a/x-type fragments,
respectively (Scheme 1), similar to the β-scission of peptide alkoxy radicals.\textsuperscript{22} Although the β-radical-induced C\textsubscript{α}-C bond cleavage is energetically favorable for many radical peptides,\textsuperscript{5,19,23} some cases of N-C\textsubscript{α} bond cleavages were also observed.\textsuperscript{17,21,24-27} Competition between the cleavages of the N-C\textsubscript{α} and C\textsubscript{α}-C bonds apparently depends on the properties of the amino-acid residues\textsuperscript{17,21,28} and the availability of a freely mobile proton.\textsuperscript{5,7,29,30} However, their exact roles on these competitive bond cleavages were not detail studied.

In this report, the β-radical-induced cleavages of the N-C\textsubscript{α} and C\textsubscript{α}-C bonds along a peptide backbone has been theoretically examined based on small radical peptide models using density functional theory (DFT). Effects of the mobile proton and the amino-acid side chains on the competitive bond cleavages have been illustrated.

**Computational Details**

The model peptides used for this theoretical examination are a single amino-acid residue (Xxx) with its amino and carboxylic sides being modified with an acetyl group and a methylamide group (Ac(Xxx)NHMe) or both with a glycine residue (GlyXxxGly). One-electron oxidation of the model molecule results in its molecular radical cations ([Ac(Xxx)NHMe]\textsuperscript{+} or [GlyXxxGly]\textsuperscript{+}). Removing one proton from them will give respective neutral peptide radicals ([Ac(Xxx)NHMe – H]\textsuperscript{−} or [GlyXxxGly – H]\textsuperscript{−}). All calculations were carried out with Gaussian 09 quantum chemical program.\textsuperscript{31} Electronic energies were calculated in the framework of DFT using the unrestricted M06-2X.\textsuperscript{32} For comparisons, some other DFT functionals\textsuperscript{33-37} as well as high-level ab initio methods including MP2,\textsuperscript{38} CCSD,\textsuperscript{39,40} and G3X(MP2)-RAD\textsuperscript{41} calculations were also performed for a few peptide models. Atomic orbitals were described by a Gaussian-type split-valence-shell 6-311++G(d,p) basis set including polarization and diffuse functions for all atoms.\textsuperscript{42,43} Lowest-energy structures of the model peptides were obtained from manual searches on their conformational
spaces with respect to the dihedral angles of the peptide backbones and the side chains.

Geometries optimized at local minima or maxima on a potential energy surface (PES) were confirmed, respectively, with zero or one imaginary vibrational frequency evaluated from harmonic vibrational analyses. All electronic energies were corrected with zero-point vibrational energies. For cases that transition structures were not able to be located either due to very flat PES or non-existence of transition state, PES scans along the reaction coordinate were performed instead. Harmonic zero-point energies were also estimated for geometries with the fragments being well-separated apart. Natural population analyses were performed to determine distributions of atomic charge and spin density.  

Cleavages of the N-C$_\alpha$ or C$_\alpha$-C bonds result in c/z-type or a/x-type fragments, respectively. Scheme 1A illustrates the homolytic cleavages of a neutral $\beta$-radical tautomer of [Ac(Ala)NHMe – H]$^\cdot$. This structure mimics a peptide radical in which the charge (proton) is sequestered by a basic residue that is far away from the $\beta$-radical center. Cleavages of its N-C$_\alpha$ and C$_\alpha$-C bonds are primarily induced by the $\beta$-radical of the amino-acid residue via the $\beta$-scission without influence from other residues that are present in real systems. They produce incipient [c]$^\cdot$ / [z – H] or [a – H] / [x]$^\cdot$ fragments, respectively.

