Metastable Decomposition of Peptide $[M + H]^+$ Ions via Rearrangement Involving Loss of the C-Terminal Amino Acid Residue

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A novel fragmentation of metastable peptide $[M + H]^+$ ions is described. Loss of the C-terminal amino acid residue is accompanied by retention of one of the carboxyl oxygens, as judged by ^{18}O -labeling. The retained ^{18}O label is located at the new C-terminus. Sequential mass spectrometric analyses indicate that the structure of the first-generation product ion is indistinguishable from that of the $[M + H]^+$ ion of the peptide with one fewer amino acid residues. Thus, for example, the metastable decompositions of ions of m/z 904 are similar whether they correspond to des-Arg⁹-bradykinin $[M + H]^+$ ions or to fragments derived from bradykinin $[M + H]^+$ ions. No corresponding rearrangements have been observed for peptides with C-terminal amide or ester functions. The mechanism of this fragmentation may be considered to be analogous to that previously suggested for fragmentations of $[M + \text{alkali metal cation}]^+$ ions. For the examples of bradykinin and related peptides, the rearrangement is strongly promoted when arginine is the amino acid residue lost. The same fragmentation is also favored by the presence of an arginine residue at or near the N-terminus. The strong influence of peptide amino acid composition, including residues remote from the C-terminus, on the prevalence of this fragmentation suggests mechanistic complexities that require further elucidation. (*J Am Soc Mass Spectrom* 1990, 1, 249-257)

The combination of fast atom bombardment (FAB) and related ionization techniques with tandem mass spectrometry (MS/MS) is making an increasingly significant contribution to the elucidation of the structures of peptides [1-4]. While the sequencing of reasonably pure samples of unmodified peptides is usually best achieved by methods based on Edman sequencing, the advantages of the MS/MS approach include the ability to sequence N-blocked peptides and other structural modifications, together with a capability for the handling of mixtures.

In MS/MS analyses, ion species selected by the first mass analyzer are allowed to fragment spontaneously (when metastable precursors are involved) or are induced to do so following collision with an inert gas such as helium or argon. The conditions under which collisional activation and decomposition occur are markedly different in different tandem instrument configurations. Thus, for example, in four-sector instruments, precursor ions subjected to collisional activation are generally of high (kiloelectron-volt) kinetic energy. In contrast, low (electronvolt) ki-

netic energy ions are the precursors in MS/MS experiments performed with instruments that incorporate an rf-only quadrupole as the decomposition region (triple quadrupoles and hybrid sector/quadrupole instruments). An adequate comparison of the different decomposition regimes involves a complex of interrelated parameters [5, 6]. For example, in addition to the influence on the maximum energy uptake per collision, the different energy regimes have a substantial effect on the time scale of the activation and decomposition processes. Thus, the contributions of metastable decay to the fragmentation pattern observed under conditions of low-energy collision-activated decomposition (CAD) in the quadrupole decomposition region of a hybrid or triple quadrupole instrument are expected to substantially exceed the contributions of the same processes to a high-energy CAD spectrum [7]. This is arguably a disadvantage in that low-energy decompositions may include prominent rearrangement processes rather than readily interpreted simple fragmentations, which are more likely to be favored following high-energy CAD. Nevertheless, the high sensitivity of detection of metastable decompositions together with some advantages of hybrids and triple quadrupoles with respect to versatility of operation encourage a thorough study of the decomposition processes observed under low-energy conditions. Furthermore, the rapid emergence of electrospray as a powerful ionization technique for biomolecules [8-10] and the simplic-

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ity of its coupling to quadrupole mass analyzers (and therefore triple quadrupoles) suggest the future importance of understanding decompositions observed under low-energy regimes.

Studies of the high-energy CAD of peptide $[M + H]^+$ ions using four-sector instruments (for example, refs. 1-3) have established the preponderance of product ions arising by cleavages of the peptide chain (designated, according to the Roepstorff convention [11], as A-, B-, or C-type ions for charge retention on the N-terminal fragment and X-, Y-, or Z-type ions for charge retention on the C-terminal fragment). Other prominent product ions arise via additional cleavage of the side chains of amino acid residues proximal to the site of peptide cleavage [12]. The quality of product ion data obtained by low-energy CAD appears to be strongly analyte-dependent; diagnostic fragments include simple sequence ions together with "midchain fragments" corresponding to chain cleavage at two points with charge retention on the excised fragment [4, 7, 13]. [Such fragments may be designated as, for example, $(A_m Y_n)$ or $(B_m Y_n)$, by extension of the simple nomenclature.] The contribution of midchain fragments (which may confuse interpretation unless recognized as such) appears to be generally minor in high-energy CAD spectra. Side-chain-specific ions, on the other hand, are observed only at higher collision energies [12, 14].

