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Radical-Induced, Proton-Transfer-Driven Fragmentations in $[b_5 - H]^{\bullet+}$ Ions Derived from Pentaalanyl Tryptophan

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Abstract

The collision-induced dissociation (CID) of $[b_5 - H]^{\bullet+}$ ions containing four alanine residues and one tryptophan give identical spectra regardless of the initial location of the tryptophan indicating that, as proposed for b_5^+ ions, sequence scrambling occurs prior to dissociation. Cleavage occurs predominantly at the peptide bonds and at the N−C_α bond of the alanine residue that is attached to the N-terminus of the tryptophan residue. The product of the latter pathway, an ion at *m/z* 240, is the base peak in all the mass spectra. With the exception of one minor channel giving a b_3^+ ion, the product ions retain both the tryptophan residue and the radical. Experiments with one trideuterated alanine established the sequences of loss of alanine residues.

Formation of identical products implies a common intermediate, a $[b_5 - H]^{\bullet+}$ ion that has a `linear` structure in which the tryptophan residue is present as an α-radical located in the oxazolone ring, structure **Ie**. Density Functional Theory calculations show this structure to be at the global minimum, 14.6 kcal mol^{-1} below the macrocyclic structure, ion **II**. Loss of CO from the $[b_5 - H]^{\bullet+}$ ions is inhibited by the presence of the radical centre in the oxazolone ring and migration of the proton from the oxazolone ring onto the peptide backbone induces cleavage of an N−C_α or peptide bond.

Three calculated structures for the ion at *m/z* 240 all have an oxazolone ring. Two of these structures may be formed from **Ie** , depending upon which proton migrates onto the peptide chain prior to the dissociation. The barrier to interconversion between these two structures requires a 1,3-hydrogen atom shift and is high (51.0 kcal mol^{-1}), but both can convert into a third isomer that readily loses CO_2 (barrier 38.7 kcal mol^{-1}). The lowest barrier to the loss of CO, the usual fragmentation path observed for protonated oxazolones, is 47.0 kcal mol^{-1}.

Keywords

Collision-induced dissociation; $[b_5 - H]^{\bullet+}$ ions; fragmentation mechanisms; Density Functional Theory; loss of CO_2 from an oxazolone; macrocyclization; isotopically-labeled alanine.

Introduction

In the gas phase, protonated peptides fragment by cleavage of an amide bond forming an oxazolone as the N-terminal fragment and a truncated peptide as the C-terminal product.[1-8] In the case of a monoprotonated peptide the relative proton affinities of the two fragments determines which fragment is observed as an ion, a b_n^+ ion if the charge is on the N-terminal fragment and a y_m^+ ion if it is on the C-terminal fragment.[7,8] b_n^+ ions undergo subsequent systematic losses of CO and an imine from the oxazolone ring, thereby creating first a_n^+ and then $b_{(n-1)}^+$ ions, and effectively losing the amino acid residue which was at the C-terminus of the b_n^+ residue. This dissociation from the C-terminus of the b_n^+ ion provides an important framework for protein sequencing in proteomics. However, recent studies have shown that the b_n^+ ions ($n \geq 5$) may also undergo a rearrangement[8-10] in which the terminal amino group attacks the carbonyl carbon of the oxazolone ring and forms a macrocyclic peptide which may subsequently ring open at a different amide bond and give a *different* oxazolone.[8-16] Dissociation of this second oxazolone again results in the loss of the residue at the C-terminus and leads to incorrect identification of the peptide sequence. Numerous examples of sequence scrambling in b_n^+ ions have been observed since the initial observation in 2003.[17]

A wider variety of fragmentation pathways is observed for peptide radical cations than for the analogous protonated peptides because dissociation reactions can be driven either by the charge or by the radical.[18-32] Charge-proximal fragmentation of peptide radical cations gives predominantly $[b_n - H]^{\bullet +}$ ions, a pathway that is analogous to the formation of b_n^+ ions from protonated peptides. In peptide radical cations containing a tyrosine or tryptophan residue, proton transfer from the β-carbon of the aromatic side chain to the backbone can induce N–C_α bond cleavage, resulting in the formation of $[z_n - H]^{\bullet +}$ and/or $[c_m + 2H]^+$ ions.[25,29] By contrast, radical-driven reactions lead to cleavage of the C_α–C bond of the backbone and of the C_α–C_β or C_β–C_γ bonds of the side chain.[22,26,27,33,34] Charge-driven fragmentation at the backbone of a peptide radical cation can be suppressed by sequestering the charge onto the side chain of an arginine residue, thereby permitting study of radical-driven cleavage of the backbone.[21,32] In general, product ion mass spectra of peptide radical cations are richer than those of protonated peptides, and this has provided additional information such as enabling differentiation of the isobaric amino acids leucine and isoleucine.[20]

In peptide radical cations, interconversions between isomers can occur both by intramolecular proton transfer and by hydrogen atom transfer (HAT). Consequently, for many peptide radical cations there are several low-energy structures. The captodative structure, where the radical is located at the α-carbon of the N-terminal residue and the proton is on the adjacent amide oxygen, is frequently at the global minimum on the potential energy surface. The stability of the captodative structure is attributed to the radical at the α-carbon of the N-terminus being sandwiched between a powerful electron-donating group (NH_2–) and a strongly electron-withdrawing group (–COH^+).[18]

Our interest in this study was to establish whether the fragmentations of $[b_5 - H]^{\bullet+}$ ions that formally have both the charge and radical located on the backbone follow similar pathways to those observed for b_5^+ ions. In particular, we were interested in whether sequence scrambling occurs, or does the radical play a role that results in other types of dissociation. If the $[b_5 - H]^{\bullet+}$ ions have the captodative structure, then the lone pair electrons on the N-terminal amine are delocalized and are less available for nucleophilic attack; similarly, if the oxazolone ring does not carry a charge, it is less susceptible to nucleophilic attack. Consequently, head-to-tail macrocyclization is less likely to occur and sequence scrambling probably will not be observed.

