Why Are B Ions Stable Species in Peptide Spectra?

Talat Yalcin, Charlotte Khouw, Imre G. Csizmadia, Michael R. Peterson, and Alex G. Harrison

Department of Chemistry, University of Toronto, Toronto, Ontario M5S 1A1, Canada

Protonated amino acids and derivatives $\text{RCH}(\text{NH}_2)\text{C}(=\text{O})\text{X} \cdot \text{H}^+$ ($\text{X} = \text{OH}, \text{NH}_2, \text{OCH}_3$) do not form stable acylium ions on loss of HX , but rather the acylium ion eliminates CO to form the immonium ion $\text{RCH}=\text{NH}_2^+$. By contrast, protonated dipeptide derivatives $\text{H}_2\text{NCH}(\text{R})\text{C}(=\text{O})\text{NHCH}(\text{R}')\text{C}(=\text{O})\text{X} \cdot \text{H}^+$ [$\text{X} = \text{OH}, \text{OCH}_3, \text{NH}_2, \text{NHCH}(\text{R}'')\text{COOH}$] form stable B_2 ions by elimination of HX . These B_2 ions fragment on the metastable ion time scale by elimination of CO with substantial kinetic energy release ($T_{1/2} = 0.3\text{--}0.5$ eV). Similarly, protonated *N*-acetyl amino acid derivatives $\text{CH}_3\text{C}(=\text{O})\text{NHCH}(\text{R}')\text{C}(=\text{O})\text{X} \cdot \text{H}^+$ [$\text{X} = \text{OH}, \text{OCH}_3, \text{NH}_2, \text{NHCH}(\text{R}'')\text{COOH}$] form stable B ions by loss of HX . These B ions also fragment unimolecularly by loss of CO with $T_{1/2}$ values of ~ 0.5 eV. These large kinetic energy releases indicate that a stable configuration of the B ions fragments by way of activation to a reacting configuration that is higher in energy than the products, and some of the fragmentation exothermicity of the final step is partitioned into kinetic energy of the separating fragments. We conclude that the stable configuration is a protonated oxazolone, which is formed by interaction of the developing charge (as HX is lost) with the N-terminus carbonyl group and that the reacting configuration is the acyclic acylium ion. This conclusion is supported by the similar fragmentation behavior of protonated 2-phenyl-5-oxazolone and the B ion derived by loss of H-Gly-OH from protonated $\text{C}_6\text{H}_5\text{C}(=\text{O})\text{-Gly-Gly-OH}$. In addition, ab initio calculations on the simplest B ion, nominally $\text{HC}(=\text{O})\text{NHCH}_2\text{CO}^+$, show that the lowest energy structure is the protonated oxazolone. The acyclic acylium isomer is 1.49 eV higher in energy than the protonated oxazolone and 0.88 eV higher in energy than the fragmentation products, $\text{HC}(=\text{O})\text{N}^+\text{H} = \text{CH}_2 + \text{CO}$, which is consistent with the kinetic energy releases measured. (*J Am Soc Mass Spectrom* 1995, 6, 1165–1174)

Protonated carboxylic acids fragment to a considerable extent by elimination of H_2O to form stable acylium ions [1–4]. In the same vein, protonation of the methyl esters of carboxylic acids leads to substantial elimination of CH_3OH that again forms stable acylium ions [1, 4–6]. By contrast, protonated α -amino acids, whether they are formed by chemical ionization (CI) [7–9], by fast-atom bombardment (FAB) [10], or by plasma desorption [9, 11], do not form stable acylium ions by elimination of H_2O . Rather the highest mass fragment observed is the immonium ion $\text{RCH}=\text{NH}_2^+$ that results from elimination of (H_2 , C, O_2) from the MH^+ ion $\text{RCH}(\text{NH}_2)\text{COOH} \cdot \text{H}^+$. Similarly, protonated α -amino acid methyl esters and amides do not appear to form stable acylium ions upon fragmentation, but once again immonium ions are observed as the highest mass fragment ions [9].

