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| <b>Author(s)</b>   | <b>Song, T; Hao, Q; Law, CH; Siu, CK; Chu, IK</b>  |
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## RESEARCH ARTICLE

# Novel C<sub>β</sub>–C<sub>γ</sub> Bond Cleavages of Tryptophan-Containing Peptide Radical Cations

Tao Song,<sup>1</sup> Qiang Hao,<sup>1,2</sup> Chun-Hin Law,<sup>1</sup> Chi-Kit Siu,<sup>2</sup> Ivan K. Chu<sup>1</sup>

<sup>1</sup>Department of Chemistry, The University of Hong Kong, Hong Kong, China

<sup>2</sup>Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, China

### Abstract

In this study, we observed unprecedented cleavages of the C<sub>β</sub>–C<sub>γ</sub> bonds of tryptophan residue side chains in a series of hydrogen-deficient tryptophan-containing peptide radical cations (M<sup>•+</sup>) during low-energy collision-induced dissociation (CID). We used CID experiments and theoretical density functional theory (DFT) calculations to study the mechanism of this bond cleavage, which forms [M – 116]<sup>+</sup> ions. The formation of an α-carbon radical intermediate at the tryptophan residue for the subsequent C<sub>β</sub>–C<sub>γ</sub> bond cleavage is analogous to that occurring at leucine residues, producing the same product ions; this hypothesis was supported by the identical product ion spectra of [LGGGH – 43]<sup>+</sup> and [WGGGH – 116]<sup>+</sup>, obtained from the CID of [LGGGH]<sup>•+</sup> and [WGGGH]<sup>•+</sup>, respectively. Elimination of the neutral 116-Da radical requires inevitable dehydrogenation of the indole nitrogen atom, leaving the radical centered formally on the indole nitrogen atom ([Ind]<sup>•</sup>–2), in agreement with the CID data for [WGGGH]<sup>•+</sup> and [W<sub>1-CH<sub>3</sub></sub>GGGH]<sup>•+</sup>; replacing the tryptophan residue with a 1-methyltryptophan residue results in a change of the base peak from that arising from a neutral radical loss (116 Da) to that arising from a molecule loss (131 Da), both originating from C<sub>β</sub>–C<sub>γ</sub> bond cleavage. Hydrogen atom transfer or proton transfer to the γ-carbon atom of the tryptophan residue weakens the C<sub>β</sub>–C<sub>γ</sub> bond and, therefore, decreases the dissociation energy barrier dramatically.

**Key words:** C<sub>β</sub>–C<sub>γ</sub> cleavages of tryptophan, Peptide radical cations, Low-energy collision-induced dissociation, Density functional theory calculations

## Introduction

Gas phase dissociation of enzymatically cleaved peptide ions is a key technology for peptide sequencing and, ultimately, protein identification [1, 2]. Dissociations of protonated even-electron peptides under the conditions of low-energy collision-induced dissociation (CID) commonly

reveal charge-directed cleavages at different amide bonds along the peptide backbone, as described by the “mobile proton” model [3–7], forming sequence-informative b or y ions [8, 9]. During low-energy CID processes, the side chains of the amino acid residue often remain intact (except for the loss of H<sub>2</sub>O or NH<sub>3</sub>), thereby introducing the analytical challenge of distinguishing isobaric residues (e.g., leucine and isoleucine) in peptides; the challenge can be overcome by using high-energy CID to induce side chain cleavages of the bonds between the β- and γ-carbon atoms of the amino acid residues [10, 11].

Radicals can also induce bond cleavages in the side chains of the amino acid residues. Such side chain cleavages, together with the formation of c and z ions resulting from the cleavages of N–C<sub>α</sub> bonds along the

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Correspondence to: Chi-Kit Siu; e-mail: chiksiu@cityu.edu.hk, Ivan K. Chu; e-mail: ivankchu@hku.hk

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peptide backbone, are common fragmentation channels for the hydrogen-rich odd-electron peptide radical cations  $[M+nH]^{*(n-1)+}$  that are generated in electron-capture dissociation (ECD) [12–15] or electron-transfer dissociation (ETD) experiments [16–18]. These characteristic side chain losses assert the presence of specific amino acid residues in peptides. For example, losses of 30, 33, 61, 129, and 87 Da are potential indicators of the presence of serine, cysteine, methionine, tryptophan, and arginine residues, respectively [14, 15, 18]. Facile distinction of isomeric leucine and isoleucine is also realized by the respective diagnostic losses of 43 and 29 Da, respectively, that result from their C $\beta$ –C $\gamma$  bond cleavages [18, 19]. At present, 11 amino acid residues can be identified by their characteristic side chain losses, which also provide additional information relating to backbone fragmentations and, thus, greatly enhance the confidence of peptide identification [20].

Many investigations have been performed recently regarding the side chain losses occurring during the dissociations of classical molecular peptide radical cations (M $^{*+}$ ), which are odd-electron peptide cations that are hydrogen-deficient relative to their even-electron protonated counterparts ( $[M+nH]^{n+}$ ). M $^{*+}$  species can be effectively generated through oxidative dissociations of transition metal/peptide complexes [21–25], laser photolysis of peptides containing photolabile tags [26], or dissociation of peptides containing labile groups [27–34]. Examinations of several model M $^{*+}$  species, containing aliphatic, aromatic, and/or basic amino acid residues, have revealed that their fragmentation behaviors are greatly governed by competition between the radical mobility within these radical cations and their fragmentations [20, 35–48]; in arginine-containing M $^{*+}$  species, where the charge is tightly sequestered by the very basic side chain of the arginine residue, radical migrations are facilitated and, as a result, radical-induced charge-remote fragmentations (such as side chain, C $\alpha$ –C bond, and N–C $\alpha$  bond cleavages) are favored [20, 37, 44–46, 49]. Abundant side chain losses from the amino acid residues most likely involve  $\alpha$ - or  $\gamma$ -centered radical intermediates.