Herein we compare the fragmentations of the closed-shell b_5^+ and the open-shell $[b_5 - H]^{\bullet+}$ ions that are the primary dissociations products of the tryptophan-containing hexapeptides $A_5W^{\bullet+}$, where W is located at all possible positions of the sequence.[29] By systematically replacing the alanine residues by a trideuterated alanine we are able to determine the order in which the residues are lost.

Experimental

Chemicals

The hexapeptides were synthesized using solid-phase chemistry.[35] Fmoc-protected amino acids and Wang resin were available from Advanced ChemTech (Louisville, KY). Cu(II)-peptide ternary complexes were prepared *in situ* by electrospraying Cu(II) perchlorate hexahydrate (Sigma-Aldrich) with the appropriate peptide and 4′-chloro-2,2′:6′,2″-terpyridine (Cl-tpy) or tpy in a 50/50 water/methanol solution.

Mass Spectrometry

All experiments were conducted on a hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap Elite, Thermo Scientific). Samples were typically 5 μM Cu(II)-tpy or -Cl-tpy and 5-10 μM peptide, and were continuously infused at a rate of 3 μL/min via a HESI-II for electrospray ionization in an Ion Max source. Standard conditions include spray voltage, 3.2-3.5 kV; sheath gas, ≤ 5 units; auxiliary gas, 0; heated capillary, 250-275 ºC; and S-lens RF, 35-68%. For MS^n, scans were accumulated 1-5 min; the dissociation of $[b_5 - H]^{\bullet+}$ required an MS^4 experiment, involving sequentially the dissociations of $[Cu^{II}(tpy)M]^{\bullet+}$, $M^{\bullet+}$ and $[b_5 - H]^{\bullet+}$. Data acquisition was made under LTQ Tune and analyzed using Xcalibur (Thermo Scientific).

Method of calculations

All calculations were performed on the Gaussian09 package of programs. [36] Geometries were optimized at the B3LYP/6-31++G(d,p) level of theory[37-43] for closed-shell systems, while the UB3LYP method was employed for open-shell systems. All optimized structures were characterized by vibrational frequency analyses as minima (no imaginary frequency) or transition states (one imaginary frequency). Intrinsic reaction coordinate (IRC) calculations[44] were performed on each transition state to determine the associated reactants and products.

Results and Discussion

Dissociation of the closed-shell b_5^+ ion.

The b_5^+ ions containing one tryptophan and four alanine residues produce identical CID spectra (Figure 1) regardless of the initial location of the tryptophan residue. This implies that the b_5^+ ions form a common intermediate, believed to be a macrocyclic peptide, prior to dissociation, as has been reported in the CID of b_5^+ ions containing four alanine and one tyrosine residue.[8-10]

The base peak in the spectra in Figure 1 is an ion at *m/z* 443 corresponding to the a_5^+ ion, formed via the loss of CO from the precursor ion. Further loss of NH_3 from the a_5^+ ion produces the $a_5{}^{*+}$ ion at *m/z* 426. Product ions at *m/z* 285 and *m/z* 400 correspond to losses of tryptophan and alanine, respectively, and the relatively high abundance of the *m/z* 285 ion indicates that the tryptophan residue is the most easily lost (recalling the ratio of 1:4 of tryptophan to alanine). This implies that when the macrocyclic ion ring opens, it preferentially forms an oxazolone with

the tryptophan residue at the C-terminus, $[AAAAW_{oxa} + H]^+$. Here the subscript "oxa" notation indicates which amino acid residue is at the C-terminus in the oxazolone ring. Experiments with peptides in which the side chain of one of the alanines was trideuterated (denoted A*) established that any alanine residue may be lost, but that located second from the C-terminal side of tryptophan in the protonated cyclic pentapeptide has the highest probability of being lost (Figure 2). Why the loss of tryptophan is the preferred route and why the loss of one particular alanine is preferred over the others will not be further explored here. The fact that the loss of *each* residue is observed indicates that the barriers to these fragmentation pathways are all very close in energy.

Dissociation of the open-shell $[b_5 - H]^{\bullet+}$ ions.

The CID spectra of the $[b_5 - H]^{\bullet+}$ ions derived from tryptophan-containing hexapeptides radical cations are also virtually identical (Figure 3); however, the dissociation patterns are strikingly different from those of the closed-shell b_5^+ ions. Notable differences are:

(i) in the spectra of the $[b_5 - H]^{\bullet+}$ ions there are almost negligible amounts of the product ions $[a_5 - H]^{\bullet+}$ at *m/z* 442, corresponding to loss of CO. By contrast, the a_5^+ ion is the base peak in the mass spectrum of the b_5^+ ion.

(ii) in the mass spectrum of the $[b_5 - H]^{\bullet+}$ ion there is *no* loss of the tryptophan residue. By contrast, loss of the tryptophan residue is dominant in the CID of the b_5^+ ions.

(iii) the second most abundant ion in the spectrum of the $[b_5 - H]^{\bullet+}$ ion is at *m/z* 328, due to the loss of two alanine residues. At all collision energies this ion is more abundant than the ion at *m/z* 399 (corresponding to loss of one alanine residue, Figure S1), and this suggests that the two alanine residues are lost *concurrently*, probably as the b_2 oxazolone structure (*vide infra*).

(iv) the most abundant fragment ion in the spectrum of the $[b_5 - H]^{\bullet+}$ ion is at *m/z* 240, due to the loss of trialaninamide. The mass spectra of the labeled peptides established that the alanine residue immediately N-terminal to the tryptophan residue is retained in the *m/z* 240 ion (*vide infra*).

Another unusual feature of the spectra of the $[b_5 - H]^{\bullet+}$ ions is that most product ions retain *both* the charge and the radical. Only the product ion at *m/z* 214, a b_3^+ ion $[AAA_{oxa} + H]^+$,

has lost the radical. Also this is the only product ion that does not contain the tryptophan residue. The spectra from the deuterium labeling experiments (Figure 4) show that the alanine residue lost in the neutral fragment when forming the $[AAA_{oxa} + H]^+$ ion is that attached to the N-terminus of the tryptophan. All this evidence strongly indicates that the radical is always retained on the tryptophan residue and the amide bond at the N-terminus of the tryptophan residue is never broken.