The failure to observe acylium ions in the CI mass spectra of α -amino acids was rationalized qualitatively by Tsang and Harrison [8], who concluded that, although the acylium ion was formed as a transient

species, the fragmentation reaction

was exothermic due to the stability of the immonium ions formed. A recent detailed study by Hoppilliard and co-workers [9] of the fragmentation of protonated leucine concluded that fragmentation of the protonated species occurred by the pathway outlined in Scheme I ($\text{R} = i\text{-Bu}$)*. Thermochemical estimates of the heats of formation of **b** and **c** showed that they were higher in energy than the protonated species **a** or the final products $\text{RCH}=\text{NH}_2^+ + \text{CO}$. Experimentally, the fragmentation reaction ($\text{R} = i\text{-Bu}$)

*The loss of H_2O is proposed to originate from a hydroxyl-protonated species formed by proton migration from the N-protonated species. This differs from the H_2O -loss reaction in simple protonated carboxylic acids where protonation is believed to occur on the carbonyl oxygen [12] and H_2O loss occurs by way of a 1,3-H shift that is symmetry forbidden and has a large energy barrier [3]. Experimentally, it was observed (Yalcin, T.; Dookeran, N. N.; Harrison, A. G., unpublished results) that there is complete randomization of all labile hydrogens in protonated amino acids prior to fragmentation.

Address reprint requests to Alex G. Harrison, Department of Chemistry, University of Toronto, Toronto, Ontario M5S 1A1, Canada.

Scheme I

was found to occur on the metastable ion time scale to give a flat-topped metastable ion peak with a substantial release of kinetic energy ($T_{1/2} = 0.39$ eV). This result is consistent with the intermediates **b** and **c** in the fragmentation sequence being higher in energy than the final products [13, 14].

In contrast to the simple amino acids and derivatives, the mass spectra of protonated peptides [both the FAB mass spectra and the collision-induced dissociation (CID) mass spectra] frequently show abundant formation of B-type ions [15-20] (Scheme II), which usually have been assumed to be acylium ions. Indeed, the B_n series of ions often provides substantial sequence information for the peptide. The question arises as to why these B ions, which are nominally acylium ions, are stable when the acylium ions formed from amino acids are unstable.* The present work explores this question experimentally through CID and metastable ion studies, which include kinetic energy release measurements, with particular emphasis on B_2 ions. In addition, ab initio calculations have been carried out to probe the potential energy surface for fragmentation of B-type ions.

* "Stable" and "unstable" are to some extent ambiguous words. In the present context we imply only that B_n ($n \geq 2$) ions exist in a potential well sufficiently deep that they survive long enough to be observed experimentally. By contrast, the B_1 ions derived from amino acids and derivatives do not appear to reside in a potential well and, therefore, are not observed. On a more quantitative scale, it appears that B ions are less stable toward further fragmentation than their Y counterparts [21].

Scheme II

Experimental

All experiments were carried out with a VG Analytical (Manchester, UK) ZAB-2FQ mass spectrometer, which has been described in detail previously [22]. Briefly, the instrument is a reversed-geometry (BE) double-focusing mass spectrometer with a third stage that consists of a deceleration lens system, a rf-only quadrupole collision cell, and a quadrupole mass analyzer. The ions studied were produced by fast-atom bombardment (FAB) by using an Ar atom beam of 7-8-keV energy with the appropriate sample dissolved in a matrix that consisted of thioglycerol-2,2'-diethiodiethanol (1:1) saturated with oxalic acid. The ion source was operated at room temperature.

To obtain relative intensities of fragment ions formed on the metastable ion time scale, the precursor ion of interest was mass-selected by the BE double-focusing mass spectrometer at 6-keV ion energy, decelerated to 20-40-eV energy, and introduced into the rf-only quadrupole cell in the absence of collision gas (base pressure $< 10^{-8}$ torr). The products of unimolecular fragmentation in the quadrupole cell were measured by scanning the final quadrupole mass analyzer. Low energy collision-induced dissociation (CID) studies were carried out in the same fashion, but with the addition of N_2 at an indicated pressure of $2-3 \times 10^{-7}$ torr to the quadrupole collision cell. These pressures corresponded to 20-30% attenuation of the precursor ion intensity. In the CID experiments the incident ion energy was typically varied from 2 to 50 eV (laboratory scale).