Facile 3-methyleneindolenine (129 Da) loss through side chain C $\alpha$ –C $\beta$  bond cleavage of the tryptophan residue is commonly observed in the dissociations of both hydrogen-rich [15, 18] and hydrogen-deficient radical peptide systems [20, 30, 36, 46]. Loss of a neutral species having a mass of 116 Da through cleavage at the C $\beta$ –C $\gamma$  bond of a tryptophan residue has also been observed in several ETD/ECD experiments [15, 18, 50], therefore, such a fragmentation potentially provides diagnostic information to directly implicate the presence of a tryptophan residue in a parent peptide. In this study, we report the first observation of this unique 116-Da neutral loss from molecular peptide radical cations. Using both experimental and theoretical methods, we have examined its chemical identity and the mechanistic details of its formation via the C $\beta$ –C $\gamma$  bond cleavages of tryptophan residues.

## Experimental

### Materials

Fmoc-protected unmodified amino acids and the Wang resin were obtained from Advanced ChemTech (Louisville, KY, USA). Fmoc-protected 1-methyl tryptophan and  $\alpha$ -methyl tryptophan were purchased from Matrix Scientific (Columbia, SC, USA). All other chemicals were obtained commercially (Aldrich or Sigma, St Louis, MO, USA; Bachem, King of Prussia, PA, USA). Oligopeptides and the Cu(II)(terpy)(NO $_3$ ) $_2$  (terpy=2,2':6',2''-terpyridine) and [Co(III)(salen)]Cl [salen=N, N'-ethylenebis(salicylideneiminato)] complexes were synthesized according to procedures described in the literature [51–53].

### Methylation of Peptides

A solution of HCl (ca. 2 M) in MeOH was prepared through the dropwise addition of acetyl chloride (800  $\mu$ L) into anhydrous MeOH (5 mL) and then stirring for 5 min at room temperature. This solution (1 mL) was added to the peptide (10 mg) and then the mixture was stirred for 3 h at room temperature. The resulting solution was dried using a SC250DDA Speedvac Plus (Thermo Electron Corporation, Waltham, MA, USA). The methylated peptide was mixed with the metal complexes in each experiment without any further purification.

### Mass Spectrometry

All mass spectrometry experiments were conducted using a quadrupole ion trap mass spectrometer (Finnigan LCQ, ThermoFinnigan, San Jose, CA, USA). The molecular peptide radical cations M $^{*+}$  were generated through one-electron oxidative dissociations of transition metal/peptide complexes [21–25]. Their abundances were optimized using  $[Cu^{II}(terpy)M]^{*2+}$  complexes (for M = GGGGW, GWGGG, WGGGG, GGGGW-OMe, and GGGFW) or  $[Co^{III}(salen)M]^{*2+}$  complexes (for M = WGGGR, GWGGR, WGGGK, GWGGK, WGGGH, GWGGH, LGGGH, W $_{\alpha-CH_3}$ GGGH, W $_{1-CH_3}$ GGGH, WGGFLR, WGGFLK, WGGFLH, WVYIHPR, WVYIFPK, and WVYIHPF). The fragmentation chemistries of those radical peptide cations were independent of the choice of the metal complexes. Samples typically comprised 600  $\mu$ M Cu(II)(terpy) or Co(III)(salen) complex and 50  $\mu$ M oligopeptide in a H $_2$ O/MeOH (50:50) solution. A syringe pump (Cole Parmer, Vernon Hills, IL, USA) was used for direct infusion of the electrospray samples (flow rate: 30  $\mu$ L/h). CID spectra were acquired using helium as the collision gas. The injection time and excitation time for CID in the ion trap were 200 and 50 ms, respectively; the amplitude of the excitation was optimized for each experiment.

### Computational Methods

Electronic energies were calculated in the framework of density functional theory (DFT) using the unrestricted (U)

hybrid functional formulated with a mixture of the Hartree-Fock exchange energy and Becke's three-parameter 1988 gradient-corrected exchange energy, and the Lee-Yang-Parr (LYP) correlation energy [54]. Atomic orbitals were described by a Gaussian-type split valence shell 6-31++G (d,p) basis set including polarization and diffuse functions for all atoms [55, 56]. Harmonic vibrational frequencies of all optimized structures were calculated to confirm that the structures were at local minima (all real frequencies) or were transition states (one imaginary frequency). The local minima associated with each transition structure were verified using the intrinsic reaction coordinate (IRC) method. Relative enthalpies at 0 K ( $\Delta H_0^\circ$ ) were calculated from the electronic energies and zero-point vibrational energies (ZPVE) obtained within the harmonic approximation. All DFT calculations were conducted using the Gaussian 03 software package [57].