Schemes 1B and C show, for examples, some similar N-C$_\alpha$ and C$_\alpha$-C bond cleavages for the $\beta$-radical tautomers of [Ac(Ala)NHMe]$^{+\cdot}$. Homolytic or heterolytic cleavages of the N-C$_\alpha$ bond of the N-side protonated tautomer will give incipient [c + H]$^{+\cdot}$ / [z – H] or [c + H] / [z – H]$^{+\cdot}$ fragments, respectively (Scheme 1B). Similarly, the incipient [a – H] / [x + H]$^{+\cdot}$ or [a – H]$^{+\cdot}$ / [x + H] fragments will be produced, respectively, from the homolytic or heterolytic cleavage of the C$_\alpha$-C bond of the C-side protonated tautomer (Scheme 1C).

In this work, the energy barriers against the N-C$_\alpha$ and C$_\alpha$-C bond cleavages induced by the $\beta$-radical to form the incipient fragments are emphasized. Table 1 summarizes the energies of the transition structures relative independently to the respective reactant.

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configurations of the β-radical tautomer of \([\text{Ac(Ala)NHMe – H}]^\cdot\) and \([\text{Ac(Ala)NHMe}]^{++}\). The values obtained from CCSD and G3X(MP2)-RAD are comparable, within 0 – 4 kcal mol\(^{-1}\).

The good agreement of these two levels of theory was also suggested in a previous study for the analog β-scission of peptide backbone by an alkoxyl radical.\(^{44}\) As previously found for radical systems,\(^{45}\) MP2 and B3LYP, respectively, overestimates (by 1 – 11 kcal mol\(^{-1}\)) and underestimates (by 4 – 10 kcal mol\(^{-1}\)) the barriers against the β-radical-induced N-C\(_\alpha\) and C\(_\alpha\)-C bond cleavages. It is interesting to note that accuracy of the pure DFT functionals, such as HCTH and PBE, is comparable with that of the B3LYP hybrid DFT functional. Both M06-2X and mPW1PW91 hybrid DFT functionals perform well; they predict energy barriers that are only around 1 – 4 kcal mol\(^{-1}\) deviated from the values obtained from CCSD and G3X(MP2)-RAD. The values obtained from M06-2X are used for the following discussions.

**Results and Discussion**

\([\text{Ac(Ala)NHMe – H}]^\cdot\) and \([\text{Ac(Ala)NHMe}]^{++}\)

Figure 1 shows the lowest-energy structures of the reactants and the transition states associated with the N-C\(_\alpha\) or C\(_\alpha\)-C bond cleavages of the β-radical tautomers of \([\text{Ac(Ala)NHMe – H}]^\cdot\) and \([\text{Ac(Ala)NHMe}]^{++}\). The energy barriers at 0 K (\(\Delta H^\circ\) in kcal mol\(^{-1}\)) are the energy of the transition structure relative to the energy of the corresponding reactant structure. Spin-density distributions are displayed as yellow surfaces with an isovalue of 0.01.

\([\text{Ac(Ala)NHMe – H}]^\cdot\). The radical of the lowest-energy structure of \([\text{Ac(Ala)NHMe – H}]^\cdot\) is localized in the \(p\) orbital of the β-carbon which can be visualized from the spin-density distribution. The N-C\(_\alpha\) and C\(_\alpha\)-C bond cleavages are straightforward, following the mechanism that involves a direct bond cleavage via the transition structures as shown in Figure 1A. The barrier against the N-C\(_\alpha\) bond cleavage (33.2 kcal mol\(^{-1}\)) is higher than that
for the Cα-C bond cleavage (24.1 kcal mol⁻¹), indicating that formation of the [c]⁺ / [z – H] fragments is less favorable than formation of the [a – H] / [x]⁺ fragments (Scheme 1A). Natural population analyses show that the natural charges on the incipient [c]⁺ fragment (CH₃CONH) in the transition structure associated with the N-Cα bond cleavage and the incipient [x]⁺ fragment (CONHCH₃) in the transition structure associated with the Cα-C bond cleavage are only slightly increased by 0.07 and 0.03, respectively, relative to the values of the corresponding fragments in the reactant structure. These insignificant changes in natural charge suggest that both N-Cα and Cα-C bonds are likely cleaved homolytically, as shown in Scheme 1A.