Kinetic energy releases associated with the unimolecular fragmentation reactions were determined by the mass-analyzed ion kinetic energy spectrometry (MIKES) technique [13]. In this technique the ion of interest was mass-selected by the magnetic sector at 6-keV kinetic energy and the products of unimolecular fragmentation in the drift region between the magnetic and electric sectors were identified in accordance with their kinetic energy by scanning the electric sector. The kinetic energy releases were determined, under conditions of good energy resolution, from the peak widths at half-height after correction for the inherent energy spread of the ion beam in accordance with the relation [14]

$$W_{\text{corr}} = (W_{\text{met}}^2 - W_{\text{mb}}^2)^{1/2} \quad (3)$$

where W_{met} is the measured width of the metastable ion and W_{mb} is the width of the parent ion main beam. The corrected half-widths were converted to $T_{1/2}$ values by using the equation developed [13] for electric sector scans. It should be noted that the time scale for unimolecular (metastable ion) fragmentation in the drift region before the electric sector (MIKES technique) is quite different from the time scale for unimolecular fragmentation in the rf-only quadrupole cell. For the former the time is 10-20 μs after ion formation

in the source, whereas the time scale pertinent to the quadrupole cell is on the order of hundreds of microseconds. The same unimolecular fragmentation reactions were observed for fragmentation that occurs in both regions. Kinetic energy releases were, of necessity, measured for fragmentation reactions that occurred before the electric sector. Metastable ion spectra reproduced in the following text were recorded for fragmentation reactions that occurred in the quadrupole cell.

The compounds used were obtained from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or Bachem Bioscience, Inc. (King of Prussia, PA). In a number of cases the *N*-acetyl derivatives of amino acids and peptides were prepared by reaction of the appropriate precursor that contained the free amino end with acetic anhydride in *t*-butanol. A portion of this reaction mixture was then added to the matrix used for FAB. The reference compound 2-phenyl-5-oxazolone was prepared by the reaction of hippuric acid with dicyclohexylcarbodiimide [23].

Results and Discussion

In our initial studies we examined the metastable ion fragmentation of a number of simple protonated amino acids and their amides and methyl esters. In all cases metastable peaks were observed for the nominal fragmentation reaction

where $\text{X} = \text{OH}, \text{NH}_2, \text{CH}_3\text{O}$. In no cases were metastable peaks observed that corresponded to elimination of HX alone. As the data in Table 1 indicate, reaction 4 yields flat-topped metastable ion peaks in all cases with $T_{1/2}$ values of 0.36–0.39 eV for protonated amino acids, 0.30–0.37 eV for the protonated amides, and 0.22–0.26 eV for the protonated methyl esters. Our $T_{1/2}$ of 0.38 eV for fragmentation of protonated leucine is in excellent agreement with the value of 0.39 eV

Table 1. Kinetic energy releases in fragmentation of protonated amino acids and derivatives

M of MH^+	Neutrals lost	$T_{1/2}$ (eV)
H-Leu-OH	$\text{CO} + \text{H}_2\text{O}$	0.38
H-Pro-OH	$\text{CO} + \text{H}_2\text{O}$	0.39
H-Phe-OH	$\text{CO} + \text{H}_2\text{O}$	0.36
H-Leu-NH ₂	$\text{CO} + \text{NH}_3$	0.30
H-Pro-NH ₂	$\text{CO} + \text{NH}_3$	0.37
H-Phe-NH ₂	$\text{CO} + \text{NH}_3$	0.30
H-Ile-NH ₂	$\text{CO} + \text{NH}_3$	0.36
H-Leu-OCH ₃	$\text{CO} + \text{CH}_3\text{OH}$	0.24
H-Pro-OCH ₃	$\text{CO} + \text{CH}_3\text{OH}$	0.24
H-Phe-OCH ₃	$\text{CO} + \text{CH}_3\text{OH}$	0.22
H-Ile-OCH ₃	$\text{CO} + \text{CH}_3\text{OH}$	0.26

reported earlier [9]. It is clear that protonated amino acid amides and methyl esters fragment by a pathway similar to that postulated for the amino acids themselves and outlined in Scheme I, and that the acylium ion initially formed fragments exothermically by elimination of CO .

