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FOCUS: DEVELOPMENT AND APPLICATION OF TOF AND TOF/TOF MS: RESEARCH ARTICLE

157 nm Photodissociation of a Complete Set of Dipeptide Ions Containing C-Terminal Arginine

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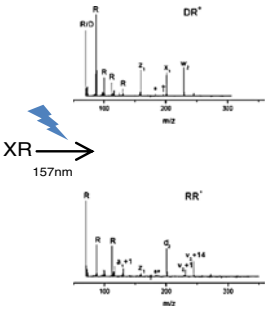

Abstract. Twenty singly-charged dipeptide ions with C-terminal arginine were photodissociated with 157 nm light and their tandem mass spectra recorded. Many of the small product ions that were observed are standard peptide fragments that have been commonly seen in VUV photodissociation studies. However, the study of a library of dipeptides containing all 20 N-terminal amino acids enabled the recognition of trends associated with the occurrence of w-, v-, and immonium ions, the observation of competition between forming N- and C-terminal fragments in dipeptide RR, and the identification of some unusual fragment ions appearing at masses of 183, 187, 196, and 197 Da. A highly accurate internal calibration of the photodissociation TOF-TOF data enabled molecular formulae for these four product ions to be derived. Their proposed structures reflect the rather high-energy nature of this fragmentation phenomenon.

Key words: Photodissociation, Peptides, Ion fragmentation

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Introduction

A number of peptide ion activation methods have been developed, including collision-induced dissociation (CID) [1–4], electron capture/transfer dissociation (ECD/ETD) [5, 6], surface-induced dissociation [7, 8], and photodissociation [9]. In the latter field, different photodissociation wavelengths have been applied, ranging from infrared [10–12] to ultraviolet [13, 14]. Ultraviolet light excites electronic transitions and provides a well-defined energy that is sufficient to induce bond cleavage [15]. Photofragmentation patterns can vary with light wavelength.

Because light having a wavelength below 200 nm is strongly absorbed by peptide backbone amide groups, it is quite suitable for activating peptide ions [16]. The absorption spectrum of polyalanine film displays two main bands around 190 and 165 nm [17]. These two absorption bands can be excited by the ArF excimer laser at 193 nm (6.4 eV) and the F_2 laser at 157 nm (7.9 eV). Peptide photodissociation with 193 nm light has been studied by several groups with different mass spectrometers [18–23]. Most spectra are dominated by a few high-energy backbone (a- and x- ions) and side chain fragments (d-, v-, and w-ions) along with several low-energy b- and y-type fragments that are similar to those observed in CID spectra. Our group has utilized 157 nm vacuum ultraviolet (VUV) light to fragment peptide ions [14, 24, 25]. Photodissociation of singly-charged arginine-containing peptide ions leads to abundant high-energy fragments. Peptides containing C-terminal arginine yield rather complete series of x-ions along with some v- and w-ions. One hundred fifty-seven nm photodissociation of peptides having N-terminal arginine yields a full set of a-type ions accompanied by some d-type fragments. In addition to even-electron a-type ions, odd-electron a+1 radical ions are also produced. Observation of these radical ions indicates that 157 nm photodissociation can involve a homolytic bond cleavage [26]. It has been demonstrated that backbone C–C(O) bonds are primarily cleaved, leading to two radicals. These unstable radical products undergo secondary fragmentation processes by losing the amide hydrogen or a carbon-centered radical from the adjacent side chain to become even-electron ions [27]. The appearance of v-, w-, and z-type ions correlates with the presence of different amino acids in the peptide sequence. However, due to the variety of sequences in random tryptic peptides, the effect that any one residue has on fragmentation propensities can be difficult to discern. A library of peptides can facilitate data analysis and the recognition of fragmentation propensity trends [28]. However, even for peptides of modest length, libraries are typically (and necessarily) incomplete because of the large number of combinations of sequences that they contain. To circumvent

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this problem, in this work we investigated the photodissociation of all 20 naturally occurring dipeptides that contain C-terminal arginine. Their spectra yielded a number of common features along with fragment ions whose appearance and mass depended on the N-terminal residue. While most of the observed product ions were familiar peptide photofragments, several unusual masses were indicative of a surprising extent of fragmentation. Deriving assignments and structures for the latter was facilitated by correlating their appearance with the N-terminal residue of the precursor from which they arose. Observations about dipeptide photodissociation spectra are summarized, and structures of some unusual fragments are proposed.

Experimental

Materials

Acetonitrile and *N*,*N*-dimethylformamide (DMF) were supplied by EMD Chemicals (Gibbstown, NJ, USA). Formic acid (FA) and trifluoroacetic acid (TFA) were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Piperidine, *N*,*N*-diisopropylethylamine and α-cyano-4-hydroxycinnamic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Amino acids, Wang resin, and DEPBT were purchased from Midwest Biotech (Erie, IL, USA).