As part of a program of study of the MS/MS of FAB-desorbed peptides using a hybrid instrument, we recently noted a novel fragmentation of $[M + H]^+$ ions derived from angiotensins II and III, observed under conditions of low-energy CAD [15]. Loss of the C-terminal amino acid residue was accompanied by retention of one of the carboxyl oxygens (as judged by ^{18}O labeling). The resulting product ion of an n -residue peptide may therefore be designated $[B'_{n-1} + \text{OH}]$. This fragmentation is formally equivalent to a fragmentation (yielding $[B_{n-1} + \text{cation} + \text{OH}]$) of alkali metal-cationized peptides observed by Renner and Spiteller [16], by Tang et al. [17], and by Grese et al. [18] and studied in detail by the last group. The same fragmentation of $[M + \text{Na}]^+$ has recently been described by Kulik et al. [19], in apparent ignorance of the earlier reports. These authors also noted a single example of the equivalent rearrangement of a peptide $[M + H]^+$ ion. Following our recognition that the principal product ion in the metastable decomposition spectrum of bradykinin $[M + H]^+$ was apparently attributable to the same rearrangement, we have undertaken a more extensive study of this fragmentation.

Experimental Procedures

The peptides bradykinin, des-Arg⁹-bradykinin, Lys¹-bradykinin, Lys⁰-bradykinin, Tyr⁰-bradykinin, angiotensin II, angiotensin III, substance P, substance P fragment 2-11, substance P Met-OH¹¹, proctolin, Leu-

enkephalin, Arg-Gly-Asp, and Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly were purchased from Sigma Chemical Company (St. Louis, MO). *N*-Methyl-bis(trifluoroacetamide) (MBTFA, Pierce, Rockford, IL), heptafluorobutyric acid (HFBA, Peninsular Chemresearch, Inc., Gainesville, FL), and phenylisothiocyanate (PITC, ICN Pharmaceuticals, Inc., Plainview, NY) were used without further purification. Acetylacetone (Fisher, Fair Lawn, NJ), triethylamine (Fisher), methyl iodide (ICN Pharmaceuticals), acetyl chloride (J. T. Baker, Phillipsburg, NJ), and pyridine (Baker) were redistilled under nitrogen prior to use. C₁₈ Sep-Paks were purchased from Waters Associates (Milford, MA).

Dimethylpyrimidylornithyl Derivatives of Arginyl Peptides

The dimethylpyrimidylornithyl (DMPO) derivatives of bradykinin, Lys¹-bradykinin, des-Arg⁹-bradykinin, and proctolin were prepared by using a procedure similar to that described by Morris and co-workers [20]. To each dry peptide sample (approximately 100 μg) were added 20 μL of water, 40 μL of ethanol, and 20 μL of triethylamine, and the samples were capped under argon and vortexed. Acetylacetone (40 μL) was added to each sample under a stream of argon, and the samples were capped under argon, vortexed, and heated at 100 $^\circ\text{C}$ for 1 h. After evaporation under nitrogen, to each oily sample were added 100 μL of water and 10 μL of glacial acetic acid, and the samples were again heated at 100 $^\circ\text{C}$ for 12 min to hydrolyze Schiff's bases. The cool samples were extracted three times with 400 μL of diethyl ether, and the aqueous samples were evaporated under a stream of nitrogen. The residual oily material was dissolved in methanol (25-50 μL) prior to FAB/MS/MS analysis, for which 1-2- μL portions were used.

^{18}O -Exchange

Bradykinin, Lys⁰-bradykinin, and proctolin were labeled at their C-termini with ^{18}O by acid-catalyzed isotopic exchange. Approximately 100 μg of each peptide was dissolved in 25 μL of ^{18}O water (98% isotopic purity, Cambridge Isotope Laboratories, Woburn, MA). Hydrochloric acid (12 M, 1 μL) was added to each sample, and the mixtures were allowed to stand at room temperature for 72 h. A portion (1-2 μL) of each of these mixtures was analyzed by FAB/MS/MS.