The identical CID spectra indicate that the $[b_5 - H]^{\bullet+}$ ions also undergo macrocyclization and ring opening to form a common intermediate *prior to* dissociation occurs as observed in the closed-shell b_5^+ systems. The loss of two alanines from the $[b_5 - H]^{\bullet+}$ ions could be explained by sequential eliminations of alanine residues from the C-terminus of an oxazolone, as found in the fragmentation of b_5^+ ions; if this were the situation, then it is puzzling as to why the loss of two alanine residues occurs more readily than the loss of just one residue. As the tryptophan residue and part of the alanine residue that is attached to its N-terminus are retained in most of the product ions, the structure $AWAAA_{oxa}^{\bullet+}$ would appear to be a good candidate for the common intermediate that dissociates. However, systematically substituting an alanine residue by a trideuterated-alanine (Figure 4) showed that the first alanine to be lost is that immediate attached to the C-terminus of the tryptophan, and that the two residues lost together are those attached to the same C-terminus of the tryptophan (in the cyclic peptide). Clearly, this is incompatible with the $AWAAA_{oxa}^{\bullet+}$ hypothesis.

An alternative explanation is that macrocyclization leads to $AAAAW_{oxa}^{\bullet+}$ (analogous to the dominant pathway for b_5^+ ions), the radical remains on the tryptophan residue in the oxazolone ring and the proton migrates from the oxazolone ring to the peptide bonds on the backbone where it initiates cleavage of amide bonds. In this mechanism, unlike in the fragmentation of the closed shell analogs, the oxazolone ring remains intact. The labeling experiments show definitively that the A* ($NH{=}CH(CD_3) + CO$) and AA_{oxa}* neutrals lost are the residues located at the N-terminus of an $AAAAW_{oxa}^{\bullet+}$ ion. Similarly, the b_3^+ ion $[AAA_{oxa} + H]^+$ at *m/z* 214 contains the three alanine residues located at the N-terminus of the $AAAAW_{oxa}^{\bullet+}$. Finally, there is formation of the base peak ion at *m/z* 240 that labeling shows to contain part of the alanine residue immediately N-terminal to the tryptophan residue (Figure 4).

On the basis of these experimental findings, we postulate that macrocyclization of the $[b_5 - H]^{\bullet+}$ ions occurs and, after reopening to an oxazolone, only one structure $AAAAW_{oxa}^{\bullet+}$ dissociates. The presence of the radical on the α-carbon of the tryptophan ring in the oxazolone induces the proton to migrate onto the peptide backbone and results in cleavage of an amide bond by the same mechanism as occurs in the fragmentation of protonated peptides. Cleavage of the second amide bond is preferred because nucleophilic attack by the carbonyl oxygen of the first amide group displaces the C-terminus as a neutral oxazolone composed of the three residues, thereby creating a b_2^+ ion from the N-terminus. Subsequent proton transfer creates the observed 'internal' $[b_3 - H]^{\bullet+}$ ion at *m/z* 328.

The product of cleavage of the third amide bond by the same mechanism results the formation of a b_3^+ ion at *m/z* 214, is also observed, but in lower abundance. This is possibly because protonation of the third amide nitrogen of $AAAAW_{oxa}^{\bullet+}$ preferentially undergoes N–C_α bond cleavage of the fourth residue, thereby creating the major product ion at *m/z* 240. The product of this cleavage has the charge and radical both located on the oxazolone ring and the $CH(CH_3)$ at the N-terminus. An alternative structure for the ion at *m/z* 240, in which the charge and radical are delocalized over the whole structure, can be created if the proton that initiates the cleavage is from the β-carbon of the tryptophan residue of $AAAAW_{oxa}^{\bullet+}$ rather than from the nitrogen of the oxazolone ring. The 1,6-proton transfer from the β-CH_2 group of the side chain of the tryptophan residue to the N of the fourth residue appears to be sterically difficult due to the rigidity imposed by the oxazolone ring, but possibly can be achieved via proton transfer to the terminal amino group or an amide oxygen closer to the N-terminus as an intermediate step. Protonation on the nitrogen of the third peptide bond then should result in facile cleavage of the N–C_α bond producing a structure in which both the charge and unpaired electron are fully delocalized over the entire C-terminal fragment.

Theoretical Investigations.

(a) Fragmentation mechanisms of the $[b_5 - H]^{\bullet+}$ ions

There are several isomers of the $[b_5 - H]^{\bullet+}$ ions and each one has many conformers. In order to make the system slightly more amenable to DFT calculations, we have examined isomers of $G_4W_{oxa}^{\bullet+}$ rather than $A_4W_{oxa}^{\bullet+}$. There are numerous potential sites in the $[b_5 - H]^{\bullet+}$ ions for

location of the radical and charge but a preliminary DFT study established that three classes of structures have the lowest energies. The favored sites for location of the radical are (a) at the α-carbon of the tryptophan residue, structure **1**; (b) at the β-carbon of the tryptophan, structure **2**; and (c) at the α-carbon of the N-terminal residue (a captodative structure), structure **3** (Figure 5). There are so many possible structures when considering isomers of these $[b_5 - H]^{\bullet+}$ ions that we opted to simplify the problem by replacing the alanine residues by glycines. Table 1 summarizes the relative energies of the $[b_5 - H]^{\bullet+}$ ions with different possible structures on the potential energy surface (PES). The structure of $GGGGW_{oxa}^{\bullet+}$ with the tryptophan residue at the C-terminus and the radical at the α-carbon of the tryptophan is at the global minimum on the PES (ion **Ie** in Scheme 1).