Peptide Synthesis

Twenty dipeptides XR (where X represents each one of the 20 amino acids) were synthesized in-house via solid phase reaction [29, 30]. Eleven mg of Wang resin with preloaded Fmoc-arginine were weighed out and placed in the cell for synthesis. One mL of DMF was added and the resin swelled for 5 min. After draining DMF, 250 μL of 20 % piperidine in DMF (vol/vol) was used to cleave the Fmoc group. The piperidine was drained after 10 min and the resin was washed five times using DMF. Nine mg DEPBT and Fmoc amino acid equivalent to a four times molar excess relative to the resin were dissolved in 250 μL of 4.2 %*N*,*N*-diisopropylethylamine in DMF (vol/vol). The solution was immediately added to the cell to attach the N terminal amino acid. The reaction took 45 min, and the mixture was stirred several times during synthesis. The resin was dried and washed five times using DMF; 250 μL of 20 % piperidine in DMF were added to cleave the Fmoc. After the resin was washed using DMF, 1 mL of 95 % TFA and 5 % methanol were transferred to the cell to cleave the peptide from the resin. The reaction took 2 h; the cleaved peptide was drained to a glass tube. The peptides were collected and stored in a freezer for future analysis.

Mass Spectrometry

Each dipeptide was diluted to 2 pmol/μL with H_2O, and 0.5 μL of this solution was spotted onto a MALDI target plate. When the spots were dry, 0.5 μL of matrix solution (10 mg/mL CHCA in 50 % ACN/50 % H_2O/0.1 % trifluoroacetic acid) was added on top of it. MALDI mass spectra were recorded with an ABI 4700 TOF-TOF mass spectrometer (Foster City, CA, USA) in positive reflectron mode. For the photodissociation experiments, an F_2 laser (Coherent Lambda Physik, Gottingen, Germany) was connected to the mass spectrometer as previously described [31]. Its timing is automatically controlled by a programmable delay generator.

Although it is possible to record post-source decay (PSD) MS-MS spectra without any photodissociation, (simply by turning the 157 nm laser off) it is not possible to record photodissociation spectra without any contribution from the post-source decay phenomenon. Nevertheless, an attempt was made to roughly subtract the PSD contribution from photodissociation spectra. Direct subtraction of the two spectra did not remove all PSD fragments since photodissociation affects the intensities of different PSD peaks to varying extents. The overall intensity of a PSD spectrum was adjusted to ensure a fairly complete subtraction. In this experiment, the y_1 ion was targeted for intensity adjustment since it was strong in all dipeptide spectra and its intensity was less affected by photodissociation than that of other ions indicating that y_1 ions were mainly formed by PSD. The intensities of y_1 ions in PSD and photodissociation spectra were compared and the resulting ratio was used to adjust the overall intensity of each PSD spectrum. In this way, the PSD contribution was subtracted from every photodissociation spectrum reported in this paper.

In an attempt to assign their structures, the masses of several observed photofragmentation product ions were measured with particularly high accuracy using the following procedure. An internal calibration was applied to each spectrum using ABI (Foster City, CA, USA) software Data Explorer 4.6. A reference file was created using the exact masses of unambiguously assigned peaks such as immonium and backbone fragment ions that are commonly formed by photofragmentation. Two-, three-, and multi-point calibrations were all attempted to check consistency. For the two-point calibration, x_1 and y_1 ions that bracket the unknown photofragmentation product ions were used, while the three-point calibration generally employed x_1, y_1, and z_1 ions. The multi-point calibration included x_1, y_1, z_1, immonium, and v- or w-ions. The three-point and multi-point calibrations were tested with and without peak intensity weighting factors. Overall, five separate calibrations were performed for each spectrum: two-point, three-point with and without weighting factors, and multi-point weighted and unweighted. Accurate masses for all spectral features were derived by averaging results from these calibrations.

Results and Discussion

Typical 157 nm photodissociation spectra of dipeptide ions that display the salient features of these data are displayed in Figures 1, 2, 3, and 4. The remaining dipeptide photodissociation spectra are in Supplemental Information. Immonium ions, particularly those associated with the C-terminal