Methyl Ester Preparation

The methyl esters of bradykinin, $^{18}\text{O}_2$ bradykinin and substance P Met-OH¹¹, were prepared by acid-catalyzed esterification. Methanolic HCl (3M) was freshly generated by slowly adding 440 μL of acetyl chloride (redistilled) to 1.56 mL of methanol under ni-

trogen at 0 °C and allowing the mixture to react for 30 min. Approximately 0.5 mL of this solution was added to each dry peptide sample (200 µg), and the mixtures were vortexed and allowed to stand at room temperature for 1 h, followed by evaporation under nitrogen to dryness. The methylated samples were redissolved in 50 µL of methanol prior to FAB/MS/MS analysis.

N-Trifluoroacetylation

Lys¹-bradykinin, bradykinin-bis-DMPO, and Leu-enkephalin were *N*-trifluoroacetylated with *N*-methylbis(trifluoroacetamide) (MBTFA). To the dry samples (1–200 µg) were added 100 µL of chloroform or pyridine and 30 µL of MBTFA, and the samples were allowed to stand at room temperature for 1–4 h. Similarly, [¹⁸O₂]bradykinin (50 µg) was dissolved in 30 µL of methanol; 200 µL of MBTFA was added, and the sample was allowed to stand at room temperature for 90 min. All samples were evaporated to dryness under nitrogen, with repeated addition of several drops of methanol followed by reevaporation under nitrogen.

Manual Edman Degradation

Des-Arg¹-bradykinin, bradykinin fragment 2-8, and substance P Met-OH¹¹ fragment 2-11 were prepared by single-stage manual Edman degradation of bradykinin, des-Arg⁹-bradykinin, and substance P Met-OH¹¹, respectively, using a modified version of the method of Tarr [21, 22]. Phenylisothiocyanate (PITC) reagent was prepared as ethanol-triethylamine-water-PITC 7:1:1:1 by volume. Approximately 100 µg of each peptide sample was dissolved in 20 µL of PITC reagent and heated at 50 °C for 10 min. The samples were then evaporated under nitrogen and dried briefly in vacuo. Water (10 µL) was added, and the samples were extracted with 200 µL of hexane-ethyl acetate 15:1, followed by extraction with 200 µL of hexane-ethyl acetate 7:1. After the aqueous samples were evaporated to dryness under nitrogen, 10 µL of 12 M HCl was added and the samples were again heated at 50 °C for 5 min, evaporated under nitrogen, and dried in vacuo for 1 h to remove excess HCl. Methanol (20 µL) was then added, and the samples were heated at 50 °C for 3 min, evaporated under nitrogen, and finally redissolved in 25 µL of methanol.

N-Trimethyl-Leu-enkephalin

Leu-enkephalin (1 mg) was dissolved in 2 mL of 50% aqueous methanol, and the pH was adjusted to 10.5 with 0.3 N methanolic KOH. Methyl iodide (5 µL) was added, and the mixture was allowed to stand at room temperature for 7 h, after which the solution was acidified with 100 µL of heptafluorobutyric acid (HFBA) [23]. The solution was then evaporated under nitrogen until the volume was reduced to approximately

0.5 mL to remove methanol, and the sample was diluted with 2 mL of aqueous 1% HFBA. The diluted sample was then loaded onto a C₁₈ Sep-Pak that had previously been charged with 10 mL of acetonitrile and washed with 10 mL of aqueous 0.1% HFBA [24]. After it was loaded, the sample was desalted with 10 mL of aqueous 0.1% HFBA and then eluted from the Sep-Pak with 4 mL of 80% acetonitrile-water-0.1% HFBA. The eluate was evaporated to dryness under nitrogen. Although the overall yield from this reaction was quite low, sufficient *N*-trimethyl-Leu-enkephalin was generated to permit MS/MS analysis.