Starting with structures containing a tryptophan α-radical for each isomer, the barriers to the loss of CO to form $[a_5 - H]^{\bullet+}$ ions (observed, but in very low abundances) are calculated to be in the range 40-53 kcal mol^{-1}; these are higher than the barriers to forming the cyclic pentapeptide by head-to-tail macrocyclization (Scheme 1). Furthermore, ring-opening of the macrocyclic structure to form $GGGGW_{oxa}^{\bullet+}$, ion **Ie**, has a low barrier (only 9.3 kcal mol^{-1} above the macrocyclic structure and 23.9 kcal mol^{-1} above **Ie**); consequently ions **Ia-Id** convert into **Ie** more readily than dissociate by loss of CO. This is consistent with the experimental observation that all the ions have the same dissociation products.

After ring opening of the cyclic pentapeptide, the proton is initially located on the nitrogen atom in the oxazolone ring of $GGGGW_{oxa}^{\bullet+}$. The radical centre in the oxazolone ring is adjacent to a carbonyl group and a protonated imine, both strong electron-withdrawing groups. A more stable structure in which the imine can function as a π-donor is achieved when the proton migrates along the peptide chain. Hence, proton migration onto the backbone is facile and promotes cleavage of the peptide bonds; the barriers to losses of one and two glycine residues and glycyltryptophan radical giving products **III**, **IV** and **V** are calculated to be 48.0, 37.1 and 41.8 kcal mol^{-1} respectively (Scheme 1, see Figure S2A for details). These dissociation reactions are charge-driven but are initiated by the radical in the oxazolone ring inducing the proton to migrate along the backbone, and are best described as **radical-induced, proton-transfer-driven fragmentation**s. The lowest barrier is for the loss of glycylglycylglycinamide (35.8 kcal mol^{-1}) creating ion $\mathbf{VI_a}$, the glycyl analogue of the ion at *m/z* 240, the ion that was found to be the base

peak. The loss of CO from **Ie** is charge-induced and requires protonation of the oxazolone nitrogen.[6-8] In $GGGGW_{oxa}^{\bullet+}$, the presence of the radical at the α-carbon of the oxazolone ring inhibits the loss of CO and the barrier to this dissociation pathway is much higher (52.8 kcal mol^{-1}); this is probably why it is observed in very low abundance.

Charge-driven cleavage of the peptide bonds as described above used the proton from the oxazolone nitrogen, as shown in structure **Ie** (Scheme 1). Another possibility is that a proton from the β-CH_2 of the tryptophan side chain of **Ie** migrates onto the backbone. The lowest barrier for transferring a proton from the β-CH_2 to a heteroatom on the backbone (34.4 kcal mol^{-1}) involved the oxygen of the second peptide bond. A subsequent 1,8-proton shift to the nitrogen of the fourth peptide bond was accompanied by cleavage of this bond and the barrier to this different tautomer of **VI**, labeled $\mathbf{VI_b}$ (Scheme 2), was calculated to be 36.3 kcal mol^{-1} (Figure S2B), essentially the same as that calculated for the barrier when the proton from the oxazolone nitrogen was used (pathway D in Scheme 1). The overall endothermicity for formation of $\mathbf{VI_b}$ was calculated to be 19.0 kcal mol^{-1}, considerably lower than that for formation of $\mathbf{VI_a}$ and that is attributed to the extensive delocalization of both the charge and radical over the π-systems of both the oxazolone and indole ring systems in $\mathbf{VI_b}$. Experimentally, the *m/z* 240 ion was found to be very stable and the ion isolated in the mass spectrometer probably has structure $\mathbf{VI_b}$.

In Figure 3, there is an additional minor product in the dissociation of $AAWAA_{oxa}^{\bullet+}$, an ion at *m/z* 329, the result of loss of $A^{\bullet}A_{oxa}$. This loss is due to a second pathway becoming competitive with the head-to-tail cyclization reaction.[15] Calculations on $GGWGG_{oxa}^{\bullet+}$ show that the barrier against 1,4-HAT from the α-carbon of the 4th residue of the initially formed $[b_5 - H]^{\bullet+}$ ion to the α-carbon of the tryptophan residue is only 40.2 $kcalmol^{-1}$ and is competitive with that for macrocyclization (37.3 kcal mol^{-1}). Further evidence for formation of $AAWA^{\bullet}A_{oxa}^{+}$ is provided by the high abundance of $[AAWAA_{oxa} - CO_2]^{\bullet+}$ relative to that observed from other $[b_5 - H]^{\bullet+}$ isomers. In the spectra of other $[b_5 - H]^{\bullet+}$ ions containing other residues we have noticed that loss of CO_2 occurs most easily from a structure with a radical adjacent to the oxazolone ring.[45] The 1,4-HAT gives $GGWG^{\bullet}G_{oxa}^{+}$ and a subsequent macrocyclization, proton transfer and ring opening gives $G^{\bullet}GGGW_{oxa}^{+}$ from which $G^{\bullet}G_{oxa}$, the glycyl analogue of $A^{\bullet}A_{oxa}$, can be eliminated.

(b) Structure and stability of the ion at *m/z* 240

The ion at *m/z* 240 was formed at low collision energies, both directly from $AAAAW_{oxa}^{\bullet+}$ and by MS^5 from the ion at *m/z* 328 (Figure 6(a)). CID of the *m/z* 240 ion gave two products, ions at *m/z* 196 (loss of CO_2) in high abundance and at *m/z* 212 (loss of CO) in very low abundance (Figure 6(b)). This is atypical behavior, as ions **VI$_a$** and **VI$_b$** both contain an oxazolone ring and dissociation of oxazolones invariably results in loss of CO and not CO_2.[3,4,6-8]