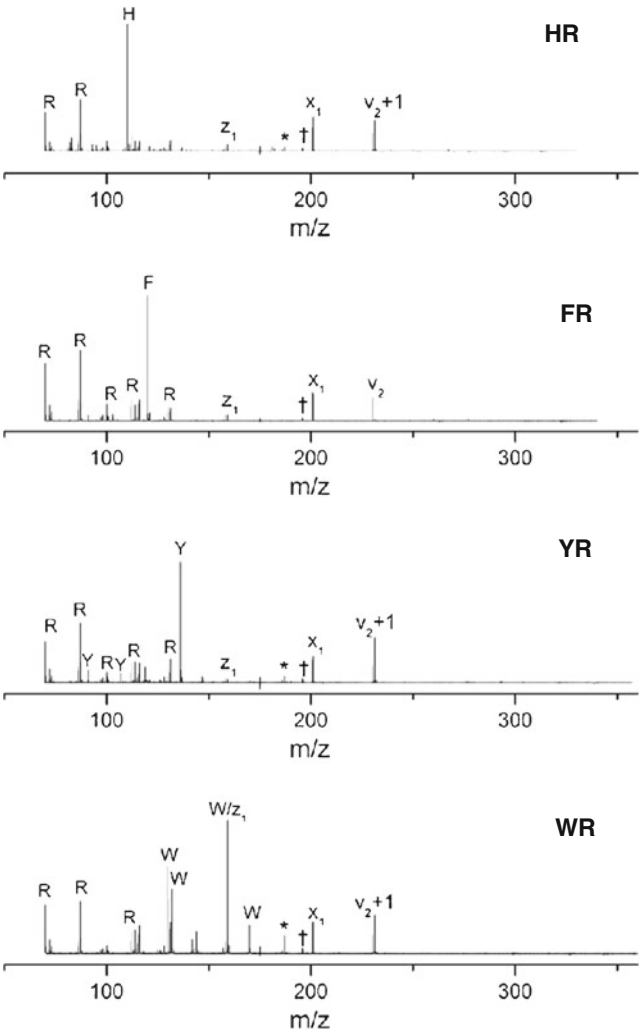


Figure 1. Photodissociation spectra of dipeptides HR, FR, YR, and WR; * labels the 187 Da peak and † labels the 196 Da peak

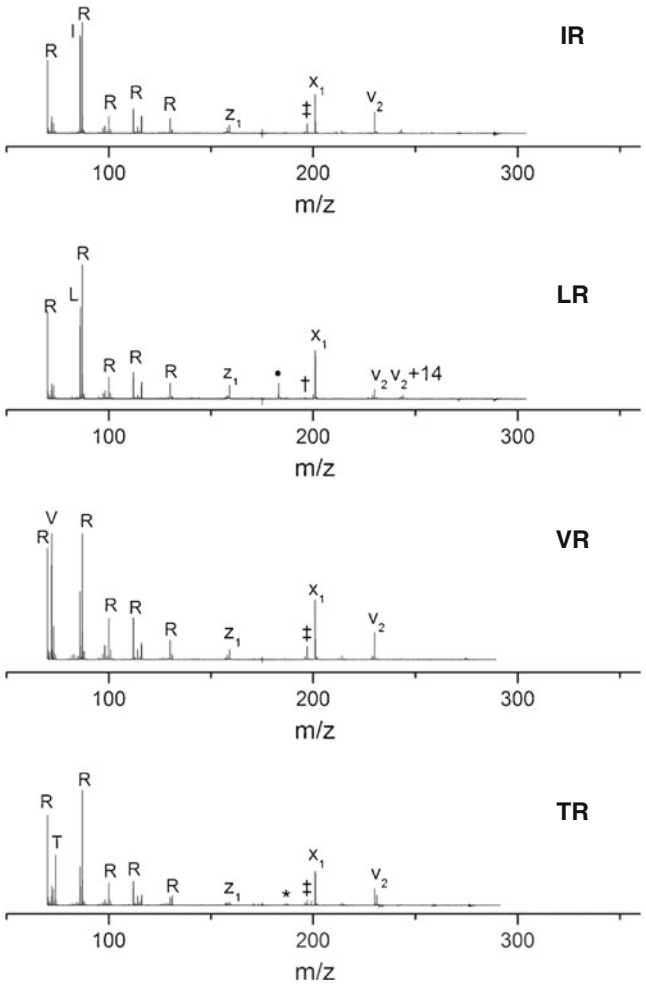


Figure 2. Photodissociation spectra of dipeptides IR, LR, VR, and TR (• labels the 183 Da peak, * the 187 Da peak, † the 196 Da peak Da, and ‡ the 197 Da peak)

arginine, appear ubiquitously in the low mass region of these spectra although for the small residue, glycine, alanine, and serine they were too light to be recorded. As evident in Figure 1, phenylalanine, histidine, tryptophan, and tyrosine yield particularly intense immonium ions, presumably because their aromatic groups absorb 157 nm vacuum ultraviolet light very strongly. Since the C-terminal arginine effectively sequesters the proton, strong x_1 ions and less intense z_1 ions result from peptide backbone cleavage. The former are commonly observed in photodissociation studies of larger peptide ions [13, 14], but the latter are relatively rare. Their appearance in these spectra is probably a consequence of depositing a rather significant activation energy (7.9 eV) into a relatively small peptide ion, so even relatively unfavored fragmentation pathways are observable. Whether v- and w-type ions appear in spectra depends on the N-terminal residue. Specific fragment ion types and other spectral idiosyncrasies are next discussed, and all results are summarized in Table 1.

v-Type Ions

Three high-energy v-type ions were observed in these spectra: v_2, v_2+1, and v_2+14. Their structures are depicted in Scheme 1.

v_2+14 ions had not been reported in previous photodissociation studies and would have been easy to ignore except for the fact that their appearance correlated with the dipeptide N-terminal residue. v_2+14 ions were observed in spectra of KR, LR, MR, NR, QR, and RR. Analysis of the library of samples thus exposed the need for an N-terminal residue with a linear alkyl side chain that can cleave between the β– and γ-carbons. This is consistent with the structure depicted in Scheme 1. v_2 and v_2+1 ions result from the loss of entire side chains, the only difference being whether a hydrogen atom is also eliminated and an N–C double bond formed. In some cases, v_2 and v_2+1 ions appear in spectra with comparable intensities. As seen in Figures 1 and 2, the v_2 ion peak is relatively intense in four cases: IR, TR, VR, and FR. Interestingly, of the 20 amino acids, only