Mass Spectrometry

Metastable decomposition analyses were performed on a VG ZAB SEQ hybrid mass spectrometer (VG Analytical Ltd., Manchester, UK) with the configuration BEqQ (B = magnetic sector, E = electric sector, q = rf-only quadrupole, Q = quadrupole mass filter). The sample was introduced with a conventional fast atom bombardment (FAB) probe. The liquid matrix used was glycerol. The primary FAB beam was xenon atoms with energies of 8 keV. The float potential of the rf-only quadrupole was set to optimize the transmission of the precursor ion (i.e., 5–10 V below the ion source potential). Precursor ion resolution was at least unity. The quadrupole mass analyzer was operated at 1–2 u resolution. The precursor ions were selected under mass spectrometer hardware control, and the quadrupole was scanned under control of the VG 11/250J data system. Product ion signals were acquired with the data system in "multichannel analyzer" (MCA) mode. Scans were generally of 10 s duration, and 5–15 scans were accumulated. Longer scan times (up to 45 s) were occasionally employed when the precursor ion signal was weak. Mass assignments of the product ion spectra were based on a simple two-point linear calibration of the quadrupole mass scale. Sequential product ion analyses employed a previously described modification that allowed independent manual control of the electric sector potentials [15]. In this mode of operation, precursor ions selected by B were allowed to decompose in the field-free region between B and E. First-generation product ions were selected by adjustment of E (mass resolution 150) and allowed, in turn, to decompose in q; final product ions were recorded by scanning Q as described above.

Results and Discussion

The spectrum of metastable decompositions of $[M + H]^+$ ions derived from bradykinin is dominated by an ion of m/z 904 [7]. Although this ion might be attributed to a Y₈^{''} structure, the spectrum of the [¹⁸O₂]-labeled analogue (Figure 1a) shows that it is shifted by 2 u to m/z 906, indicating retention of only one

Figure 1. Metastable decomposition spectra of $[\text{M} + \text{H}]^+$ ions derived by FAB of (a) $[^{18}\text{O}_2]\text{bradykinin}$ (with an inset showing the expanded region of the $[\text{B}'_8 + \text{OH}]$ ion); (b) the N-terminal TFA derivative of $[^{18}\text{O}_2]\text{bradykinin}$.

of the C-terminal carboxyl oxygens. A minor ion of m/z 908 is also observed in the spectrum of the labeled analogue and is attributed to the Y''_8 fragment. In the spectrum of the N-trifluoroacetyl (TFA) derivative of $[^{18}\text{O}_2]\text{bradykinin}$ (Figure 1b), the m/z 908 ion is unchanged, whereas m/z 906 is shifted to m/z 1002 (consistent with the ion's assignment as a fragment retaining the N-terminus). Thus the ion of m/z 904 in the metastable decomposition spectrum of bradykinin may be attributed almost entirely to the $[\text{B}'_{n-1} + \text{OH}]$ fragmentation (in this case, $[\text{B}'_8 + \text{OH}]$) previously observed in the low-energy CAD spectrum of angiotensins II and III [15].

A number of bradykinin analogues and other peptides have been analyzed to assess the prevalence of this rearrangement of $[\text{M} + \text{H}]^+$ ions and to determine the influence of structural variations on the relative abundance of the rearrangement product. Table 1 lists the peptides included in this study and indicates the abundance (relative to total product ion current and to the corresponding B_{n-1} ion) of the $[\text{B}'_{n-1} + \text{OH}]$ ion in each case. All the data shown in Table 1 refer to decompositions of metastable precursor ions; the float potential of the rf-only quadrupole (determining the residence time of the precursor ion in the decomposition region) was set to maximize the transmission of the precursor ion (generally 5–10 V below ion source potential). The estimates of relative abundances shown in Table 1 are necessarily approximate. Replicate analyses, however, suggested good reproducibility. Thus, the abundance of the rearrangement ion (as a percentage of total product ion current) for the example of bradykinin was determined to be 60 ± 2 (SD; $n = 5$).

Several bradykinin analogues were analyzed to assess the effect of minor structural variations on the prominence of this metastable decomposition. No equivalent rearrangement ion (corresponding to either $[\text{B}'_8 + \text{OH}]$ or $[\text{B}'_8 + \text{OCH}_3]$) was observed during analysis of bradykinin methyl ester (Table 1). Removal of the C-terminal arginine residue (Arg^9) of bradykinin markedly reduced the abundance of the rearrangement ion (Figure 2a), whether this was expressed as a percentage of the total product ion signal or relative to the corresponding simple B_{n-1} ion (Table 1). This suggests that loss of an arginine residue is particularly favored for this rearrangement.