The fragmentation of protonated peptides is quite different in that B ions, which are conventionally assigned an acylium structure, are observed as stable species. This is illustrated by the example of two simple tripeptides: H-Gly-Leu-Gly-OH and H-Leu-Gly-Gly-OH. The FAB mass spectra of these two tripeptides show (Table 2) MH^+ as the base peak and appreciable intensities for the B_2 ion that arise by elimination of neutral glycine from MH^+ . Figures 1 and 2 illustrate the low energy CID of these two protonated tripeptides, where the results are presented in the form of breakdown graphs that express the percent fragment ion abundances as a function of the center-of-mass collision energy. In both cases the dominant fragmentation route of MH^+ involves loss of neutral glycine to form a stable B_2 ion of m/z 171. The low intensity m/z 86 ion observed in Figure 1 represents the immonium ion derived from leucine and may arise by further fragmentation of the A_2 ion, m/z 143.

On the metastable ion time scale fragmentation of protonated tripeptides occurs, at least in part, by elimination of the C-terminus amino acid to give a stable B_2 ion. Several examples are given in Table 3. In all cases the metastable peak is Gaussian in shape with kinetic energy releases ($T_{1/2}$) in the range 0.02–0.03 eV. In addition, protonated dipeptides and derivatives such as the amides and methyl esters show fragmentation on the metastable ion time scale to give stable B_2 ions, reaction 5.

As illustrated by the data in Table 3, these fragmentation reactions, which involve elimination of H_2O , NH_3 , or CH_3OH from the MH^+ ion, show Gaussian shape metastable peaks with kinetic energy releases ($T_{1/2}$) in the range 0.02–0.03 eV. This behavior of $\text{RCH(NH}_2\text{)C(=O)NHCH(R')C(=O)X} \cdot \text{H}^+$ ions dif-

Table 2. FAB mass spectra of H-Gly-Leu-Gly-OH and H-Leu-Gly-Gly-OH

Ion	Intensity as % of base peak (m/z)	
	H-Gly-Leu-Gly-OH	H-Leu-Gly-Gly-OH
MH^+	46 (246)	42 (246)
B_2	18 (171)	9 (171)
A_2	52 (143)	3 (143)
Y_2	15 (189)	6 (133)
A_1		100 (86)
Other	100 (86)

Figure 1. Breakdown graph for protonated H-Gly-Leu-Gly-OH.

fers from that of $\text{RCH}(\text{NH}_2)\text{C}(=\text{O})\text{X} \cdot \text{H}^+$ ions, where the initial elimination of HX is followed by elimination of CO to give the immonium ion (reaction 3) in an overall reaction that yields a flat-topped metastable peak. Clearly the B_2 ion formed from the dipeptide derivatives has a much greater stability than the

Table 3. Kinetic energy releases in formation of B_2 ion

M of MH^+	HX lost	m/z , B_2 ion	$T_{1/2}$ (eV)
H-Gly-Leu-Gly-OH	H-Gly-OH	171	0.028
H-Gly-Leu-NH ₂	NH ₃	171	0.027
H-Gly-Leu-OH	H ₂ O	171	0.029
H-Ala-Ala-Gly-OH	H-Gly-OH	143	0.026
H-Ala-Ala-Ala-OMe	H-Ala-OMe	143	0.035
H-Ala-Ala-OMe	CH ₃ OH	143	0.022
H-Ala-Ala-Ala-OH	H-Ala-OH	143	0.025
H-Gly-Ala-Ala-OH	H-Ala-OH	129	0.031
H-Gly-Ala-Gly-OH	H-Gly-OH	129	0.024
H-Gly-Ala-NH ₂	NH ₃	129	0.025
H-Leu-Gly-Gly-OH	H-Gly-OH	171	0.033
H-Leu-Gly-NH ₂	NH ₃	171	0.032

acylium ion that would be formed from the amino acid derivatives.

In a number of cases the B_2 ions formed from the tripeptides and the dipeptide derivatives were of sufficient intensity in the FAB mass spectrum that they could be mass-selected and their metastable ion fragmentation reactions studied by tandem mass spectrometry. In all cases metastable fragmentation by elimination of CO was observed and, as summarized in Table 4, these metastable peaks were flat-topped with kinetic energy releases ($T_{1/2}$) of ~ 0.5 eV, except for the B_2 ion derived from H-Leu-Gly-X compounds, where a lower $T_{1/2}$ of 0.34–0.38 eV was measured.