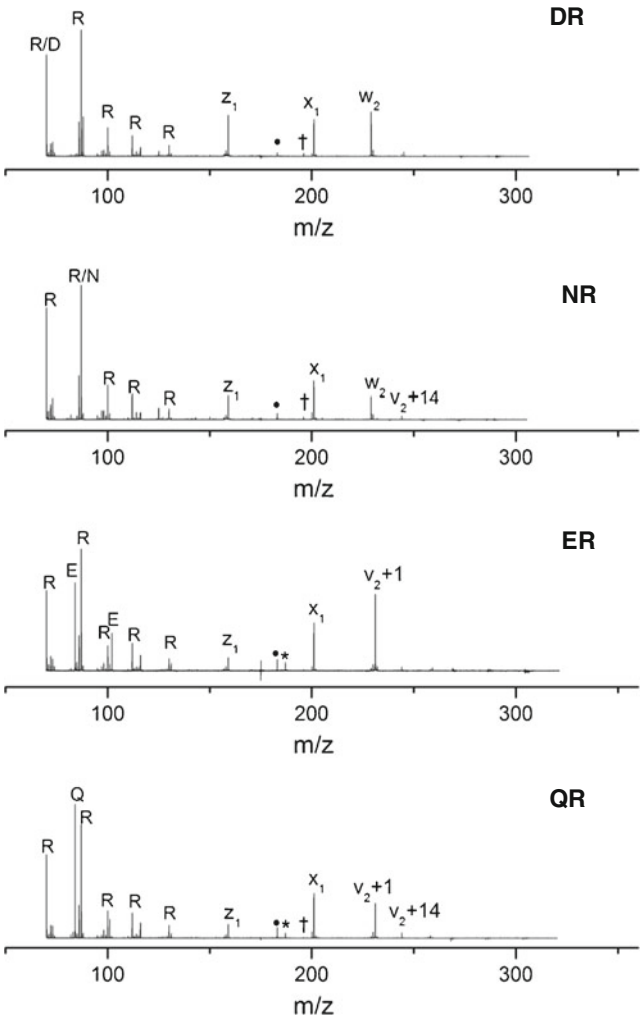


Figure 3. Photodissociation spectra of dipeptides DR, NR, ER, and QR (• labels the 183 Da peak, * labels the 187 Da peak and † the 196 Da peak)

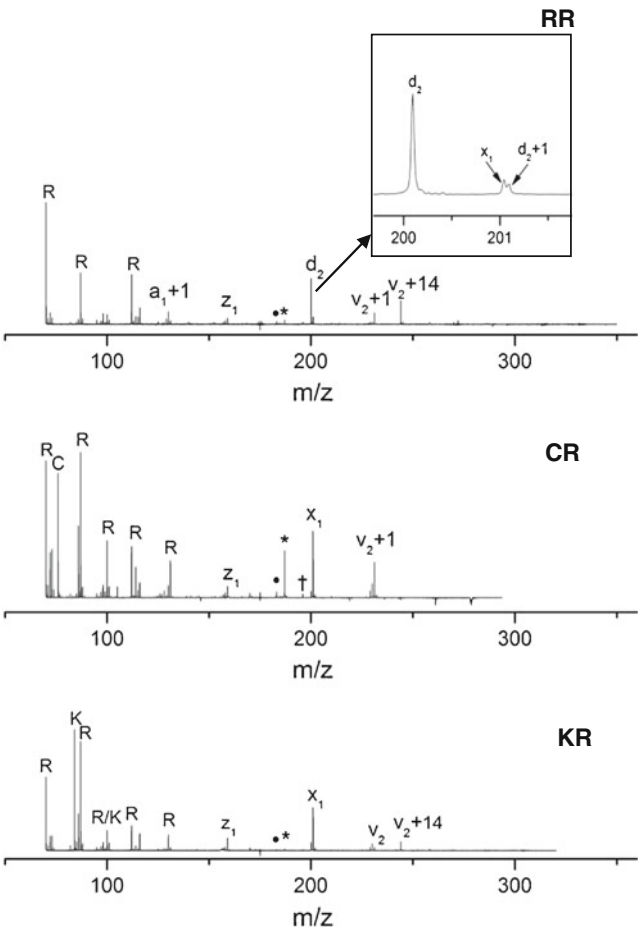


Figure 4. Photodissociation spectra of dipeptides RR, CR, and KR (• labels the 183 Da peak, * the 187 Da peak, and † the 196 Da peak)

isoleucine, threonine, and valine have tertiary β carbons in their side chains. In the 157 nm photodissociation process, 7.9 eV of energy is deposited into an ion. Formation of a relatively stable tertiary radical is energetically less costly, leaving more energy in the system to enable other processes, such as H atom loss and double bond formation. In the case of phenylalanine, loss of the side chain is facilitated by formation of the stable toluene molecule. In conclusion, when a more stable side chain species is lost, the propensity to eliminate a hydrogen atom and thereby form v ions appears to be favored. When a less stable side chain radical results (i.e., with ER, CR, QR, and RR), the molecule remains in its radical form and v+1 ions are observed. Several dipeptides (HR, MR, WR, TR, SR, and YR) seem to be intermediate cases for which both v and v+1 ions are produced.