It is reasonable that the cleavage of the N-Cα bond is energetically less favorable because the electronegative nitrogen atom of the incipient [c]⁺ fragment will accommodate only one electron from the homolytically cleaved polar N-Cα bond. The resulting spin is largely located on this nitrogen atom as a σ-radical, illustrated by the spin-density distribution of its transition structure. On the other hand, the Cα-C bond is relatively non-polar and homolytic cleavage of which can transfer the unpaired electron from the β-carbon atom to the incipient [x]⁺ fragment, in which the spin can delocalize between its amide carbon and oxygen atoms in an orbital with a significant CO π* character, similar to the isoelectronic RCN⁻ analog.⁴⁶,⁴⁷

\[\text{[Ac(Ala)NHCH₃]}⁺\]. The β-radical tautomer of [Ac(Ala)NHMe]⁺⁺ contains a mobile proton in the vicinity of the β-radical center. It is common that such a proton can locate at the amide oxygen atoms along a peptide backbone.⁴⁸,⁴⁹ Three tautomers with different protonation modes on the amide oxygen atoms are considered and their lowest-energy structures are also shown in Figure 1. They contain the proton located on the N-terminal amide (N-side protonation), the C-terminal amide (C-side protonation), or bridged between these two
amides (bridged protonation). As in the neutral [Ac(Ala)NHMe – H]⁺ analog, the unpaired electrons of these three β-radical tautomers of [Ac(Ala)NHMe]⁺⁺ are also localized in the \( p \) orbital of the β-carbon.

The N-C$_\alpha$ bond cleavage is also accompanied with a significant redistribution of spin density from the β-carbon to the amide nitrogen atom of the incipient [c+H]⁺⁺ fragment (Scheme 1B) in the transition structure as a localized σ-radical (Figure 1). The barriers against the N-C$_\alpha$ bond cleavage for all protonation modes are similar (29.4 – 32.0 kcal mol$^{-1}$), which are also close to the value for the neutral analog (33.2 kcal mol$^{-1}$). In contrast, the mode of protonation has a greater effect on the barrier against the C$_\alpha$-C bond cleavage, which increases from 21.0 kcal mol$^{-1}$ for the N-side protonation to 27.3 kcal mol$^{-1}$ for the bridged protonation. For the C-side protonation, the barrier is further increased to 31.1 kcal mol$^{-1}$, so that the C$_\alpha$-C bond cleavage becomes less favorable than the N-C$_\alpha$ bond cleavage (29.4 kcal mol$^{-1}$). This effect can also be attributed to the relative stability of the radical in the transition structures. For the N-side protonation, the spin density can be again distributed between the C-terminal amide oxygen and carbon atoms in the \( \pi^*(CO) \)-like orbital. For the C-side protonation, the spin density is mainly localized on the amide carbon of the incipient [x + H]⁺⁺ fragment. It is likely because the protonated amide oxygen atom in the case of C-side protonation can hold its electrons tighter and, consequently, have less ability to share the unpaired electron from the amide carbon atom. Similar effect is also observed for the bridged-protonation mode in which the proton that is originally closer to the C-terminal amide in the reactant structure is transferred to the N-terminal side to provide better delocalization of the spin density in the \( \pi^*(CO) \)-like orbital of the incipient [x]⁺ fragment.

It is worth to note that the proton plays a significant role in the competition between the cleavages of the N-C$_\alpha$ and C$_\alpha$-C bonds by influencing the spin-density distribution (vide supra). The proton can also affect the nature of bond cleavages. While both N-C$_\alpha$ and C$_\alpha$-C
bond cleavages in [Ac(Ala)NHMe – H]’ and [Ac(Ala)NHMe]+ are mainly homolytic in
nature, some degrees of heterolytic character are observed in the N-Ca bond cleavage for the
N-side protonation (Scheme 1B) and the Ca-C bond cleavage in the C-side protonation
(Scheme 1C). The natural charge on the N-terminal fragment in the transition structure of the
N-Ca bond cleavage for the N-side protonation is 0.19 smaller than that in the reactant.
Similar decrease in natural charge by 0.22 is also determined for the C-terminal fragment in
the case of the Ca-C bond cleavage for the C-side protonation. It is reasonable that the proton
can facilitate the heterolytic bond cleavage by drawing the bonding electron pair to the
direction where it is located. This effect becomes more significant if the alanine is replaced
by other amino acids that are able to better stabilize the β-radical (vide infra).