Perhaps more surprising, a pronounced reduction in the prominence of the rearrangement was observed when the N-terminal arginine residue (Arg^1) of bradykinin was removed by Edman degradation (Table 1). Further diminution of the significance of the rearrangement followed removal of the second arginine residue (Arg^2). Conversion of bradykinin to the bis-dimethylpyrimidylornithyl derivative (DMPO; Scheme I) reduced the abundance of the $[\text{B}'_8 + \text{OH}]$ ion; further reduction was observed after N-terminal trifluoroacetylation. Similarly, DMPO derivatization of the Arg^1 residue in des- Arg^9 -bradykinin markedly reduced the abundance of the rearrangement ion by comparison with the underivatized peptide. Thus, the structure of the peptide N-terminus is apparently influential in determining the relative abundance of this C-terminal rearrangement ion derived from bradykinin and its analogues. This unexpected conclusion was further strengthened by analysis of Lys^1 -bradykinin, where the N-terminal arginine residue is substituted

Table 1. Decomposition of metastable peptide $[M + H]^+$ ions to yield $[B'_{n-1} + OH]^+$ rearrangement ions

Peptide	Sequence ^a	Derivative ^b	$[M + H]^+$ <i>m/z</i>	$[B'_{n-1} + OH]^+$		
				<i>m/z</i>	<i>i/Σ_i</i> (%) ^c	<i>i/i(B'_{n-1})</i> ^d
Bradykinin	RPPGFSPFR	—	1060	904	60	13
		$[^{18}O_2]$	1064	906	60	13
		Methyl ester	1074	918 ^e	n.d. ^f	n.d. ^f
		DMPO	1188	968 ^g	6	1
		$[^{18}O_2]$ DMPO	1192	970	6	1
		DMPO, <i>N</i> -TFA	1286	1064	2	0.5
		$[^{18}O_2]$ methyl ester ^h	1076	918 ^e	n.d.	n.d.
Lys ¹ -bradykinin	KPPGFSPFR	—	1160	1002	53	28
		DMPO	1032	876	9	4
		Bis- <i>N</i> -TFA	1096	876	3	1
Lys ⁰ -bradykinin	KRPPGFSPFR	—	1224	1068	n.d.	n.d.
		$[^{18}O_2]$	1188	1032	60	16
Tyr ⁰ -bradykinin	YRPPGFSPFR	—	1192	1034	60	16
Des-Arg ⁹ -bradykinin	RPPGFSPF	—	1223	1067	60	18
		DMPO	904	757	10	5
Des-Arg ¹ -bradykinin	PPGFSPFR	—	968	821	0.5	0.5
Bradykinin, fragment 2-8	PPGFSPF	—	904	748	6	3.5
Angiotensin III	RVYIHPF	—	748	601	1.5	0.5
Angiotensin II	DRVYIHPF	—	931	784	7	3.5
Substance P	RPKPQQFFGLM (NH ₂)	—	1046	899	8	2
Substance P, fragment 2-11	PKPQQFFGLM (NH ₂)	—	1347	1217	n.d.	n.d.
Substance P Met-OH ¹¹	RPKPQQFFGLM	—	1191	1061	n.d.	n.d.
Substance P Met-OH ¹¹ , frag. 2-11	PKPQQFFGLM	—	1348	1217	2	2
		Methyl ester	1362	1231	n.d.	n.d.
Tridecapeptide	RRLLIEDAEYAARG	—	1192	1061	2	0.1
Proctolin	RYLPT	—	1519	1462	n.d.	n.d.
Tripeptide	RGD	—	649	548	8	5
		$[^{18}O_2]$	653	550	8	5
		DMPO	713	612	1	1
Leu-enkephalin	YGGFL	—	347	232	6	.5
		DMPO	411	296	3	.25
			556	443	1.5	.03

^a Single-letter nomenclature for amino acids.^b $[^{18}O_2]$, label incorporated in C-terminal carboxyl group; DMPO, dimethylpyrimidylornithyl; *N*-TFA, *N*-trifluoroacetyl.^c Relative abundance of rearrangement ion expressed as a percentage of the total product ion current.^d Abundance of the rearrangement ion expressed relative to the corresponding B_{n-1} ion.^e *m/z* values quoted are for $[B'_{n-1} + OCH_3]^+$ ions; $[B'_{n-1} + OH]^+$ ions were also undetectable in these examples.^f n.d., not detected.^g $[B'_3 + OH]$ isobaric with Y_8^{+} ; relative abundance values for the rearrangement ion calculated from data for the $[^{18}O_2]$ analogue.^h Acid-catalyzed methyl ester formation results in loss of one ^{18}O .