## Results and Discussion

### Peptides Containing Non-Basic Residue

The CID spectrum of [GGGGW] $^{*+}$  (Figure 1a) features a predominant ion at  $m/z$  388, which corresponds to a loss

of CO $_2$  from the C-terminal carboxylic group, and its subsequent neutral loss of 116 Da at  $m/z$  272, presumably from the cleavage at the side chain C $_{\beta}$ –C $_{\gamma}$  bond of the tryptophan residue, and a typical fragment ion of [c $_4$  + 2H] $^{*+}$  at  $m/z$  246 [36, 37]. Cleavage at the C $_{\alpha}$ –C $_{\beta}$  bond of the tryptophan residue to lose 3-methyleneindolenine with a mass of 129 Da is known, but loss through C $_{\beta}$ –C $_{\gamma}$  cleavage is unprecedented. It is noteworthy that we did not observe the direct loss of 116 Da from the CID of [GWGGG] $^{*+}$  (Figure 1b) and [WGGGG] $^{*+}$  (Figure 1c), where the tryptophan residue is, respectively, located in the middle and at the N-terminus of the peptide sequence; they fragmented predominantly via typical N–C $_{\alpha}$  bond cleavages at tryptophan residues [36, 37], forming [z $_4$  – H] $^{*+}$  (Figure 1b) and [z $_5$  – H] $^{*+}$  (i.e., NH $_3$  loss) (Figure 1c) species. Absence of the direct 116 Da loss from the CID of [GGGGW] $^{*+}$ , [GWGGG] $^{*+}$ , and [WGGGG] $^{*+}$  strongly suggests that the formation of an  $\alpha$ -carbon-centered radical at the tryptophan residue in [GGGGW – CO $_2$ ] $^{*+}$  plays an essential role prior to subsequent C $_{\beta}$ –C $_{\gamma}$  bond cleavage [46]. We observed no analogous fragmentations from the protonated [GGGGW + H] $^{*+}$  species, from which the loss of H $_2$ O and the formation of b/y ions were predominant (Figure S1). It seems clear

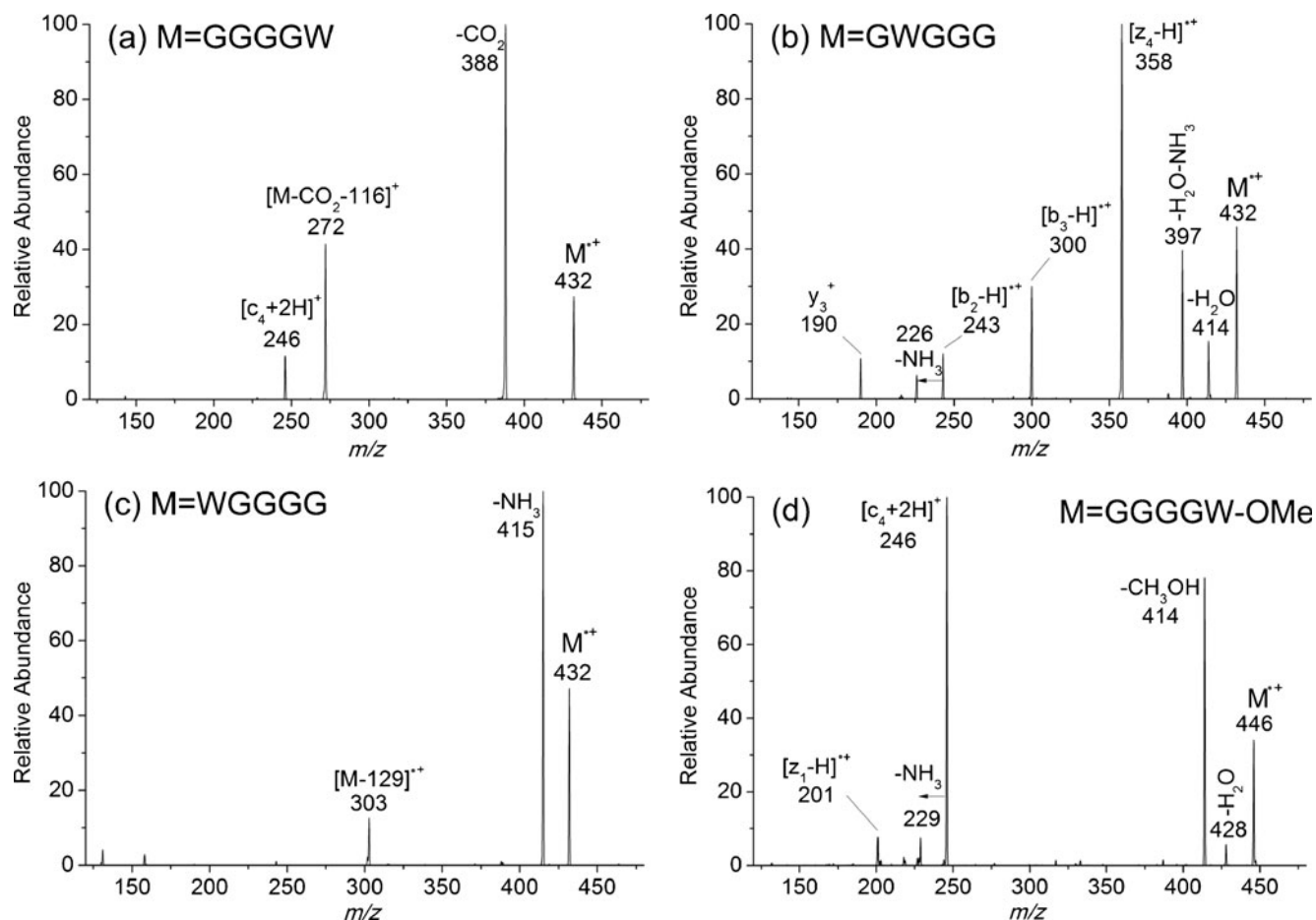


Figure 1. CID spectra of (a) [GGGGW] $^{*+}$ ; (b) [GWGGG] $^{*+}$ ; (c) [WGGGG] $^{*+}$ ; (d) [GGGGW-OMe] $^{*+}$