Thus, we have a situation where dipeptide derivatives $\text{RCH}(\text{NH}_2)\text{C}(=\text{O})\text{NHCH}(\text{R}')\text{C}(=\text{O})\text{X}$, when protonated, fragment by loss of HX to give a B_2 ion with a kinetic energy release in the metastable ion fragmentation that is quite small ($T_{1/2} = 0.02$ – 0.03 eV). The resultant B_2 ions fragment, on the metastable ion time scale, by elimination of CO with a large kinetic release ($T_{1/2}$ in the range 0.3–0.5 eV). This result suggests a potential energy profile for fragmentation of B_2 such as shown in Figure 3, where a stable ion (S) passes through a reacting configuration (RC) that is higher in energy than the final products $\text{A}_2 + \text{CO}$. The relatively large kinetic energy release results because some of the energy released in the fragmentation step

Figure 2. Breakdown graph for protonated H-Leu-Gly-Gly-OH.

Table 4. Kinetic energy releases in fragmentation reaction $\text{B}_2 \rightarrow \text{A}_2 + \text{CO}$

Peptide deriv.	Nominal B_2 ion	$T_{1/2}$ (eV)
H-Gly-Leu-Gly-OH	H-Gly-Leu	0.49
H-Gly-Leu-NH ₂	H-Gly-Leu	0.48
H-Ala-Ala-Gly-OH	H-Ala-Ala	0.57
H-Ala-Ala-Ala-OMe	H-Ala-Ala	0.54
H-Ala-Ala-Ala-OH	H-Ala-Ala	0.51
H-Gly-Ala-Ala-OH	H-Gly-Ala	0.50
H-Gly-Ala-Gly-OH	H-Gly-Ala	0.50
H-Gly-Ala-NH ₂	H-Gly-Ala	0.53
H-Leu-Gly-Gly-OH	H-Leu-Gly	0.34
H-Leu-Gly-NH ₂	H-Leu-Gly	0.38

Figure 3. Potential energy profile for reaction that leads to a large kinetic energy release.

(the reverse activation energy) is partitioned into kinetic energy of the separating fragments [13, 14]. The question then becomes what is the stable structure S and what is the structure of the reacting configuration RC that leads to elimination of CO in an exothermic fragmentation reaction. The simplest postulate is that the reacting configuration is the acylium ion $\text{RCH}(\text{NH}_2)\text{C}(=\text{O})\text{NHCH}(\text{R}')\text{CO}^+$. As a candidate structure for the stable configuration S, we first considered the possibility that, on formation of B_2 , cyclization occurred through interaction with the N-terminus amino group as indicated in Scheme III for the H-Leu-Gly-X and H-Gly-Leu-X systems. This possibility can be tested experimentally because the cyclic intermediate can be prepared, as indicated in Scheme III, by protonation of the corresponding diketopiperazine: cyclo-Leu-Gly. (It may well be assumed that the added proton is mobile and that the same cyclic intermediate or mixture of intermediates is formed in all cases.)

Figure 4 compares the metastable ion mass spectrum for the m/z 171 (B_2) ion derived from H-Gly-Leu-Gly-OH and from H-Gly-Leu- NH_2 with the metastable ion mass spectrum for the m/z 171 ion derived by protonation of cyclo-Leu-Gly. Figure 5 shows the same comparison for the m/z 171 (B_2) ion derived from H-Leu-Gly-Gly-OH and from H-Leu-Gly- NH_2 . All spectra refer to decomposition reactions

Figure 4. Comparison of metastable ion mass spectra (quadrupole cell) of B_2 ion from H-Gly-Leu-X $\cdot \text{H}^+$ and protonated cyclo-Leu-Gly.

that occur in the quadrupole cell in the absence of collision gas. Clearly, the metastable ion mass spectra for the B_2 ions derived from the acyclic peptides are significantly different from the spectrum derived from the protonated cyclic dipeptide or diketopiperazine. Thus, we conclude that cyclization to a protonated diketopiperazine has not occurred on formation of the B_2 ion. This conclusion was reached previously by Wesdemiotis and co-workers [24] from a comparison of the high energy CID mass spectrum of the B_2 ion derived from protonated trialanine with the CID mass spectrum of the MH^+ ion of cyclo-Ala-Ala (3,6-dimethyl-2,4-piperazinedione).