by the less strongly basic lysine; the rearrangement ion was observed at reduced abundance. Addition of lysine or tyrosine residues to the N-terminus of intact bradykinin had no effect on the prevalence of the rearrangement ion (Table 1). The influence of the amino acid composition remote from the site of rearrangement does not appear simply to reflect the propensity for charge localization distant from the C-terminus. Thus, metastable decomposition of the molecular cation of *N*-trimethyl leucine-enkephalin (a quaternary ammonium derivative) gave the $[B'_4 + OH]$ rearrangement ion with an abundance (relative to the total product ion current) of 1.5%, which is similar to

that observed for decomposition of leucine-enkephalin $[M + H]^+$ ions.

The reverse-geometry sector/quadrupole hybrid instrument configuration (BeqQ) provides the facility for sequential mass spectrometry (MS/MS/MS) using the decomposition regions between B and E and in q [25, 26]. Our previous sequential product ion analyses of angiotensin III established that low-energy fragmentation of the $[B'_6 + OH]$ first-generation product ion yielded principally the $[B'_5 + OH]$ fragment [15]. This finding suggested that the product of rearrangement possessed a structure corresponding to the $[M + H]^+$ ion of the peptide with one fewer amino

Scheme 1

acid residues. This conclusion was further substantiated in the present work by the direct comparison of the metastable decomposition spectra of ions of m/z 904 corresponding to either des-Arg⁹-bradykinin $[\text{M} + \text{H}]^+$ ions formed in the ion source or $[\text{B}_8' + \text{OH}]$ ions formed in the second field-free region (between B and E) by decomposition of metastable bradykinin $[\text{M} + \text{H}]^+$ ions. The spectra obtained (Figure 2), which include a wealth of structural information, show remarkable similarities in fragmentation (bear in mind the likely differences in internal energies of the m/z

904 species) and provide a strong indication of identity of structure.

The metastable decomposition product ion spectrum of bradykinin bis-DMPO $[\text{M} + \text{H}]^+$ ions (Figure 3) included a number of diagnostic fragment ions of comparable abundance, in contrast to the corresponding spectrum of the native peptide, which was dominated by the $[\text{B}_8' + \text{OH}]$ ion (Figure 1a; Table 1). The spectrum illustrates the remarkable extent of structural information that may be obtained, without recourse to collisional activation of precursor ions, when metastable

Figure 2. Metastable decomposition spectra of ions of m/z 904 corresponding to (a) des-Arg⁹-bradykinin $[\text{M} + \text{H}]^+$ ions formed in the ion source and (b) $[\text{B}_8' + \text{OH}]$ ions formed in the second field-free region by decomposition of bradykinin $[\text{M} + \text{H}]^+$ ions. The structural assignments are indicated.

Figure 3. Metastable decomposition product ion spectrum of $[M + H]^+$ ions derived by FAB of bradykinin bis-DMPO. The structural assignments are indicated.

decompositions are allowed to occur on the time scale determined by the hybrid MS/MS experiment. Both B and Y'' series ions are prominent in the spectrum of the derivatized peptide. The product ion of m/z 968 is attributable to the $[B'_8 + OH]$ ion and/or the isobaric Y''_8 structure. The corresponding product ion spectrum derived from $[M + H]^+$ ions of the bis-DMPO derivative of $[^{18}O_2]$ bradykinin included m/z 970 ($[B'_8 + OH]$) and m/z 972 (Y''_8) fragments in the ratio 1:6.

A number of peptides unrelated in structure to bradykinin have also been analyzed (Table 1) to assess the occurrence of the same rearrangement ion ($[B'_{n-1} + OH]$). The ion is not observed in the metastable decomposition spectra of the $[M + H]^+$ ions of substance P or its 2-11 fragment, both of which incorporate C-terminal amide groups. The rearrangement is observed, albeit at low abundance, with the C-terminal carboxyl analogues. As observed for bradykinin, methyl ester formation at the C-terminus of these analogues abolishes the rearrangement; that is, neither $[B'_{n-1} + OH]$ nor $[B'_{n-1} + OCH_3]$ ions are observed. The $[B'_{n-1} + OH]$ ion was not observed in the product ion spectrum of $[M + H]^+$ ions of the tridecapeptide RRLIEDAEYAARG. This parallels the observation of Grese et al. [18] with $[M + Li]^+$ ions, where no rearrangement was found with peptides containing an arginine residue at the $n - 1$ position.