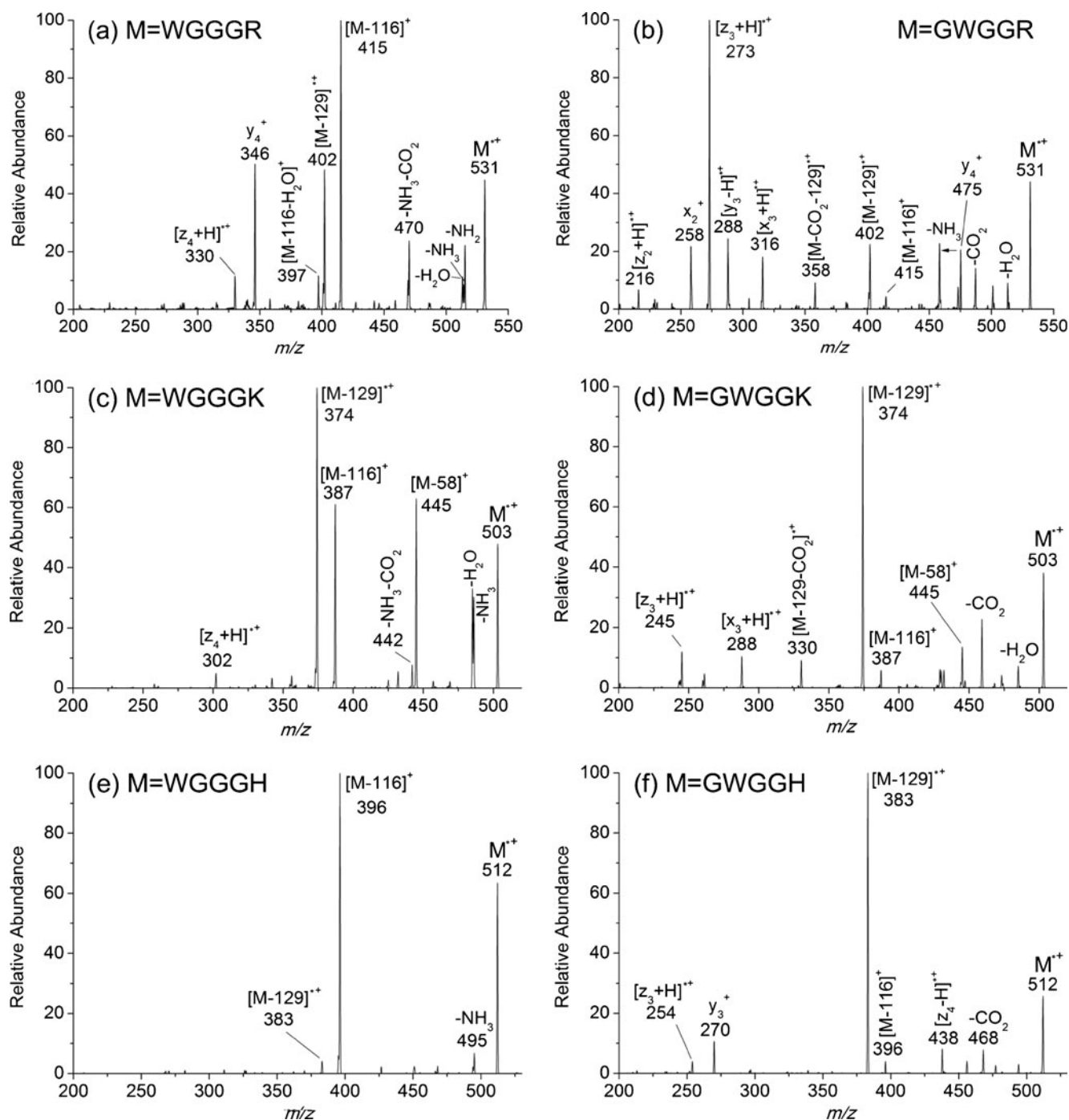


Figure 2. CID spectra of (a) [WGGGR] $^{*+}$ ; (b) [GWGGR] $^{*+}$ ; (c) [WGGGK] $^{*+}$ ; (d) [GWGGK] $^{*+}$ ; (e) [WGGGH] $^{*+}$ ; (f) [GWGGH] $^{*+}$

that propagation of the radical is inefficient among the  $\alpha$ -carbon centers along the backbones of peptide radical cations containing only glycine and tryptophan residues, thereby suppressing some of the competitive fragmentation reactions [42], allowing the [GGGGW – CO $_2$ ] $^{*+}$  fragment ion, possessing its initial radical site at the C-terminal  $\alpha$ -carbon atom of the tryptophan residue, to readily undergo subsequent C $\beta$ –C $\gamma$  bond cleavage. Through C-terminal methylation, we experimentally eliminated the possibility of forming the  $\alpha$ -carbon-

centered radical through CO $_2$  loss; the CID spectrum of [GGGGW–OMe] $^{*+}$  revealed neither CO $_2$  loss nor its consecutive 116-Da loss (Figure 1d).

### Peptides Containing Basic Residue

We indirectly confirmed the prerequisite for the  $\alpha$ -carbon-centered radical to be located at the tryptophan residue to trigger C $\beta$ –C $\gamma$  bond cleavages through examination of the arginine-containing [WGGGR] $^{*+}$  and