\[\text{[Ac(Xxx)NHMe – H]}’ \text{ and [Ac(Xxx)NHMe]+}\]

Table 2 summarizes the energy barriers against cleavages of the N-Ca and Ca-C bonds
in the β-radical tautomers of [Ac(Xxx)NHMe – H]’ and [Ac(Xxx)NHMe]+, where Xxx is
any natural amino acids except glycine that does not have a β-carbon. Optimized structures
and spin-density distributions for Xxx = Trp is shown in Figure 2 and some other systems are
also available in Supporting Information. In general, the results discussed above for Xxx =
Ala are similar for many systems of other amino acids.

\[\text{[Ac(Xxx)NHMe – H]}’ \text{ (Neutral).} \]

The N-Ca bond cleavage of [Ac(Xxx)NHMe – H]’ for all
Xxx is less competitive than the Ca-C bond cleavage by 2.9 – 22.3 kcal mol⁻¹ (except Ser and
Thr, for which N-Ca bond cleavage was also experimentally observed previously²⁴,²⁸). The
less favorable N-Ca bond cleavage can also be rationalized by the formation of the less stable
σ-radical localized on the nitrogen atom of the incipient [c]’ fragment in its transition
structure, as compared with the more stable and delocalized radical in the π*(CO)-like orbital
of the incipient [x]’ fragment in the transition structure of the Cα-C bond cleavage. The changes of natural charge of the incipient [c]’ or incipient [x]’ fragment from the reactant to the transition structures are also insignificant, indicating that both bond cleavages are also homolytic in nature induced by the β-radical.

**[Ac(Xxx)NHMe]** with N-side or bridged protonation. The competition between the N-Cα and Cα-C bond cleavages in [Ac(Xxx)NHMe]** depends largely on the protonation modes. In general, for the N-side and bridged protonations, the barriers against the N-Cα bond cleavage are also higher than those for the Cα-C bond cleavage by 0.8 – 12.2 kcal mol⁻¹ (except Ser, Thr, Trp, Tyr, His, Lys, Val). In the transition structures for all these amino acid residues, the unpaired electron is again largely localized on the nitrogen atom of the incipient [c+H]** fragment, resulting from the homolytic cleavage of the N-Cα bond. As discussed for the case of Xxx = Ala, these transition structures are destabilized by this localization of electron spin. Thus, the Cα-C bond cleavage is alternatively favored. The mobile proton can also promote some degrees of heterolytic N-Cα bond cleavage, albeit again insignificant as in the case for Ala. It is reasonable because the charge and radical at the α- and β-carbons of the incipient [z-H]** fragment resulting from a heterolytic N-Cα bond cleavage (Scheme 1B) can only be weakly stabilized by an aliphatic side chain or are even destabilized if an electron withdrawing side chain is present.

**[Ac(Xxx)NHMe]** with C-side protonation and basic amino acids. For the C-side protonation, the N-Cα bond cleavage prevails over the Cα-C bond cleavage with the barriers of the former being 1.4 – 12.9 kcal mol⁻¹ lower than those of the latter (except Xxx = Ile, Pro, Asp, Asn, Glu, Gln, Lys, Arg, His, and Met). It is again because the energy barriers against the Cα-C bond cleavage are significantly increased as compared with the case of the neutral
[Ac(Xxx)NHMe – H]⁺ analogs due to the formation of a more localized, but less stable, radical on the amide carbon atom of the incipient [x + H]⁺ fragment. It is interesting to note that there are quite a number of exceptions, of which the amino acid residues are relatively more basic. Many of them also contain a basic side chain that can sequester the mobile proton which apparently favors the Cα-C bond cleavage.²³,⁵⁰ In these cases, the energies of the transition structures associated with the Cα-C bond cleavage are largely reduced because the delocalization of electron spin in the π*(CO)k-like orbital is available again, as the case for [Ac(Xxx)NHMe – H].²³