Additional examples of the occurrence of the $[B'_{n-1} + OH]$ ion include peptides with three and five amino acid residues (Table 1). Figure 4 shows the

metastable decomposition spectrum of the $[M + H]^+$ ions of proctolin (RYLPT). In addition to the $[B'_4 + OH]$ ion of m/z 548 and a variety of simple cleavage products, a second-generation rearrangement ion of m/z 451 ($[B'_3 + OH]$) is present. The corresponding spectrum obtained for $[^{18}O_2]$ proctolin (not shown) indicates that the $[B'_4 + OH]$ was shifted principally by 2 u to m/z 550. The incorporation of ^{18}O into the second-generation rearrangement ion ($[B'_3 + OH]$) derived from proctolin $[M + H]^+$ ions was consistent with the location of $^{18}O_1$ in the C-terminal carboxyl group of the first-generation product ($[B'_4 + OH]$).

In order to assess parallels between the formation of $[B'_{n-1} + OH]$ and $[B_{n-1} + Na + OH]$ [16-18] ions, the product ion spectra of metastable $[M + H]^+$ and $[M + Na]^+$ ions were compared for several peptides (Table 2). The rearrangement ion is predominant in the product ion spectrum of leucine-enkephalin $[M + Na]^+$ [15, 18] but of minor importance in the spectrum of the products of $[M + H]^+$. Conversely, $[B_{n-1} + cation + OH]$ is of greater relative abundance in the product ion spectrum of $[M + H]^+$ than in that of $[M + Na]^+$ for bradykinin. Evidently, the structural features that predispose toward these nominally similar rearrangements are not the same.

It is difficult to determine the precise mechanism of formation of $[B'_{n-1} + OH]$ ions (if, indeed, a single mechanism applies); nevertheless, the pertinent experimental observations (from the present study and our previous preliminary work [15]) may be summarized

Figure 4. Metastable product ion spectrum of proctolin $[M + H]^+$ ions. The structural assignments are indicated; the origin of the m/z 587 peak is not yet known.

Table 2. Rearrangement ions by metastable decomposition of $[M + H]^+$ and $[M + Na]^+$

Precursor ion	Rearrangement ion: m/z (rel. abundance)*	
	$[B'_{n-1} + OH]$	$[B_{n-1} + Na + OH]$
Bradykinin		
$[M + H]^+$	904(60)	—
$[M + Na]^+$	—	926(20)
$[^{18}O_2]$ bradykinin		
$[M + H]^+$	906(60)	—
$[M + Na]^+$	—	928(20)
Angiotensin III		
$[M + H]^+$	784(7)	—
$[M + Na]^+$	—	806(5)
Leu-enkephalin		
$[M + H]^+$	443(2)	—
$[M + Na]^+$	—	465(90)
Leu-enkephalin <i>N</i> -TFA		
$[M + H]^+$	539(1)	—
$[M + Na]^+$	—	561(75)

* Expressed as a percentage of the total product ion current.

as follows.

1. Formation of the product ion occurs with retention of one of the C-terminal carboxyl oxygens, as judged by several ^{18}O -labeling experiments.
2. The structure of the rearrangement ion is apparently the same as that of the $[M + H]^+$ ion of the peptide with one fewer amino acid residues. Thus, the fragmentation of $[B'_{n-1} + OH]$ ions derived from bradykinin $[M + H]^+$ (determined by MS/MS/MS) parallels the fragmentation of des-Arg⁹-bradykinin $[M + H]^+$ ions. Furthermore, the evidence from analyses of $[^{18}O_2]$ angiotensin III [15] and $[^{18}O_2]$ proctolin indicates that the original ^{18}O label is located at the new C-terminus.
3. Formation of the rearrangement ion is observed (in the examples studied here) only for peptides with a C-terminal carboxyl group.
4. The prevalence of this rearrangement is dependent on which amino acid residue is lost and also on other structural features, including the composition of the peptide remote from the C-terminus.