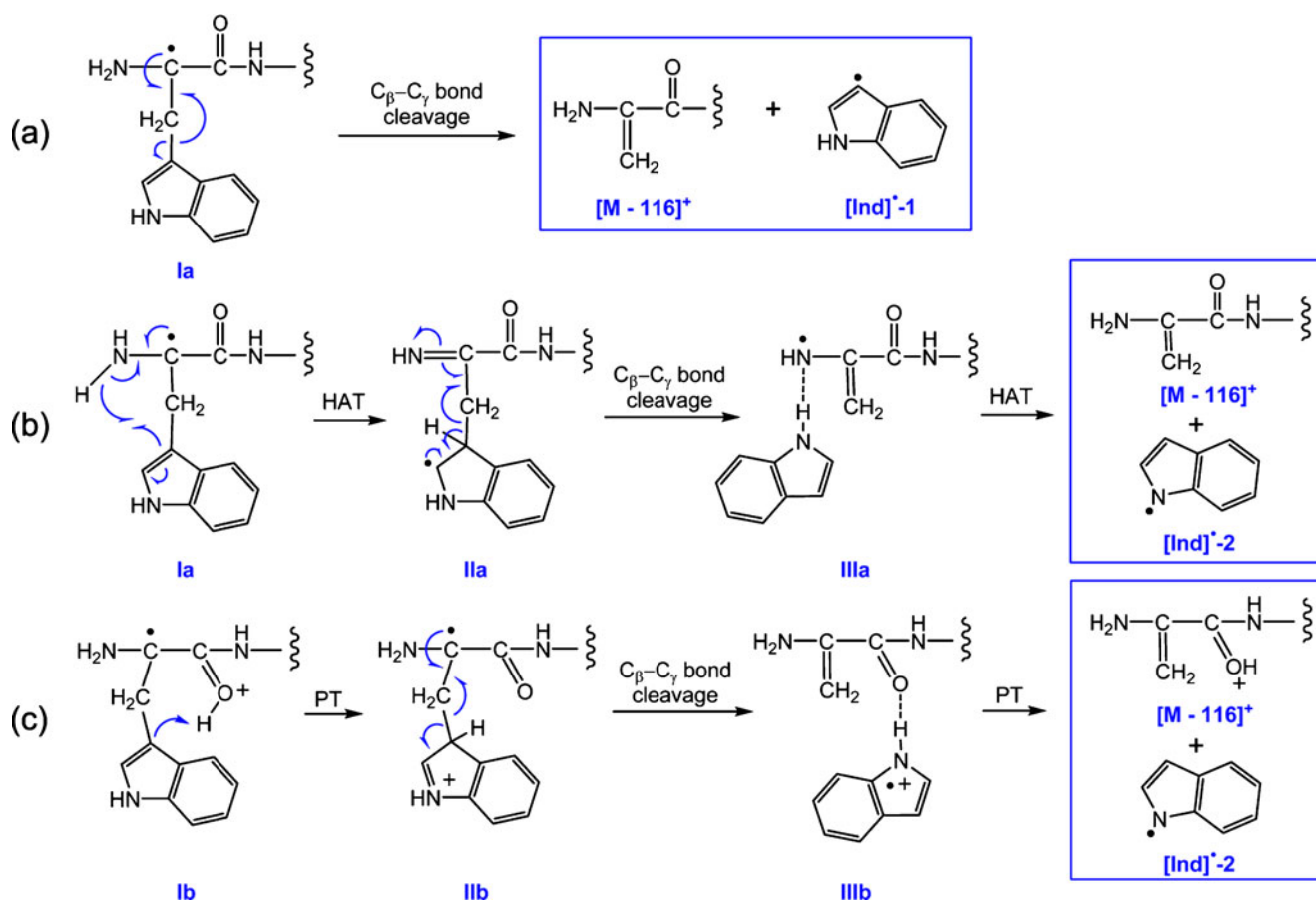
(43 Da) from leucine through C<sub>β</sub>–C<sub>γ</sub> side chain cleavages of the (iso)leucine residues, allowing the two peptides to be distinguished. We speculate that the C<sub>β</sub>–C<sub>γ</sub> bond cleavages of tryptophan residues described herein follow a similar mechanism as that for leucine residues, generating product ions with the same structure: that is, [M – 116]<sup>+</sup> and [M – 43]<sup>+</sup> ions from C<sub>β</sub>–C<sub>γ</sub> bond cleavages of the tryptophan and leucine residues, respectively, as displayed in Scheme 1a and Scheme S1a. This hypothesis is supported by the identical CID spectra of [WGGGR – 116]<sup>+</sup> and [LGGR – 43]<sup>+</sup> (Figure 4), originally derived from [WGGGR]<sup>•+</sup> and [LGGR]<sup>•+</sup>, respectively.

### Identity of the 116-Da Neutral Species

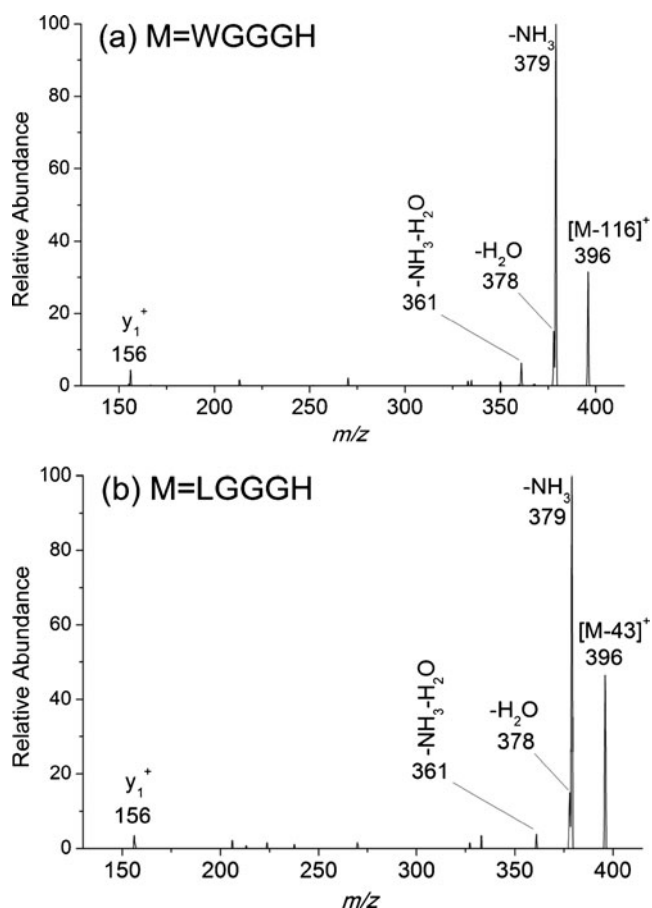
The neutral molecules that are lost from the homolytic C<sub>β</sub>–C<sub>γ</sub> bond cleavages of the α-carbon-centered radicals of leucine and isoleucine are the 2-propyl radical ([2-prop]<sup>•</sup>) and the ethyl radical ([eth]<sup>•</sup>), respectively (Scheme S1). A similar pathway (Scheme 1a) for 3-indolyl radical ([Ind]<sup>•</sup>-1) formation from tryptophan residue is not favorable, judging from its radical stabilization energy (RSE), which we evaluated employing a widely used method at the ROMP2/6-311+G(3df,2p)//UB3LYP/6-31G(d) level [58] (details relating to the calculation for each radical fragment are available in Table S1 of the supplementary material); the RSE of [Ind]<sup>•</sup>-1 of –16.3 kcal/mol