[Ac(Xxx)NHMe]⁺⁺ with aromatic amino acid residues. The N-Cα bond cleavage is more favorable than the Cα-C bond cleavage for most systems with Xxx = aromatic amino acids, especially for Tyr and Trp; the barriers associated with the N-Cα bond cleavage in [Ac(Tyr)NHMe]⁺⁺ and [Ac(Trp)NHMe]⁺⁺ are always lower than those in the neutral [Ac(Tyr)NHMe – H]⁺ and [Ac(Trp)NHMe – H]⁺ analogs by 8 – 15 kcal mol⁻¹, regardless of the protonation modes. Figure 2 shows the lowest-energy structures of the β-radical tautomers of [Ac(Trp)NHMe]⁺⁺ with different protonation modes and the corresponding transition structures associated with the N-Cα and Cα-C bond cleavages. Unlike most of the transition structures of the N-Cα bond cleavage for other amino acids, less spin density is transferred to the nitrogen atom of the N-terminal fragment (Figure 2). For the N-side protonation, the natural charge of the N-terminal fragment in the reactant structure (0.7) decreases significantly to that in the transition structure (0.2). These results suggest that the N-Cα bond cleavage in the N-side protonated [Ac(Trp)NHMe]⁺⁺ is heterolytic in nature, yielding incipient [c + H] fragment (c.f. Scheme 1B). The radical in the complementary incipient [z – H]⁺ fragment can be well-delocalized with the aromatic indole ring. Heterolytic N-Cα bond cleavages are also observed for the bridged- and C-side protonations; the heterolytic N-Cα...
cleavages are accompanied by a proton shift from the C-terminal side to the N-terminal side, also forming the incipient [c + H] / [z – H]⁺⁺ fragments (Figures 2C and D).

**Effects of the mobile proton and the aromatic ring toward the selectivity of N-Cα and Cα-C bond cleavages of the β-radical tautomers of tripeptides [GlyXxxGly – H]⁺ and [GlyXxxGly]⁺⁺, Xxx = Ala or Trp**

As discussed in the previous section, the conjugated aromatic ring plays a significant role in reducing the energy barrier against the heterolytic N-Cα bond cleavage induced by the mobile proton. This effect is further verified using two larger tripeptide systems GlyAlaGly and GlyTrpGly (Table 3). In general, the energy barriers against both N-Cα and Cα-C bond cleavages for the tripeptide systems are not deviated much from the smaller models. The Cα-C bond cleavage is favorable for both [GlyAlaGly – H]⁺ and [GlyAlaGly]⁺⁺ with energy barriers ranging from 21.9 to 31.2 kcal mol⁻¹, while the energy barriers against the N-Cα bond cleavage are ranging from 32.3 to 36.8 kcal mol⁻¹. It is worth to note that the increase of energy barrier against the Cα-C bond cleavage for the Cα-side protonation of [GlyAlaGly]⁺⁺ can also be rationalized by the reduced ability of the spin delocalization between the carbon and oxygen atoms of the C-terminal amide by the mobile proton. Figures 3A and C show the spin distribution of reactant and transition structures for [GlyAlaGly – H]⁺ and [GlyAlaGly]⁺⁺, respectively. Both the N-Cα and Cα-C bonds are cleaved homolytically, indicated by the spin density that is significantly located on the nitrogen atom of the N-terminal amide bond and the CO of the C-terminal amide in the respective transition structures.