w Ions

In previous photodissociation studies of larger peptides, w-type ions were observed following side chain losses from various amino acids. In fact, they were found to be particularly useful for distinguishing the isobaric amino acids leucine and isoleucine [13, 14, 31]. With these larger peptides, it was postulated that w_n ions were produced by secondary fragmentation of $x_{n+1}+1$ ions [18, 31]. However, by this mechanism it should not be possible to form w_N ions from a precursor that contains only N residues. Liu et al. recently studied a library of 10–12 residue peptides having just six N-terminal amino acids (Y, N, G, F, E, and A) [28]. w_N Ions were only observed with peptides having N-terminal asparagine. In the present work, we were able to investigate whether w_2 fragments could be formed from dipeptide ions having any other N-terminal residue. In fact, photodissociation of only two dipeptide ions, DR and NR, yielded w_2 ions. However, as shown in Figure 3, these peaks are of significant intensity. The similarity of aspartic acid and asparagine suggests that their side chains are involved in the w_2 photofragment ion production. The side chains are of similar length, and both can form a hydrogen bond with the N-terminal amine. As shown in Scheme 2, elimination of neutral molecules from a six-membered ring can lead to production of w- ions. The acidity of the aspartic side chain

Table 1. Summary of Dipeptide Photodissociation Fragments

	Immonium ion from N-terminal residue	x_1	v_2	v_2+1	v_2+14	w_2	z_1	183 Da (•)	187 Da (*)	196 Da (†)	197 Da (‡)
AR		√					√				
GR		√					√		√		
PR	√	√					√	√			
KR	√	√	√		√		√	√	√		
LR	√	√	√		√		√	√		√	
FR	√	√	√				√			√	
IR	√	√	√				√			√	√
TR	√	√	√				√		√	√	√
VR	√	√	√				√			√	√
SR		√		√			√	√		√	
CR	√	√		√			√	√	√	√	
ER	√	√		√			√	√	√		
QR	√	√		√	√		√	√	√	√	
HR	√	√		√			√		√	√	
MR	√	√		√	√		√	√	√		
WR	√	√		√			√		√	√	
YR	√	√		√			√		√	√	
DR	√	√				√	√	√		√	
NR	√	√			√	√	√	√		√	
RR	√	a_1 √	d_2 √	√	√		√	√	√		

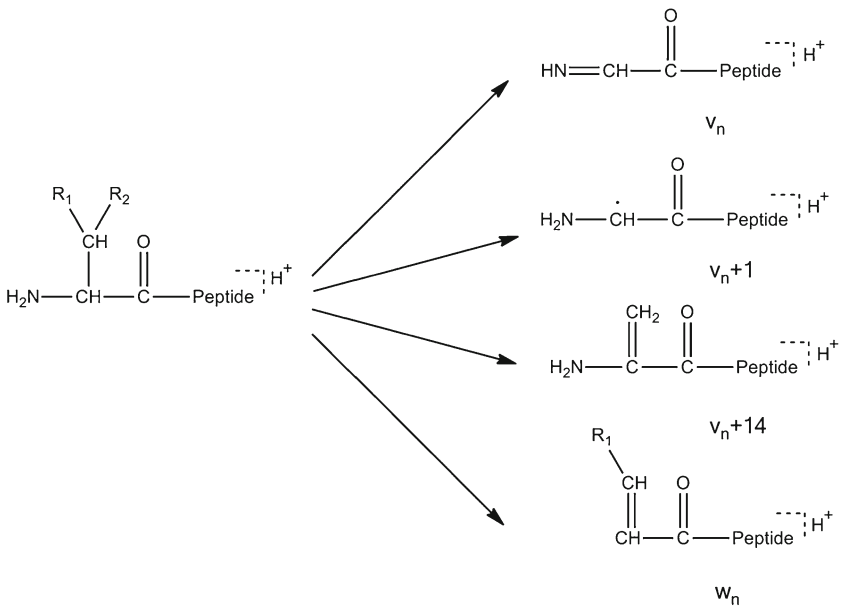
may facilitate this process, enhancing the fragmentation for DR relative to NR.