is significantly lower than those of [eth]<sup>•</sup> and [2-prop]<sup>•</sup> (3.3 and 5.6 kcal/mol, respectively; Table 2). The energy barrier against the C<sub>β</sub>–C<sub>γ</sub> bond cleavage of tryptophan following the mechanism presented in Scheme 1a, evaluated at the UB3LYP/6-31++G(d,p) level of theory for the α-tryptophyl-methylamino radical as a model system (Figure S3b), is 50.8 kcal/mol, substantially higher than those against the C<sub>β</sub>–C<sub>γ</sub> bond cleavage of the α-leucyl analogue (31.9 kcal/mol, Figure S3a) and the backbone cleavages for peptide radical cations (<40.0 kcal/mol) [42, 43]. Taken together, although the product ions are the same, our theoretical calculations suggest that the C<sub>β</sub>–C<sub>γ</sub> bond cleavages of tryptophan residues are not as simple as the direct bond cleavages of leucine or isoleucine residues; as a result, [Ind]<sup>•</sup>-1 might not be the structure of the lost 116-Da neutral species.

One notable difference between leucine and tryptophan is that the former has an aliphatic side chain, while the latter has an aromatic indole ring with a labile hydrogen atom on the nitrogen atom at position 1 of the indole ring (<sup>1</sup>N). This labile hydrogen atom might be involved in the side chain loss, resulting in a much more stable 1-indolyl radical ([Ind]<sup>•</sup>-2) with an RSE of 10.1 kcal/mol, comparable with those of [2-prop]<sup>•</sup> (5.6 kcal/mol) and [eth]<sup>•</sup> (3.3 kcal/mol) (Table 2). We used the model [W<sub>1-CH<sub>3</sub></sub>GGGH]<sup>•+</sup>, with the hydrogen atom on <sup>1</sup>N replaced by a methyl group, to examine the role played by this



Scheme 1. Possible mechanisms for the C<sub>β</sub>–C<sub>γ</sub> cleavage of tryptophan residue examined in this study



**Figure 4.** CID spectra of (a)  $[M - 116]^+$  from  $[WGGGH]^{\bullet+}$ ; (b)  $[M - 43]^+$  from  $[LGGGH]^{\bullet+}$

hydrogen atom. As revealed in Figure 5, CID of  $[W_{1-CH_3}GGGH]^{\bullet+}$  did not produce the  $[M - 130]^+$  ion, which would be the analogue of  $[M - 116]^+$  resulting from the C<sub>β</sub>–C<sub>γ</sub> bond cleavage of the tryptophan residue. This experiment demonstrates that dehydrogenation of the indole nitrogen atom is an inevitable step in the loss of the 116-Da neutral species.

Interestingly, the most abundant ion from  $[W_{1-CH_3}GGGH]^{\bullet+}$  was the  $[M - 131]^+$  species, which also originated from the

C<sub>β</sub>–C<sub>γ</sub> bond cleavage of  $W_{1-CH_3}$  with the loss of 131 Da corresponding to neutral 1-methyl-1*H*-indole. This finding suggests that the hydrogen atom on the indole nitrogen atom, although it could change the form of the final product, was not an indispensable factor for the C<sub>β</sub>–C<sub>γ</sub> bond cleavage. The counterpart of the  $[M - 131]^{\bullet+}$  ion in the CID of  $[WGGGH]^{\bullet+}$  is the  $[M - 117]^{\bullet+}$  species, which is not observable in Figure 2e, indicating that the product of  $[M - 116]^+$  plus  $[Ind]^{\bullet-2}$  is more favorable than that of  $[M - 117]^{\bullet+}$  plus 1-methyl-1*H*-indole. The formation of  $[Ind]^{\bullet-2}$  requires the transfer of one hydrogen atom to the γ-carbon atom of the tryptophan residue (or position 3 of the indole ring). This process could occur either before or after C<sub>β</sub>–C<sub>γ</sub> bond cleavage. The aforementioned calculations revealed that the direct C<sub>β</sub>–C<sub>γ</sub> bond cleavage of a tryptophan residue is associated with a very high energy barrier relative to that of a leucine residue. In addition to the stabilities of the products, this high energy barrier might also result from the stronger sp<sup>3</sup>–sp<sup>2</sup> type of C<sub>β</sub>–C<sub>γ</sub> bond of tryptophan (greater s character) than the sp<sup>3</sup>–sp<sup>3</sup>-type bond of leucine (less s character), consistent with the shorter C<sub>β</sub>–C<sub>γ</sub> bond length of tryptophan (1.514 Å) than that of leucine (1.555 Å), as indicated in Figure S3. Therefore, it is highly possible that one hydrogen atom transfers to the γ-carbon atom of the tryptophan side chain prior to cleavage of the C<sub>β</sub>–C<sub>γ</sub> bond, which transforms the γ-carbon atom from sp<sup>2</sup> to sp<sup>3</sup> hybridization, thereby weakening the C<sub>β</sub>–C<sub>γ</sub> bond. The C<sub>β</sub>–C<sub>γ</sub> bond cleavage followed by HAT from the indole nitrogen atom to the peptide backbone in the ion–molecule complex results in the  $[M - 116]^+$  ion and  $[Ind]^{\bullet-2}$ . Because there is no labile hydrogen atom on the indole ring of  $[W_{1-CH_3}GGGH]^{\bullet+}$ , during CID, the 1-methyl-1*H*-indole produced in the course of the C<sub>β</sub>–C<sub>γ</sub> bond cleavage cannot undergo HAT back to the peptide backbone; as a result, the  $[M - 131]^{\bullet+}$  ion is formed instead of the HAT product  $[M - 130]^{\bullet+}$ .