In addition to the N-terminus amino group, the compounds we have examined contain a remote carbonyl group associated with the N-terminus amino acid and it is possible that stabilization of the incipient B_2 ion occurs by interaction with this carbonyl group. (Such an interaction has been suggested by Wesdemiotis and co-workers [24] and by Hunt and co-workers [25]. Tang et al. [26] also have proposed that B ions can have more than one structure with the "cyclic" form being more stable, although the cyclic structure was not clearly identified.) To probe for such an interaction experimentally we have eliminated the terminal amino group while retaining the carbonyl group by examination of the fragmentation reactions of protonated N-acetyl peptide and amino acid derivatives. Table 5 presents the FAB mass spectra of two N-acetyl dipeptides: Ac-Leu-Gly-OH and Ac-Ala-Ala-OH. In both

Scheme III

Figure 5. Comparison of metastable ion mass spectra (quadrupole cell) of B_2 ion from $H\text{-Leu-Gly-X}\cdot H^+$ and protonated cyclo-Leu-Gly.

cases significant ion signals are observed that correspond to loss of the C-terminus amino acid as a neutral to give the B ion with the nominal structure $\text{CH}_3\text{C}(=\text{O})\text{NHCH(R)}\text{CO}^+$ (m/z 156 for Ac-Leu-Gly-OH and m/z 114 for Ac-Ala-Ala-OH). Furthermore, as Figures 6 and 7 show, CID of the MH^+ ion yields the same ion as the dominant low energy fragmentation channel for Ac-Leu-Gly-OH, whereas protonated Ac-Ala-Ala-OH shows a more complicated breakdown graph that includes formation of the B ion as a significant fragment.

A variety of protonated species of the type $\text{CH}_3\text{C}(=\text{O})\text{NHCH(R)}\text{C}(=\text{O})\text{X}\cdot H^+$ were studied, where X was an amino acid, NH_2 , OH, or OCH_3 . In all cases metastable peaks were observed that corre-

Table 5. FAB mass spectra of Ac-Leu-Gly-OH and Ac-Ala-Ala-OH

Ion	Intensity as % base peak (m/z)	
	Ac-Leu-Gly-OH	Ac-Ala-Ala-OH
MH^+	45 (231)	100 (203)
B	45 (156)	80 (114)
A	51 (128)	52 (86)
Y_1		39 (90)
$A - \text{CH}_2\text{CO}$	100 (86)	
$MH^+ - \text{CH}_2\text{CO}$		10 (161)

Figure 6. Breakdown graph for protonated Ac-Leu-Gly-OH.

sponded to elimination of HX and formation of stable B-type ions. As indicated by the data in Table 6, these metastable peaks were Gaussian in shape with low kinetic releases ($T_{1/2}$ in the range 0.02–0.04 eV). In a number of cases the FAB mass spectra showed sufficient intensity of these B-type ions that they could be mass-selected and their metastable ion decomposition reactions could be studied. In all cases unimolecular elimination of CO was observed to give flat-topped metastable peaks with kinetic energy releases ($T_{1/2}$) in the range 0.44–0.53 eV (Table 7).

Figure 7. Breakdown graph for protonated Ac-Ala-Ala-OH.

derivatives as structures **d** and **e**. To provide further evidence we have undertaken ab initio calculations, described in the next section, on the simplest possible B ion, nominally $\text{HC}(=\text{O})\text{NHCH}_2\text{CO}^+$, and its fragmentation products.

Theoretical Calculations

The ab initio calculations were carried out by using the GAUSSIAN 92 series of programs [28] that employed restricted Hartree-Fock (RHF) formalism. Fully optimized geometries were calculated initially at the 3-21G split valence basis set [29] level of theory. These geometrical parameters were used subsequently for input to calculations of the fully relaxed geometries, total energies, and harmonic frequencies at the HF/6-31G** level of theory. Figures 9, 10, and 11 show the structures of the species for which calculations were performed, whereas Table 8 lists the total energies calculated at the 3-21G and 6-31G** levels of theory.

With full geometry optimization for the nominal ion $\text{HC}(=\text{O})\text{NHCH}_2\text{CO}^+$, the global energy minimum found was for the cyclic structure **g**.^{*} To obtain an energy for the acyclic structure **h** it was necessary to hold the torsional angle $\angle \text{C}_1\text{C}_3\text{-N}_2\text{C}_5$ at 180° . Under these conditions one imaginary frequency resulted, which indicated that the structure corresponded to a saddle point or transition state not a stable structure on the potential energy hypersurface.