z Ions

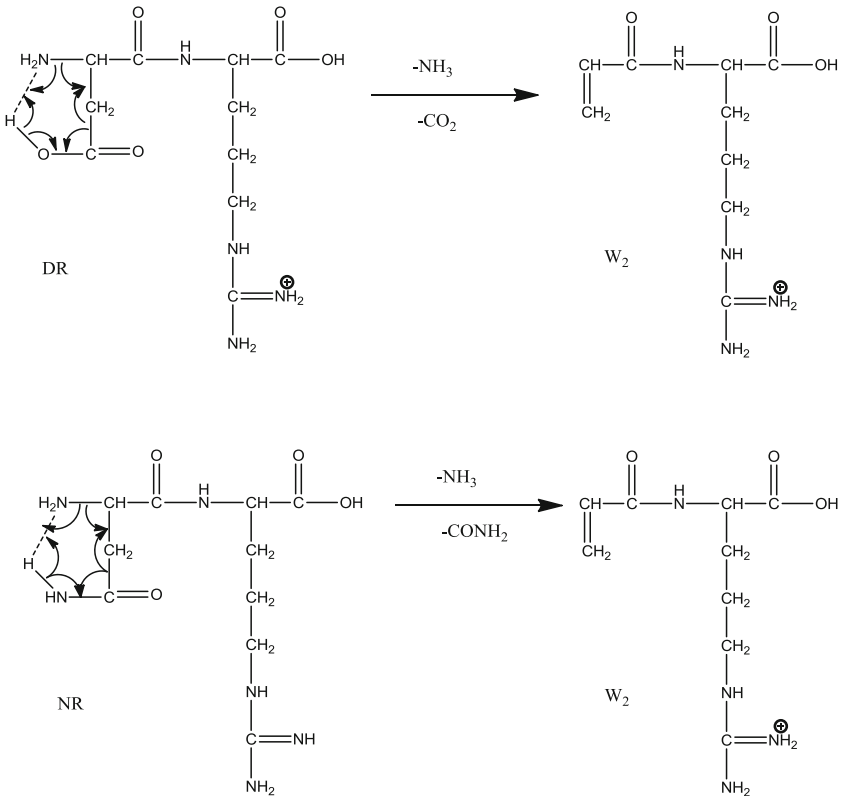
z-Type ions are generated by breaking the N – C bond in a peptide backbone. z_1 ions appear in all 20 dipeptide spectra but with significant intensity in only four: DR, ER, NR, and QR (as shown in Figure 3). Aspartic acid, glutamic acid, asparagine, and glutamine all have a carbonyl group in their side chains. As depicted in Scheme 3, the carbonyl oxygen can interact with the H atom on the nitrogen nearby to form a hydrogen bond in a seven-member ring structure. This weakens the N – C bond outside the ring and facilitates its cleavage following photoexcitation. Because this process requires a less-favored eight-member ring for the glutamic acid and glutamine side chains, the intensities of z ions are somewhat lower in photodissociation spectra of ER and QR.

Dipeptide RR

Of the 20 dipeptides, RR is quite exceptional since it is the one case for which there is an N-terminal arginine. The resulting photodissociation spectrum contains both N-terminal and C-terminal fragments as shown in Figure 4. C-terminal fragment ions dominate the spectra of all the other XR dipeptides, since for these molecules a C-terminal arginine sequesters the proton. In the case of RR, there are



Scheme **1**. Formation of different side-chain loss photodissociation product ions

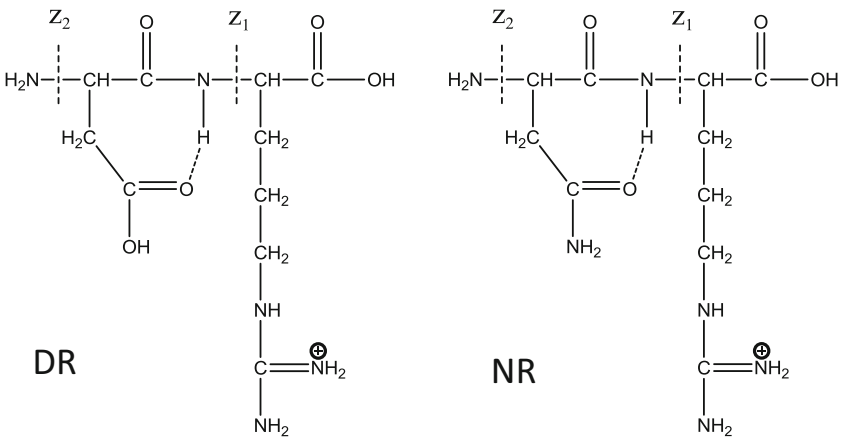


Scheme **2**. Proposed processes for the generation of w_2 ions from dipeptides DR and NR

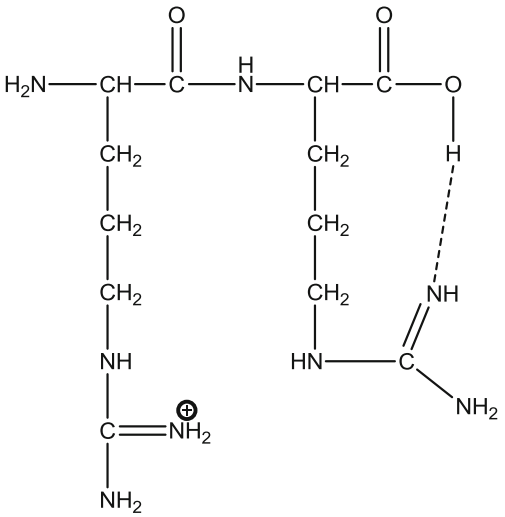
two arginine side chains and the proton could easily reside on either. It is possible that the C-terminal carboxylic acid forms a salt bridge with the C-terminal arginine side chain as shown in Scheme 4 making the N-terminal arginine in this dipeptide slightly more basic. However, the difference in intensities between N- and C-terminal fragments is not sufficient to justify further speculation. As shown in the enlarged section, the 201 Da product ion peak appears to be split. The x_1 ion should have a mass of 201.0982 Da while the ^{13}C peak associated with the d_2 ion is expected to occur at 201.1540 Da. These values are consistent with the observed splitting.