### Alternative Mechanisms for the C<sub>β</sub>–C<sub>γ</sub> Bond Cleavage

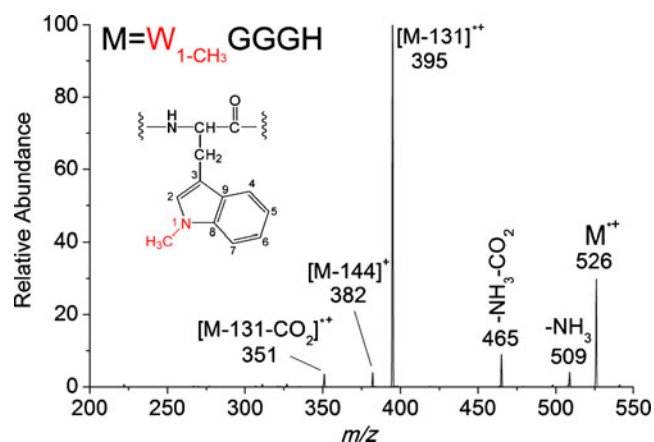
Our present experimental observations for the facile C<sub>β</sub>–C<sub>γ</sub> bond cleavages of N-terminal tryptophan residues can be

**Table 2.** Relative Energies and Radical Stabilization Energies (RSE)<sup>a</sup> in kcal/mol for Possible 116 Da Neutral Losses, Produced by C<sub>β</sub>–C<sub>γ</sub> Bond Cleavage of Tryptophan. The RSE Values for 29 and 43 Da Neutral Losses from Isoleucine and Leucine, Respectively, are also Shown

|                 | Tryptophan           |                      | Isoleucine         | Leucine               |
|-----------------|----------------------|----------------------|--------------------|-----------------------|
|                 |                      |                      |                    |                       |
|                 | [Ind] <sup>•-1</sup> | [Ind] <sup>•-2</sup> | [eth] <sup>•</sup> | [2-prop] <sup>•</sup> |
| Relative Energy | 0.0                  | -29.0                | -                  | -                     |
| RSE             | -16.3                | 10.1                 | 3.3                | 5.6                   |

<sup>a</sup>Please refer to the Table S1 of the supplementary material for the details regarding the calculation of RSE.





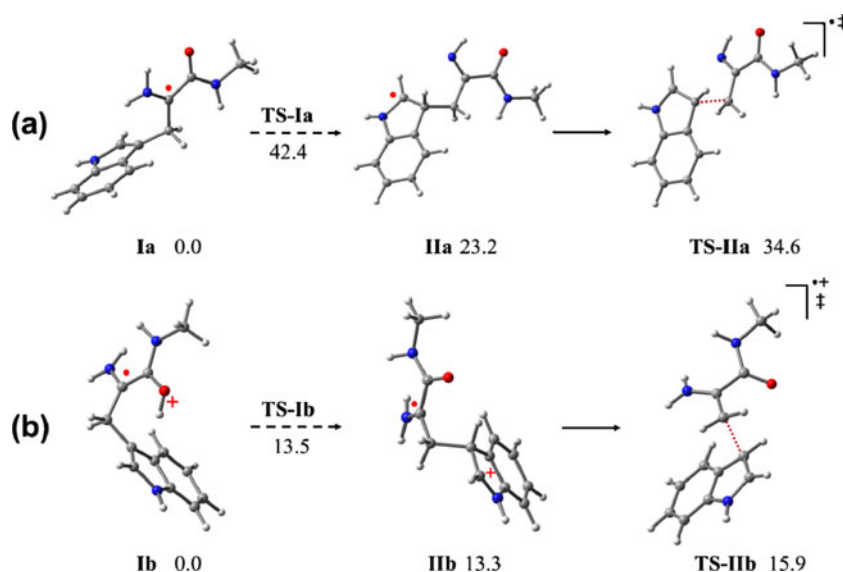
**Figure 5.** CID spectrum of  $[W_{1-CH_3}GGGH]^{*+}$ . “ $W_{1-CH_3}$ ” represents a modified tryptophan residue with the 1-hydrogen of indole ring substituted by a methyl group, which prevents the dehydrogenation of the indole nitrogen of tryptophan