The calculations fully support the hypothesis that

^{*}Calculations also were performed at the 3-21G level for the B_2 ion, nominally $\text{H}_2\text{NCH}_2\text{C}(=\text{O})\text{NHCH}_2\text{CO}^+$. The global energy minimum was found to correspond to the protonated oxazolone (**e**, Scheme IV; $\text{R} = \text{R}' = \text{H}$), which indicates that the presence of the terminal amino group does not alter the nature of the cyclization reaction that occurs.

Figure 9. Molecular structure of protonated oxazolone **g**.

Geometrical parameters

$r_{21} = 1.4329$	$\text{C}_3\text{C}_1\text{N}_2 = 110.5712$
$r_{13} = 1.4844$	$\text{O}_4\text{C}_3\text{C}_1 = 178.317$
$r_{34} = 1.0944$	$\text{C}_5\text{N}_2\text{C}_1 = 114.2553$
$r_{52} = 1.3802$	$\text{H}_6\text{N}_2\text{C}_5 = 121.8629$
$r_{62} = 0.9952$	$\text{O}_7\text{C}_5\text{N}_2 = 120.3467$
$r_{57} = 1.1841$	$\text{H}_8\text{C}_5\text{N}_2 = 114.0247$
$r_{85} = 1.0854$	$\text{Alfa} = 113.5587$
$r_{91} = 1.0894$	$\text{O}_4\text{C}_3\text{C}_1\text{N}_1 = 180.0^\circ$
$r_{101} = 1.0894$	$\text{C}_5\text{N}_2\text{C}_1\text{C}_3 = 180.0^\circ$

Figure 10. Molecular structure of acyclic acylium ion **h**.

B_2 ions, on formation, undergo cyclization and fragment by loss of CO, in accordance with the mechanism shown in Scheme V. Figure 12 schematically shows a cross section of the potential energy hypersurface as the potential energy profile for this fragmentation reaction as derived from the calculated energies. The acyclic acylium ion is calculated to be 1.49 eV higher in energy than the cyclic protonated oxazolone and 0.88 eV higher in energy than the final fragmentation products $\text{HC}(=\text{O})\text{N}^+\text{H}=\text{CH}_2 + \text{CO}$. This latter energy difference is consistent with the substantial kinetic energy releases ($T_{1/2} = 0.3\text{--}0.5$ eV) we have measured for unimolecular elimination of CO from the B ions studied.

Conclusions

The essential conclusion of the present study is that the B_2 ion observed as a stable species in peptide mass

Geometrical parameters

$r_{21} = 1.2709$	$\text{C}_3\text{N}_2\text{C}_1 = 121.7234$	$r = 1.1138$
$r_{32} = 1.4755$	$\text{N}_2\text{C}_3\text{O}_4 = 119.1523$	
$r_{34} = 1.1628$	$\text{H}_5\text{C}_3\text{N}_2 = 112.4894$	
$r_{35} = 1.0826$	$\text{H}_6\text{N}_2\text{C}_1 = 119.8404$	
$r_{26} = 1.0056$	$\text{H}_8\text{C}_1\text{N}_2 = 119.3515$	
$r_{81} = 1.0764$	$\text{H}_7\text{C}_1\text{N}_2 = 119.3515$	
$r_{71} = 1.0764$		

Figure 11. Molecular structure of fragmentation products.

Table 8. Total calculated energies

Species ^a	Energy (Hartree)	
	HF/3-21G	HF/6-31G**
g	-318.064769	-319.869665
h	-318.004654	-319.814845
i	-205.940195	-207.109059
j	-112.093299	-112.737877

^a g = protonated oxazolone (cf. Figure 9); h = acyclic acylium ion (cf. Figure 10); i = immonium ion (cf. Figure 11); j = carbon monoxide (cf. Figure 11).

spectra is not an acyclic acylium ion but has cyclized by interaction with the N-terminus carbonyl function to form a protonated substituted oxazolone. This protonated oxazolone fragments by ring opening to an acyclic acylium ion that exothermically eliminates CO. A considerable part of this exothermicity is released as kinetic energy of the separating fragments and leads to flat-topped metastable ion peaks.