Sequence-Independent Fragment Ions

In addition to the familiar types of immonium and backbone ions present in these photofragmentation mass spectra, four small peaks between 180 and 200 Da appeared in several cases. Because the four were not observed in our PSD data (with one exception noted below), we believe that they are



Scheme **3**. Proposed structures of dipeptide DR and NR ions



Scheme **4**. Proposed structure of dipeptide RR that would lead to N-terminal fragments

high-energy fragment ions generated by photodissociation. To confirm that they were not in any way associated with the matrix, a few dipeptide ions were also generated by electrospray ionization and photodissociated inside a linear ion trap apparatus that has previously been described [32, 33]. Singly-charged precursor ions yielded the same four unusual fragment ions observed in photodissociation TOF-TOF experiments. While these peaks were generally low in intensity (in some cases extremely low), they were nevertheless intriguing: their masses were independent of the dipeptide from which they derived, but whether or not they appeared was indeed sequence-dependent. For example, a 183 Da fragment (labeled in Figures 3 and 4 with a dot) is formed in the photodissociation of dipeptides whose N-terminal residue contains a linear alkyl side chain (KR, SR, RR, LR, CR, DR, NR, ER, QR, MR, and PR). A 197 Da fragment (labeled with a double dagger in Figure 2) only appears in spectra of dipeptides with tertiary β carbons (TR, IR, and VR). A 196 Da peak (labeled with a single dagger in Figures 1, 2, 3, and 4) arises from dipeptides having either an aromatic side chain or an alkyl side chain (CR, QR, DR, FR, YR, WR, HR, NR, TR, IR, LR, SR, and VR). Finally, a 187 Da peak (labeled with an asterisk) is observed with significant intensity in CR and WR spectra and with much lower intensity in many other spectra. Table 1 summarizes when these four ions are observed.

Multiple tentative structures for the four unique photofragments just discussed can immediately be proposed, but without firm justification. Fortunately, most of the possible structures are associated with different elemental compositions. In order to determine the likeliest structures, an attempt was made to derive very accurate masses for the fragments. In TOF-TOF experiments, the masses of peptide fragment ions are often reported with error tolerances on the order of 0.1 Da. However, it seemed likely that the masses of our four unknown photofragment ions could be measured far more accurately than this. For one reason, their masses are smaller than those of most peptide fragments. Secondly, every dipeptide photodissociation spectrum contains a number of other small ion fragments (immonium, x_1, v_1, z_1 ions etc.) whose identities and, therefore, masses are known with little ambiguity. These peaks bracket the masses of our four unknown fragments and can serve as excellent internal mass calibrants. We, therefore, expected that it might be possible to measure the masses of the unknown fragments with an accuracy that would enable us to clearly establish their chemical formulae. The procedure for accomplishing this is outlined in the section 2 above. Two-, three-, and multi-point calibrations were performed. The resulting calibration curves enabled us to match the expected masses of our internal calibrants to within 0.0045 Da. In addition, a simple test was performed using five known fragment ions (x_1, y_1, z_1, and two arginine immonium fragments) as calibrants and another arginine immonium fragment as an "unknown". Nineteen sets of data were analyzed and the mass derived for the "unknown" arginine fragment was found to be within 0.0016 Da of its expected value. Using the same set of calibrants, we applied two-, three-, and multi-point calibrations to all of the spectra in which these peaks were observed. Averaging these results, the masses of our four unusual photofragments were determined to be 183.127±0.003, 187.142±0.003, 196.071±0.002, and 197.141±0.001 Da. The ABI Data Explorer software can generate a list of all possible molecules that contain carbon, hydrogen, nitrogen, oxygen, and sulfur and that have masses within some tolerance of a selected input mass. (The maximum number of atoms of each element can be further restricted based on the elemental composition of the dipeptides). Using this software, all molecular formulae whose masses are within 0.01 Da of the above four values are listed in Table 2. For one case, 197 Da, the procedure yielded a single formula whose mass matched the experimental mass to within 0.001 Da. For the other three cases, multiple possible formulae that are listed in Table 2 were derived. However, most of these formulae were unreasonable because they contained too few carbons, nitrogens, or oxygens to be products of our dipeptide ion precursors. The most reasonable possibilities are underlined in Table 2. For the 187.142 mass, another problem arose. The formula proposed by the software that contained the most reasonable distribution of atoms, $C_9H_{19}N_2O_2$, actually includes more carbons and hydrogens than are present in the GR ion, which is one of the precursors that yields this fragment. However, it was found that the Data Explorer software considers only even-electron species when matching formulas with masses. By expanding our search to odd-electron species, we determined that a radical ion, $C_7H_{17}N_5O^{\bullet}$, provides an excellent mass match to our 187 Da peak and is consistent with the numbers of atoms of each element found in all dipeptides that produced this fragment ion.