rationalized in terms of two proposed mechanisms (Scheme 1b and c). Scheme 1b depicts a charge-remote radical-driven cleavage, in which HAT from the amino group of the tryptophan residue to the  $\gamma$ -carbon atom of its side chain, followed by homolytic cleavage of the C $_{\beta}$ –C $_{\gamma}$  bond, gives a molecule–ion complex (IIIa) formed from the radical peptide backbone and the 1H-indole; subsequent HAT from the indole nitrogen atom to the amino nitrogen atom generates the neutral 116-Da molecule. For an M $^{*+}$  species containing a 1-methyl-tryptophan residue, the dissociation of IIa will give  $[M - 131]^{*+}$  directly, instead of the ion molecule complex (IIIa). Scheme 1c depicts a charge-assisted radical-driven cleavage, which involves proton transfer (PT) from the peptide backbone to the  $\gamma$ -carbon atom of the tryptophan residue, thereby weakening the C $_{\beta}$ –C $_{\gamma}$  bond; subsequent heterolytic cleavage of this bond,

followed by PT from the indole nitrogen atom to the backbone, could also result in the loss of the neutral species having a mass of 116 Da.

To examine these two mechanisms, we performed DFT calculations using the  $\alpha$ -tryptophylmethylamino radical and radical cation, respectively, as model systems. Figure 6 presents the key structures for the considered mechanisms; all related structures are provided in Figure S4. For the mechanism depicted in Scheme 1b, the isomer IIa, resulting from HAT from the amino group to the  $\gamma$ -carbon atom of the tryptophan residue through TS-Ia, is 23.2 kcal/mol higher in enthalpy higher than the low-lying conformer Ia. The barrier against the homolytic cleavage of the C $_{\beta}$ –C $_{\gamma}$  bond of IIa is 11.4 kcal/mol (34.6 kcal/mol relative to the lowest-energy isomer Ia), substantially lower than that for direct C $_{\beta}$ –C $_{\gamma}$  bond cleavage of tryptophan (50.8 kcal/mol). A similar mechanism involving a HAT from the amide group has also examined; its barrier is higher (by 4.5 kcal/mol) than that against the mechanism depicted in Scheme 1b (Figure S5). The reaction is even more favorable through Scheme 1c; PT from the amide oxygen atom gives the isomer IIb, which is only 13.3 kcal/mol higher in enthalpy than that of the low-lying conformer Ib. The heterolytic cleavage of the C $_{\beta}$ –C $_{\gamma}$  bond of IIb has a barrier of only 2.6 kcal/mol (15.9 kcal/mol relative to Ib). The calculations for these model systems suggest that the mechanism depicted in Scheme 1c is favored as long as there is a free proton available in the system. When the peptide radical cations contain highly basic residues (e.g., arginine), the charge-remote process—the mechanism depicted in Scheme 1b—would become possible for the C $_{\beta}$ –C $_{\gamma}$  bond cleavages of tryptophan residues.

Parenthetically, the CID spectrum of  $[W_{1-CH_3}GGGH]^{*+}$  provides mechanistic insight regarding the C $_{\alpha}$ –C $_{\beta}$  bond cleavage of tryptophan. The  $[M - 143]^{*+}$  ion, the counterpart of  $[M - 129]^{*+}$  in the CID of  $[WGGGH]^{*+}$ , was not observed in



**Figure 6.** Some critical conformers and transition structures of  $\alpha$ -tryptophylmethylamino (a) radical and (b) radical cation involved in the elimination of neutral 116 Da radical via mechanisms shown in Scheme 1b and c, respectively. Relative enthalpies at 0 K are in kcal/mol. All related structures are shown in Figure S4

the CID spectrum of [W<sub>1-CH<sub>3</sub></sub>GGGH]<sup>•+</sup>, indicating that removal of the hydrogen atom from the indole nitrogen atom is an essential step for the loss of the neutral species having a mass of 129 Da. This result is consistent with the experimental findings for tryptophan-containing peptide radical cations with the initial radical site located on the indole nitrogen atom [30]. Similar to the C<sub>β</sub>–C<sub>γ</sub> bond cleavage, the C<sub>α</sub>–C<sub>β</sub> bond cleavage can occur without the participation of the indole nitrogen atom, instead forming the product ion [M – 144]<sup>+</sup> during the CID of [W<sub>1-CH<sub>3</sub></sub>GGGH]<sup>•+</sup>.

## Conclusion

Loss of a neutral species having a mass of 116 Da, generated from the C<sub>β</sub>–C<sub>γ</sub> bond cleavage of tryptophan, has been observed in the dissociations of a series of tryptophan-containing peptide radical cations; this feature could be a diagnostic fragmentation revealing the presence of tryptophan residues in peptides. To the best of our knowledge, this fragmentation has not been observed previously in the CID spectra of peptide radical cations generated from the dissociative oxidation of metal complexes. Both experimental and theoretical analyses revealed that the α-carbon-centered radical of the tryptophan residue is a crucial intermediate. Furthermore, dehydrogenation of the indole nitrogen atom is a prerequisite for the loss of the 116-Da neutral species, but it is not an inevitable step for the C<sub>β</sub>–C<sub>γ</sub> bond cleavage. From RSE calculations and comparisons of the CID spectra of [WGGGH]<sup>•+</sup> and [W<sub>1-CH<sub>3</sub></sub>GGGH]<sup>•+</sup>, we confirmed the identity of the neutral 116-Da species to be [Ind]<sup>•</sup>-2, with the radical positioned on the indole nitrogen atom. DFT calculations of the proposed mechanisms revealed that HAT or PT to the γ-carbon atom of the tryptophan residue weakened the C<sub>β</sub>–C<sub>γ</sub> bond, thereby decreasing the dissociation energy barriers dramatically.

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