DFT calculations established that there are three relatively low-lying structures for the ion at *m/z* 240, ions **VI$_a$**, **VI$_b$** and **VI$_c$** (Scheme 2). Structure **VI$_b$** has both the charge and radical delocalized over the whole molecular framework and consequently has the lowest energy. In **VI$_a$** the β-CH_2 breaks the conjugation between the oxazolone and the indole and the charge and unpaired spin are again delocalized, but only over the indole ring. In the third structure, **VI$_c$**, the conjugation is broken by the hydrogen on the α-carbon, resulting in a distonic ion with the charge delocalized over the indole and the spin delocalized over the oxazolone and the exocyclic $(CH_3)CH$ moiety. The two mechanisms for formation of the *m/z* 240 ion, depending on which proton migrates onto the backbone, yield structures **VI$_a$** and **VI$_b$** and the barrier to interconversion between these structures by a 1,3-HAT (51.0 kcal mol^{-1} relative to **VI$_a$)** is slightly higher than those to dissociation. However, the barriers to conversion into the third isomer, **VI$_c$** (37.8 kcal mol^{-1} from **VI$_a$** and 41.7 kcal mol^{-1} from **VI$_b$**) are lower than those to dissociation. Furthermore, the barrier to loss of CO_2 from **VI$_c$** is only 38.7 kcal mol^{-1}, essentially the same as the barriers to its formation from both **VI$_a$** and **VI$_b$**; consequently, it appears that **VI$_c$** is the intermediate through which loss of CO_2 occurs from the *m/z* 240 ion. The barriers to loss of CO, the usual pathway by which oxazolones fragment, are higher ranging from 47.0 to 54.1 kcal mol^{-1}.

Conclusion

The collision-induced spectra of b_5^+ions comprised of one tryptophan and four alanine residues are all identical, regardless of the initial site of the tryptophan residue. The major fragmentation pathway is the loss of the tryptophan residue and this is interpreted in terms of

formation of a cyclic protonated pentapeptide that preferentially ring opens to form a `linear` ion in which the tryptophan residue is located in the oxazolone ring.

The $[b_5 - H]^{\bullet+}$ ions also gave identical CID spectra, but a major difference from those of the b_5^+ ions was that most product ions retain the tryptophan (and also the radical). The similarity in the spectra implies that the $[b_5 - H]^{\bullet+}$ ions also form a common intermediate prior to dissociation. DFT calculations showed that the cyclic pentapeptide radical cation, formed by the same type of `head-to-tail` cyclization as found for the closed-shell b_5^+ ions, preferentially ring opens to a `linear` ion with the tryptophan at the C-terminus. Dissociation of this `linear` ion follows a very different path from that of its b_5^+ analog. There is very little loss of CO to form a $[a_5 - H]^{\bullet+}$ ion. Instead, the presence of both the charge and the radical centre formally localized on adjacent atoms in the oxazolone ring induces a proton, either from the N in the oxazolone ring or from the β-CH_2 on the side chain of the tryptophan residue, to migrate onto the heteroatoms along the peptide backbone. The subsequent cleavage of a peptide bond or the N−C_α bond of the fourth residue is charge-driven, but is initiated by the interaction between the radical and the charge in the oxazolone ring. The resulting reactions are then best described as *radical-induced, proton-transfer-driven fragmentations*.

The base peak in the spectra for the $[b_5 - H]^{\bullet+}$ ions is an ion at *m/z* 240 that contains (tryptophan residue - H) along with remnants of the alanine residue located at the N-terminus of the tryptophan residue. DFT calculations provided three possible structures for this ion; all contain an oxazolone ring and differ only in the location of the two hydrogen atoms distributed over the N, the C_α and the C_β of the tryptophan residue. Two of these ions, $\mathbf{VI_a}$ and $\mathbf{VI_b}$, are the products of backbone cleavage and are stabilized by extensive delocalization. The third, $\mathbf{VI_c}$, is a distonic ion with the charge delocalized over the aromatic rings, but with the radical localized in the oxazolone ring; this third structure is the common intermediate through which dissociation by the loss of CO_2 occurs. The barriers to the loss of CO from these ions are all high, again showing the influence of the radical on the dissociation pathway.

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Table 1. Enthalpies (ΔH^o_0, kcal mol^{-1}) and free energies (ΔG^o_{298}, in parenthesis) of $[b_5 - H]^{\bullet+}$ ions calculated at the B3LYP/6-31++G(d,p) level. All energies are relative to structure **1** of $GGGGW_{oxa}^{\bullet+}$

structure	**1**	**2**	**3**
$WGGGG_{oxa}^{\bullet+}$	4.4 (4.1)	15.1 (14.0)	1.1 (-1.0)
$GWGGG_{oxa}^{\bullet+}$	12.5 (12.4)	18.8 (18.1)	4.4 (2.7)
$GGWGG_{oxa}^{\bullet+}$	13.0 (13.2)	11.7 (11.3)	7.3 (5.3)
$GGGWG_{oxa}^{\bullet+}$	4.4 (2.6)	9.1 (9.0)	8.1 (6.6)
$GGGGW_{oxa}^{\bullet+}$	0.0 (0.0)	8.8 (7.7)	7.8 (8.3)

1. α-radical at tryptophan.

2. β-radical at tryptophan.

3. captodative structure at N-terminus.

Captions for Schemes

Scheme 1. Conversion of different isomers of the $[b_5 - H]^{\bullet+}$ ions into structure **Ie** via the cyclic pentapeptide ion **II** and the pathways to subsequent dissociation. The enthalpies (ΔH^o_0) and free energies (ΔG^o_{298}, in parentheses), both in kcal mol^{-1}, are relative to $GGGGW_{oxa}{}^{\bullet+}$ and were calculated at the B3LYP/6-31++G(d,p) level.

Scheme 2. Interconversion between the three isomers of the *m/z* 240 ion, structures $\mathbf{VI_a}$, $\mathbf{VI_b}$ and $\mathbf{VI_c}$. The enthalpies (ΔH^o_0) and free energies (ΔG^o_{298}, in parentheses), both in kcal mol^{-1}, are relative to ion $\mathbf{VI_a}$.

Captions for Figures

Figure 1. CID spectra of closed-shell b_5^+ ions derived from A_5W^+ with tryptophan at different positions.

Figure 2. CID spectra of closed-shell b_5^+ ions $[AAAWA_{oxa} + H]^+$ containing one trideuterated alanine residue at different locations. A* denotes the residue carrying the three deuterium atoms.