Although even with their derived molecular formulae it is not possible to establish definitive structures for the four

Table 2. Calibrated Accurate Masses and Their Matching Formulas

Nominal mass (Da)	Accurate measured mass (Da)	Possible formulae/masses (Da)
183	183.127±0.003	$C_3H_{15}N_6O_3$, 183.121
CR, DR, ER, LR,		$C_7H_{15}N_6$, 183.136
MR, NR, PR, QR, KR, SR, RR		**$C_8H_{15}N_4O$, 183.124**
		$C_{11}H_{19}S$, 183.121
187	187.142±0.003	$C_5H_{15}N_8$, 187.141
CR, GR, HR, KR, MR, QR, RR, TR, WR, YR, ER,RR		**$C_7H_{17}N_5O$, 187.143**
		$C_9H_{19}N_2O_2$, 187.144
		$C_{10}H_{19}O_3$, 187.133
		$C_{11}H_{23}S$, 187.152
		$C_{14}H_{19}$, 187.148
196	196.071±0.002	$C_3H_{10}N_5O_5$, 196.068
CR, DR, IR, LR,		**$C_8H_{10}N_3O_3$, 196.072**
NR, TR, VR,QR,SR		$C_{10}H_6N_5$, 196.062
FR, YR, WR, HR		$C_{13}H_{10}NO$, 196.076
197	197.141±0.001	**$C_9H_{17}N_4O$, 197.140**
IR, TR, VR		

The formulae in bold and underlined are the most reasonable ones for their masses

high energy ions just discussed, it is certainly possible to propose likely structures. These are presented in Figures S1–S3 in Supplemental Information. The 196 Da structure is the most difficult one to pin down, and several possibilities are depicted. Exact locations of the unpaired electron in the 187 Da structure or of double bonds in other structures cannot be ascertained. Nevertheless, definite similarities in the four structures lead to some confidence in their validity. Because the four masses are independent of the dipeptide sequences, side chains of the N-terminal residue must be lost from each structure. Because the charge is presumed to be located on the arginine, its side chain is assumed to be largely or completely intact. Likewise, the dipeptide backbone is presumed to be partly intact. Thus, to match the molecular formulae derived from the accurate mass measurements, it is simply necessary to cleave off the N-terminal amine and/or the C-terminal carboxylic acid groups along with an appropriate number of hydrogens. Although, as just noted, the masses of the postulated structures are independent of sequence, it is noteworthy that the side chains of the proposed 183 and 197 Da fragments are consistent with precursors having secondary and tertiary β-carbons. This lends further credence to the proposed structures. Likewise, the 187 Da structure could easily be formed from a wide array of different precursor ions. Every one of these proposed structures demonstrates that the fragmentation of these dipeptides is a rather explosive process. This is not surprising considering that 7.9 eV of energy is being introduced into a rather compact peptide ion.

The four unusual fragment ions observed in the 190–200 Da region were identified as high energy fragments because they appeared in photodissociation, not PSD spectra. There was one exception to this: dipeptide GR yielded a relatively intense 197 Da PSD fragment ion. The simplest explanation for the appearance of this peak is that it corresponds to MH – NH_3 – H_2O. This would be a reasonable PSD product ion, particularly since MH – NH_3 and MH – H_2O fragment ions also appear in the PSD spectrum. Fortunately, the molecular formula of the MH – NH_3 – H_2O ion is $C_8H_{13}N_4O_2$, which differs from the $C_9H_{19}N_4O$ formula determined above for a 197 Da fragment ion. Its theoretical mass is 197.1033 Da, which differs by only 0.005 Da from the 197.098 Da mass experimentally measured for this peak. We, therefore, accurately calibrated the GR PSD spectrum using immonium, y_1, Y_1, y_1-NH_3, and MH-NH_3 fragment ions as internal calibrants and obtained an accurate experimental mass of 197.098 Da for this PSD fragment. This confirmed its identity as MH – NH_3 – H_2O and not our high-energy photofragment ion.

Conclusions

Twenty dipeptides with C-terminal arginine were synthesized, MALDI-ionized in a TOF-TOF mass spectrometer, and photodissociated with 157 nm light. Owing to the C-terminal arginine, x_1 product ions were observed in the resulting spectra in all cases. Dipeptide RR also contains somewhat more intense N-terminal a-type and d-type fragments, indicating that the N-terminal arginine sequesters a proton slightly more effectively than the C-terminal arginine; v- and w-type ions are generated from certain amino acids and the observations can be rationalized. Four unusual, high-energy fragment ions were produced at masses not predictable using conventional peptide fragmentation schemes. By internally calibrating the photofragmentation TOF-TOF data, their masses were measured to a few thousandths of a Da, leading to credible molecular formulae for each. Structures compatible with these formulae suggest, not surprisingly, that multiple bonds of the small dipeptide ion shatter following absorption of 7.9 eV photons.

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