As the smaller models for Xxx = Trp, the N-Cα cleavage is more competitive than the Cα-C bond cleavage for [GlyTrpGly]⁺⁺ in which a mobile proton is present. The energy barriers against the N-Cα bond cleavage is ranging between 21.8 and 24.9 kcal mol⁻¹, which are lower than the energy barriers against the Cα-C bond ranging between 28.8 and 33.3 kcal
mol$^{-1}$. Figure 3D shows the lowest-energy structure of the β-radical tautomer of [GlyTrpGly]$^{+}$ (bridged protonation) and the transition structures associated with its N-C$_{a}$ and C$_{a}$-C bond cleavages. The N-C$_{a}$ bond cleavage is again heterolytically induced by the mobile proton and the spin is largely delocalized with the indole ring in the incipient C-terminal [z$_{2}$ – H]$^{+}$ fragment. The barrier against this heterolytic N-C$_{a}$ bond cleavage (24.9 kcal mol$^{-1}$) is significantly reduced as compared with the homolytic cleavages of [GlyAlaGly – H]$^{+}$ (34.1 kcal mol$^{-1}$), [GlyAlaGly]$^{+}$ (33.7 kcal mol$^{-1}$), and [GlyTrpGly – H]$^{+}$ (37.7 kcal mol$^{-1}$). Hence, it is further confirmed that in the presence of a conjugated aromatic ring and mobile proton, the β-radical-induced backbone fragmentation favors the N-C$_{a}$ bond cleavage.$^{17,21}$ In the absence of the mobile proton, i.e. [GlyTrpGly – H'], the C$_{a}$-C bond is cleaved instead.$^{5,7,19,30}$

**Conclusion**

The selectivity of the N-C$_{a}$ and C$_{a}$-C bond cleavages along a peptide backbone induced by the β-radical tautomer of peptide radical cations has been theoretically studied using the M06-2X/6-311++G(d,p) level of theory, which gives accuracy comparable with CCSD and G3X(MP2)-RAD. Model peptides, including [Ac(Xxx)NHMe – H]', [Ac(Xxx)NHMe]$^{+}$, [GlyAlaGly – H]', [GlyAlaGly]$^{+}$, [GlyTrpGly – H]', and [GlyTrpGly]$^{+}$, were examined. The energy barriers against the cleavages of these two types of bond depend largely on the ability of the radical being stabilized in the transition structures and the availability of the mobile proton in the vicinity of the β-radical center. The N-C$_{a}$ bond cleavages, leading to the c/z-type fragments, are in general less favorable than the C$_{a}$-C bond cleavages, leading to the a/x-type fragments. The energy barriers against the former are around 3 – 23 kcal mol$^{-1}$ higher than the latter for systems without a mobile proton in the vicinity of the β-radical center (except Ser and Thr). It is because, firstly, the homolytic cleavage of the more polar N-C$_{a}$ bond is less favorable than that of the less polar C$_{a}$-C bond.
Secondly, a less stable $\sigma$-radical localized on the nitrogen atom of the incipient [c]$^+$ fragment from the N-C$_\alpha$ bond cleavage is formed, while the C$_\alpha$-C bond cleavage can form a more stable radical delocalized in the $\pi^*(CO)$-like orbital of the incipient [x]$^+$ fragment. In the presence of a proton N-terminal to the $\beta$-radical center, the energy barriers of the N-C$_\alpha$ bond cleavage are slightly reduced. It is because the proton can induce some degrees of heterolytic cleavage character for the polar N-C$_\alpha$ bond. If the mobile proton is located at the C-terminal amide of the $\beta$-radical center, the C$_\alpha$-C bond cleavages would be significantly suppressed; the barriers against the C$_\alpha$-C bond cleavage are around 1 – 13 kcal mol$^{-1}$ higher than those against the N-C$_\alpha$ bond cleavage (except for basic amino-acid residues). It is because the radical in the incipient [x+H]$^{+\bullet}$ fragment resulting from the homolytic C$_\alpha$-C bond cleavage is now more localized as a $\sigma$-radical on the carbon atom of its amide group. For aromatic amino acids, the heterolytic N-C$_\alpha$ bond cleavage will be largely facilitated by the availability of the mobile proton at the N-terminal side and the high ability of radical delocalization with a sufficiently large aromatic ring, such as the indole ring in Trp and the phenoxy ring in Tyr. Therefore, in peptide radical cations without basic residues, the N-C$_\alpha$ bond cleavages induced by the $\beta$-radical tautomer of Trp and Tyr are often preferred.