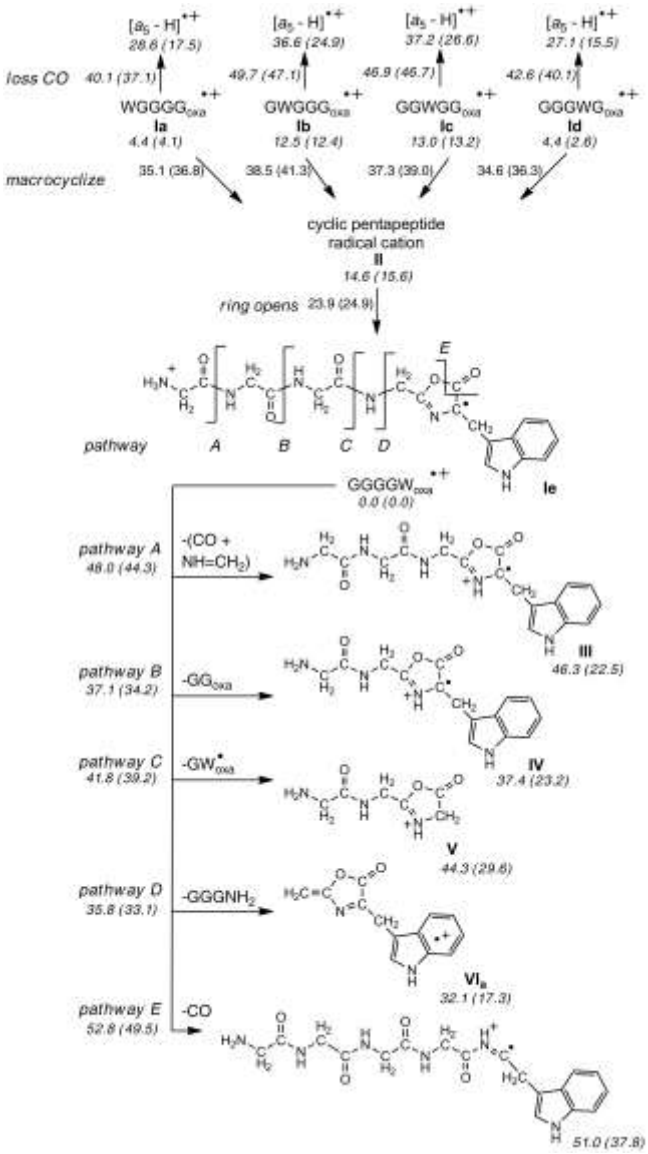
Figure 3. CID spectra of open-shell $[b_5 - H]^{\bullet+}$ ions derived from $A_5W^{\bullet+}$ with the tryptophan at different positions.

Figure 4. CID spectra of open-shell $[b_5 - H]^{\bullet+}$ ions with the initial structures (A) $AAAWA_{oxa}^{\bullet+}$ and (B) $AAAAW_{oxa}^{\bullet+}$ containing one trideuterated alanine residue at different locations. A* denotes the residue carrying the three deuterium atoms.

Figure 5. Possible types of structures of $[b_5 - H]^{\bullet+}$ ions; for convenience the tryptophan residue is taken to be in the oxazolone ring. Note that istructures **Ia**-**Id** in Scheme 1, unlike in **Ie**, the proton is on the nitrogen of the oxazolone ring.

Figure 6. (A) Spectrum of the ion at *m/z* 328 ($AAAAW_{oxa}^{\bullet+}$ minus two alanine residues) showing the formation of the ion at *m/z* 240; (B) Spectrum of the *m/z* 240 ion.

Scheme 1.



Scheme 2

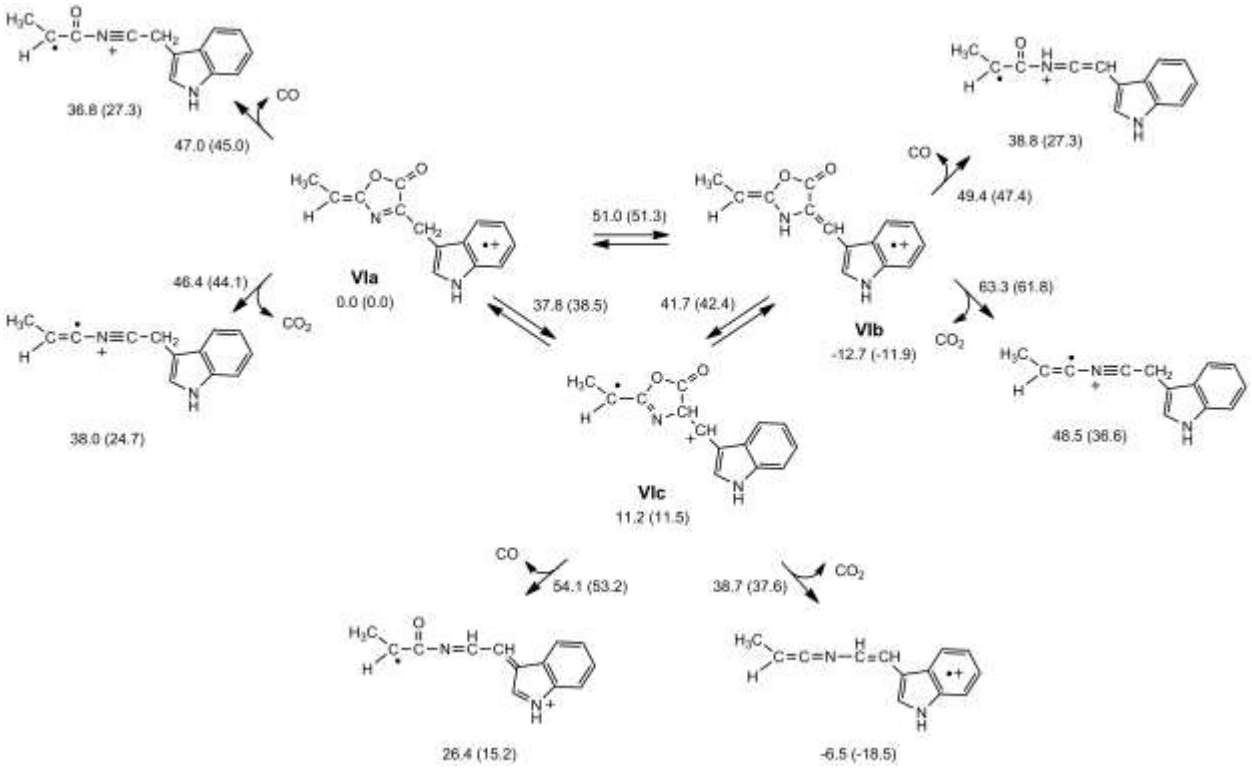


Figure 1.