We believe that the larger stable B ions, B₃, B₄, and so forth, also have cyclic structures although it is not clear whether cyclization has occurred by interaction

Scheme V **Figure 12.** Calculated potential energy profile for fragmentation of B ion.

with the next adjacent carbonyl function or with a more remote carbonyl function. This question is under study in our laboratory.

Acknowledgments

The authors are indebted to the Natural Sciences and Engineering Research Council (Canada) for financial support and to VG Analytical/Fisons Instruments, Inc. for the loan of the FAB source and fast-atom gun.

References

- Weinkam, R. J.; Gal, J. *Org. Mass Spectrom.* **1976**, *11*, 188.
- Ichikawa, H.; Harrison, A. G. *Org. Mass Spectrom.* **1978**, *13*, 389.
- Middlemiss, N. E.; Harrison, A. G. *Can. J. Chem.* **1979**, *57*, 2827.
- Harrison, A. G.; Kallury, R. K. M. *Org. Mass Spectrom.* **1980**, *15*, 277.
- Weinkam, R. J. *J. Am. Chem. Soc.* **1974**, *96*, 1032.
- Tsang, C. W.; Harrison, A. G. *J. Chem. Soc. Perkin Trans. 2* **1975**, 1718.
- LeClerq, P. M.; Desiderio, D. M. *Org. Mass Spectrom.* **1973**, *7*, 515.
- Tsang, C. W.; Harrison, A. G. *J. Am. Chem. Soc.* **1976**, *98*, 1301.
- Bouchoux, G.; Bourcier, S.; Hoppilliard, Y.; Mauriac, C. *Org. Mass Spectrom.* **1993**, *28*, 1064.
- Kulik, W.; Heerma, W. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 419.
- Bouchonnet, S.; Denhez, J. P.; Hoppilliard, Y.; Mauriac, C. *Anal. Chem.* **1992**, *64*, 743.
- Benoit, F. M.; Harrison, A. G. *J. Am. Chem. Soc.* **1977**, *99*, 3980.
- Cooks, R. G.; Beynon, J. H.; Caprioli, R. M.; Lester, G. R. *Metastable Ions*; Elsevier: New York, 1973.
- Holmes, J. L.; Terlouw, J. K. *Org. Mass Spectrom.* **1980**, *15*, 383.
- Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
- Hunt, D. F.; Yates, J. R., III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6233.
- Biemann, K.; Martin, S. *Mass Spectrom. Rev.* **1987**, *6*, 75.
- Biemann, K. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99.
- Biemann, K. In *Mass Spectrometry Methods in Enzymology*, Vol. 193; McCloskey, J. A., Ed.; Academic Press: San Diego, CA, 1990; Chaps. 18, 25.
- Biemann, K. In *Biological Mass Spectrometry Present and Future*; Matsuo, T.; Caprioli, R. M.; Gross, M. L.; Seyama, Y., Eds.; Wiley: New York, 1993.
- Tang, X.-J.; Boyd, R. K. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 651.
- Harrison, A. G.; Mercer, R. S.; Reiner, E. J.; Young, A. B.; Boyd, R. K.; March, R. E.; Porter, C. J. *Int. J. Mass Spectrom. Ion Processes* **1986**, *76*, 13.
- (a) Strukov, I. T. *Zh. Obshch. Khim.* **1959**, *29*, 2359 (*Chem. Abstracts* **1960**, *54*, 9889); (b) Grahl-Nielsen, O.; Solheim, E. *Anal. Chem.* **1975**, *47*, 333.
- Cordero, M. M.; Houser, J. J.; Wesdemiotis, C. *Anal. Chem.* **1993**, *65*, 1594.
- Arnott, D.; Kottmeir, D.; Yates, N.; Shabanowitz, J.; Hunt, D. F. *Proceedings of the 42nd ASMS Conference on Mass Spectrometry*; Chicago, June, 1994; p 470.
- Tang, X.-J.; Thibault, P.; Boyd, R. K. *Anal. Chem.* **1993**, *65*, 2824.

27. Rao, R. S.; Filler, R. In *Oxazoles. The Chemistry of Heterocyclic Compounds*, Vol. 45; Turchi, I. J., Ed.; Wiley: New York, 1986; Chap. 4.
28. Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. A.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian 92; Gaussian: Pittsburgh, PA, 1992.
29. Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939.