**Supporting Information**

Relative energies of different tautomers in Table 2; optimized structures and spin density distributions of some systems; full author list of reference 31.

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References


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<td>B3LYP</td>
<td>26.7 18.6 8.1</td>
<td>23.3 16.1 7.2</td>
</tr>
<tr>
<td>BLYP</td>
<td>18.9 12.5 6.4</td>
<td>16.5 11.4 5.1</td>
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<tr>
<td>HCTH</td>
<td>24.3 15.7 8.6</td>
<td>20.7 14.6 6.1</td>
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<tr>
<td>PBE</td>
<td>25.2 16.6 8.6</td>
<td>23.6 16.3 7.3</td>
</tr>
<tr>
<td>MP2</td>
<td>42.3 32.8 9.5</td>
<td>33.9 28.7 5.2</td>
</tr>
<tr>
<td>G3X(MP2)-RAD</td>
<td>31.2 23.6 7.6</td>
<td>32.6 20.3 12.3</td>
</tr>
<tr>
<td>CCSD (with ZPE from M06-2X)</td>
<td>31.7 26.6 5.1</td>
<td>32.8 22.5 10.3</td>
</tr>
</tbody>
</table>

**Table 1.**

Energy barriers ($\Delta H^\circ_0$ in kcal mol$^{-1}$) against the N-C$_a$ and C$_a$-C bond cleavages for [Ac(Ala)NHMe – H]$^\dagger$ and [Ac(Ala)NHMe]$^{\ddagger}$. 
<table>
<thead>
<tr>
<th>Amino Acid (Xxx)</th>
<th>[Ac(Xxx)NHMe - H]*</th>
<th>[Ac(Xxx)NHMe]+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>N-side Protonation</td>
</tr>
<tr>
<td>N-C(_a)</td>
<td>C(_a)-C</td>
<td>(\Delta)</td>
</tr>
<tr>
<td>Ala</td>
<td>33.2</td>
<td>24.1</td>
</tr>
<tr>
<td>Val</td>
<td>29.5</td>
<td>21.7</td>
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<tr>
<td>Leu</td>
<td>30.9</td>
<td>22.8</td>
</tr>
<tr>
<td>Ile</td>
<td>29.8</td>
<td>22.7</td>
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<tr>
<td>Pro</td>
<td>38.4</td>
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<tr>
<td>Asp</td>
<td>39.7</td>
<td>25.6</td>
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<tr>
<td>Asn</td>
<td>42.4</td>
<td>20.1</td>
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<td>Glu</td>
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<td>24.3</td>
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<tr>
<td>Gln</td>
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<td>22.3</td>
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<tr>
<td>Lys</td>
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<tr>
<td>Arg</td>
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<td>28.2</td>
</tr>
<tr>
<td>His</td>
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<td>30.6</td>
</tr>
<tr>
<td>Phe</td>
<td>37.0*</td>
<td>31.9</td>
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<tr>
<td>Tyr</td>
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<td>29.7</td>
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<tr>
<td>Trp</td>
<td>35.1</td>
<td>31.4</td>
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<tr>
<td>Met</td>
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<td>24.6</td>
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<tr>
<td>Cyc</td>
<td>30.7</td>
<td>26.1</td>
</tr>
<tr>
<td>Ser</td>
<td>22.4</td>
<td>22.9</td>
</tr>
<tr>
<td>Thr</td>
<td>20.0</td>
<td>29.1</td>
</tr>
</tbody>
</table>

Table 2.

Energy barriers (\(\Delta H^0\) in kcal mol\(^{-1}\)) against the N-C\(_a\) and C\(_a\)-C bond cleavages for [Ac(Xxx)NHMe – H]\* and [Ac(Xxx)NHMe]\+, evaluated at the UM06-2X/6-311++G(d,p) level. a Energy estimated from PES scan.
Table 3.

Energy barriers ($\Delta H^0_0$ in kcal mol$^{-1}$) against the N–C$_\alpha$ and C$_\alpha$–C bond cleavages for [GlyXxxGly – H]$^+$ and [GlyXxxGly]$^{++}$ (Xxx = Ala or Trp), evaluated at the UM06-2X/6-311++G(d,p) level. * Energy estimated from PES scan.