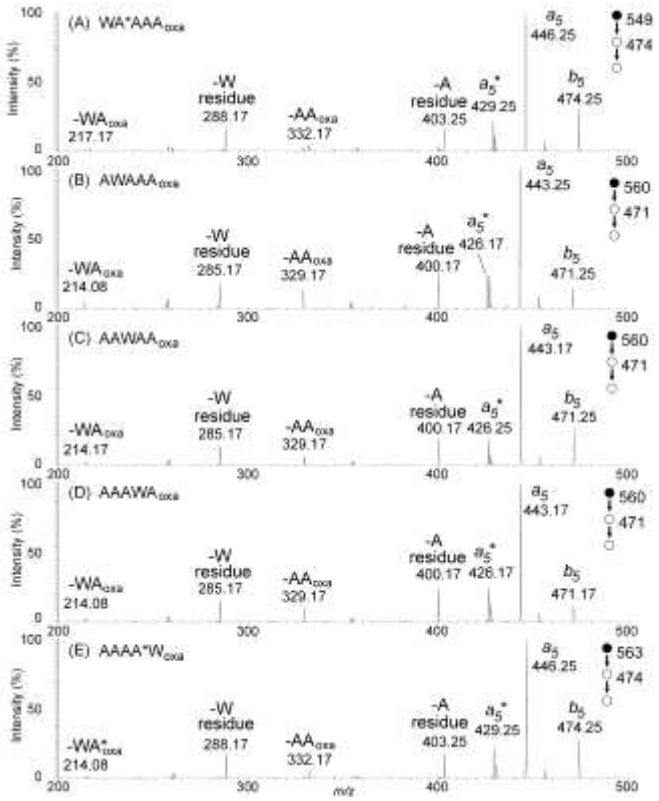


Figure 2.

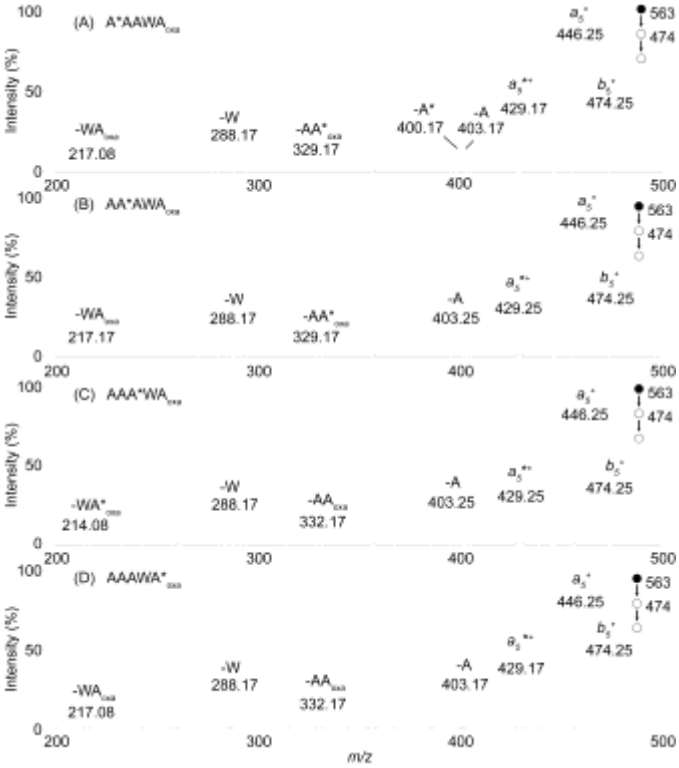


Figure 3.

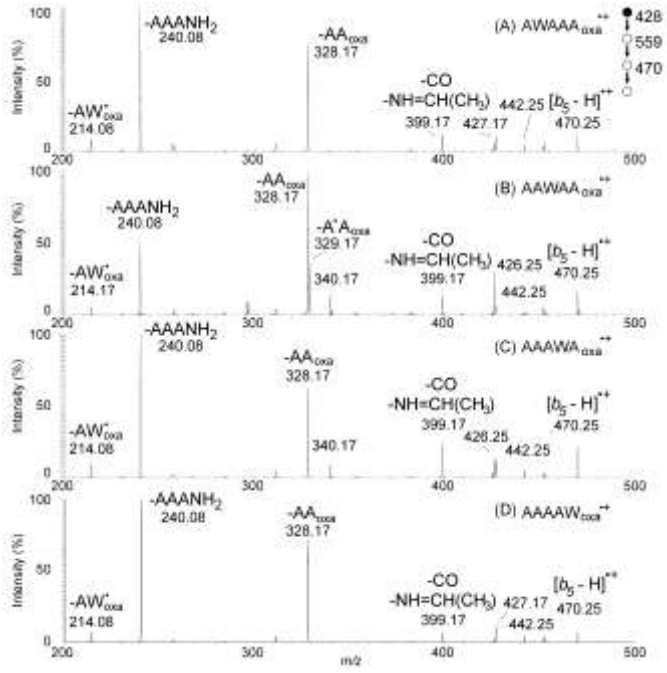


Figure 4.