In seeking to explain the $[B_{n-1} + Na + OH]$ rearrangement ion observed as a decomposition product of peptide $[M + Na]^+$ ions, Tang et al. [17] proposed chelation of the metal ion between two carbonyl oxygens. To accommodate the failure to observe rearrangement of methyl ester $[M + Na]^+$ ions, Grese et al. [18] proposed an alternative mechanism involving decomposition of the metal salt of the peptide in which the site of protonation was remote from the C-terminus. The $[B'_{n-1} + OH]$ rearrangement ion derived from peptide $[M + H]^+$ ions may formally be considered to arise via the mechanism shown in Scheme II, which resembles the mechanism proposed by Grese et al. [18] for the fragmentation of peptide $[M + Li]^+$

ions. This mechanism is consistent with the observations of predominant retention of a single ^{18}O label and its location on the new C-terminus. It is further consistent with the apparent requirement for a C-terminal carboxyl group (as opposed to a methyl ester or amide functionality). The promotion of the rearrangement by a C-terminal arginine might be attributed to stabilization (possibly via a cyclic structure)

Scheme II

of the major neutral fragment that is lost. Others of our observations, however, are not readily explained by the simple mechanism shown in Scheme II, suggesting a need for caution in drawing detailed mechanistic conclusions. Thus, the apparent influence of the N-terminal structure (or at least of structural features remote from the site of rearrangement) would appear to require intramolecular interactions that involve folding of the peptide chain.

Despite the uncertainties of mechanistic interpretation, recognition of this rearrangement ion in the metastable decomposition and low-energy CAD spectra of peptide $[M + H]^+$ ions may contribute significantly to structural analysis, via ready identification of the C-terminal amino acid residue. Continuing development of strategies (incorporating isotopic labeling and selective derivatizations) for use in conjunction with metastable and low-energy CAD analyses of peptides and related compounds will provide improved facilities for the characterization of unknowns.

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References

1. Biemann, K.; Martin, S. A. *Mass Spectrom. Rev.* **1987**, *6*, 1-76.
2. Biemann, K.; Scoble, H. A. *Science* **1987**, *237*, 992-998.
3. Carr, S. A.; Anderegg, R. J.; Hemling, M. E. In *The Analysis of Peptides and Proteins by Mass Spectrometry*; McNeal, C. J., Ed.; Wiley: New York, 1988; p 95.
4. Hunt, D. F.; Yates, J. R.; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233-6237.
5. Dawson, P. H.; Douglas, D. J. In *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley: New York, 1983; p 125.
6. Todd, P. J.; McLafferty, F. W. In *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley: New York, 1983; p 149.
7. Gaskell, S. J.; Reilly, M. H. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 188-191.
8. Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675-679.
9. Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249-256.
10. Barinaga, C. J.; Edmonds, C. G.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 160-164.
11. Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
12. Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1989**, *86*, 137-149.
13. Gaskell, S. J.; Reilly, M. H.; Porter, C. J. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 142-145.
14. Alexander, A. J.; Thibault, P.; Boyd, R. K. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 30-34.
15. Thorne, G. C.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 217-221.
16. Renner, D.; Spiteller, G. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 75-77.
17. Tang, X.; Ens, W.; Standing, K. G.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791-1799.
18. Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835-2842.
19. Kulik, W.; Heerma, W.; Terlouw, J. K. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 276-279.
20. Morris, H. R.; Dickinson, R. J.; Williams, D. H. *Biochem. Biophys. Res. Commun.* **1973**, *51*, 247-255.
21. Tarr, G. E. In *Methods of Protein Microcharacterization*; Shively, J. E., Ed.; Humana Press: Clifton, NJ, 1986; p 155.
22. Mathews, W. R.; Runge, T. A.; Haroldsen, P. E.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 314-319.
23. Sommer, H. Z.; Lipp, H. I.; Jackson, L. L. *J. Org. Chem.* **1971**, *36*, 824-828.
24. Schaaper, W. M. M.; Voskamp, D.; Olieman, C. J. *Chromatogr.* **1980**, *195*, 181-186.
25. Louris, J. N.; Wright, L. G.; Cooks, R. G.; Schoen, A. E. *Anal. Chem.* **1985**, *57*, 2918-2924.
26. Guevremont, R.; Wright, J. L. C. *Rapid Commun. Mass Spectrom.* **1987**, *1*, 12-13.