<table>
<thead>
<tr>
<th>Amino Acid (Xxx)</th>
<th>[GlyXxxGly - H]$^+$</th>
<th>[GlyXxxGly]$^{++}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>N-side Protonation</td>
</tr>
<tr>
<td></td>
<td>N-C$_\alpha$</td>
<td>C$_\alpha$-C</td>
</tr>
<tr>
<td>Ala</td>
<td>34.1</td>
<td>24.0</td>
</tr>
<tr>
<td>Trp</td>
<td>37.7</td>
<td>27.1</td>
</tr>
</tbody>
</table>
Scheme 1.

Some cleavages of the N-Cα and the Cα-C bond of the β-radical tautomer of (A) neutral [Ac(Ala)NHMe - H]* and molecular radical cation [Ac(Ala)NHMe]•+ with (B) N-side protonation and (C) C-side protonation.
Figure 1. Lowest-energy structures and transition structures associated with the N-C$_\alpha$ or C$_\alpha$-C bond cleavages for the $\beta$-radical tautomer of (A) neutral [Ac(Ala)NHMe – H]$^+$ and the molecular radical cation [Ac(Ala)NHMe]$^{+\cdot}$ with (B) N-side protonation, (C) bridged protonation, or (D) C-side protonation. Geometry optimizations and spin-density distribution were calculated at the UM06-2X/6-311++G(d,p) level. The energy barrier (with zero-point correction at 0 K ($\Delta H^0_0$ in kcal mol$^{-1}$)) of each bond-cleaving pathway is relative to the lowest-energy structure of the corresponding reactant.
Figure 2. Lowest-energy structures and transition structures associated with the N-C\(_{\alpha}\) or C\(_{\alpha}-C\) bond cleavages for the \(\beta\)-radical tautomer of (A) neutral [Ac(Trp)NHMe - H]\(^+\) and the molecular radical cation [Ac(Trp)NHMe]\(^+\) with (B) N-side protonation, (C) bridged protonation, or (D) C-side protonation. Geometry optimizations and spin-density distribution were calculated at the UM06-2X/6-311++G(d,p) level. The energy barrier (with zero-point correction at 0 K (\(\Delta H^0\) in kcal mol\(^{-1}\))) of each bond-cleaving pathway is relative to the lowest-energy structure of the corresponding reactant.
Figure 3. Lowest-energy structures and transition structures associated with the N-Cα or Cα-C bond cleavages for the β-radical tautomer of the neutral (A) [GlyAlaGly – H]⁺ and (B) [GlyTrpGly – H]⁺ and the molecular radical peptide (C) [GlyAlaGly]⁺⁺ and (D) [GlyTrpGly]⁺⁺. The geometry optimizations and spin-density distribution were calculated at the UM06-2X/6-311++G(d,p) level. The energy barrier (with zero-point correction at 0 K (∆H°₀ in kcal mol⁻¹)) of each bond-cleaving pathway is relative to the lowest-energy structure of the corresponding reactant.
(A) [\text{Ac(AIa)NMe}]^+ \text{NHMo}^- 
Neutral

Transition Structure for N-C\text{\_}2 Cleavage

Reactant

Transition Structure for C\text{\_}6-C Cleavage

33.2

24.1

31.5

21.0

32.0

27.3

(D) [Ac(AIa)NMe]^+ 
C-side Protonation

29.4

31.1
Transition Structure for N–Cα Cleavage | Reactant | Transition Structure for Cα–C Cleavage
--- | --- | ---
(A) [Ac(Trp)NHMe - H+] Neutral | 35.1 | 31.4
(B) [Ac(Trp)NHMe]++ N-side Protonation | 20.2 | 26.9
(C) [Ac(Trp)NHMe]++Bridged Protonation | 21.8 | 36.4
(D) [Ac(Trp)NHMe]++ C-side Protonation | 20.9 | 33.8

209x193mm (300 x 300 DPI)