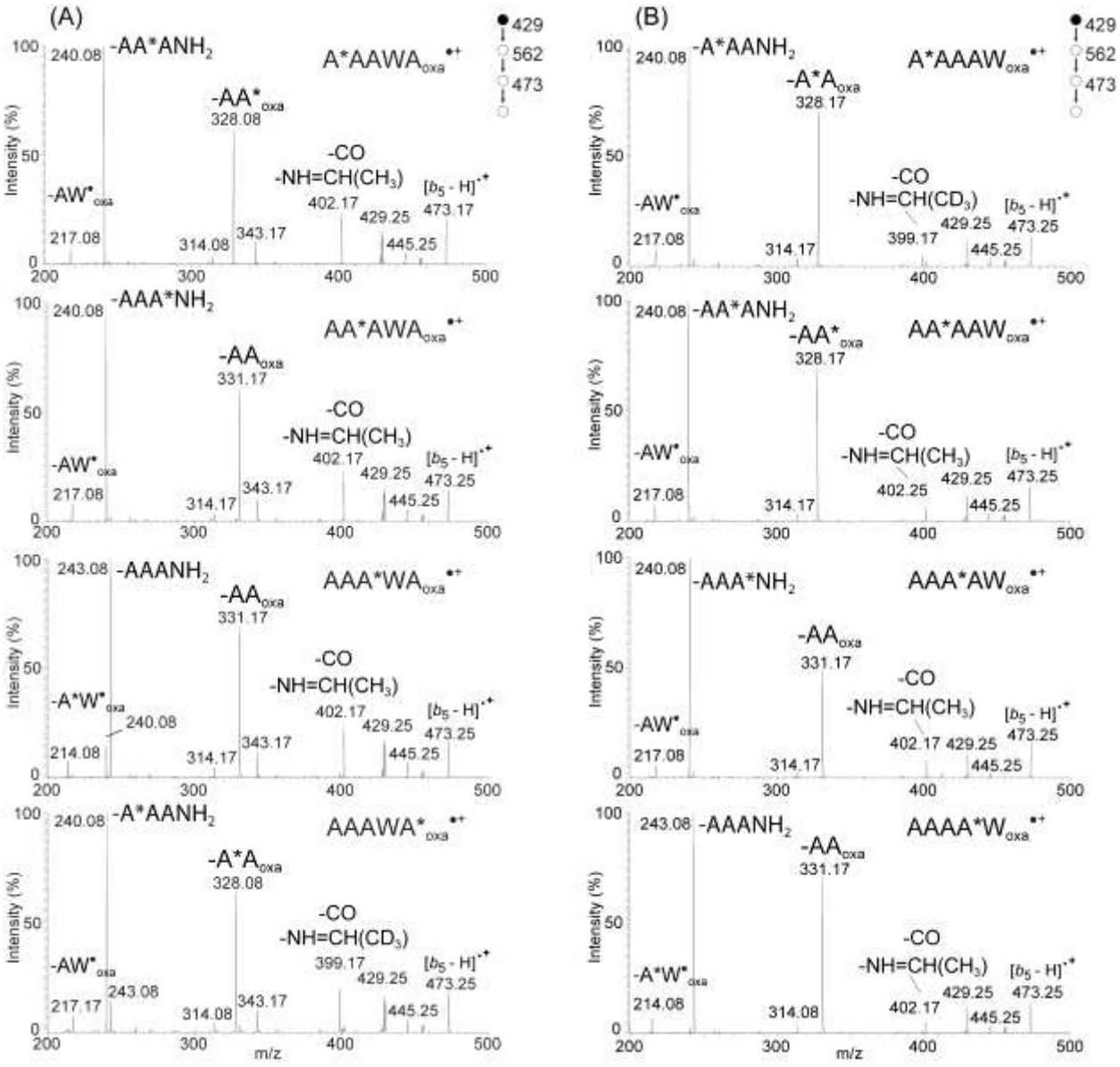


Figure 5.

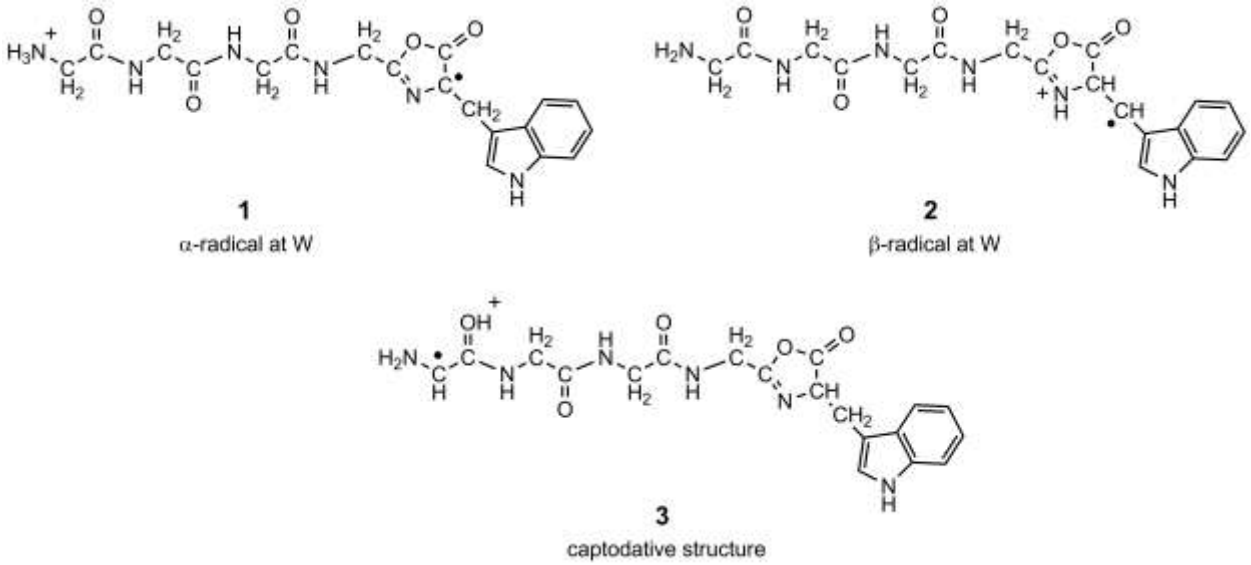


Figure 6.