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Identification of a New Fragment Ion Type in the Collision-Induced Dissociation Spectra of Peptides: Formation of a_2-16 Ions

K. R. Thornburg, K. L. Schey, and D. R. Knapp

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, South Carolina, USA

The identification of an ion observed in the high-energy collision-induced dissociation spectra of several model peptides is reported. The ion, observed at m/z 99 for the peptide pentaalanine (Ala_5) and designated a_2-16, is shown to have an elemental formula of C_5H_9NO by high-resolution peak matching. The precursor ion spectrum of the a_2-16 ion and product ion spectra of the a_2 and the a_2 + 1 ions for Ala_5 suggest that this ion is formed by the loss of 17 u (presumably NH_3) from the a_2 + 1 ion and, to a lesser extent, by loss of 28 u (presumably CO) from the b_2-16 ion. On the basis of the data presented and other experimental evidence, a structure and mechanism for the formation of the a_2-16 ion is proposed. *(J Am Soc Mass Spectrom 1993, 4, 424–427)*

Tandem mass spectrometry of peptide $[M + H]^+$ ions generated by fast-atom bombardment or liquid secondary ionization mass spectrometry (LSIMS) has become an important analytical tool for the primary sequence analysis of peptides and proteins [1]. Bond cleavage along the peptide backbone generates a series of ions from which the amino acid sequence of the peptide can be determined [2]. Additional confidence in the sequence assignment can be gained by identifying side-chain-specific sequence ions, internal acyl fragment ions, amino acid immonium ions, and side-chain losses from the intact $[M + H]^+$ ion [2–5]. Still further confirmation can be obtained by identifying ions resulting from the loss of H_2O and NH_3 from backbone cleavage ions and the loss of CO from internal acyl fragment ions [2]. Even with assignment of all of these ion types, significant peaks often remain unassigned. Efforts are ongoing to characterize unassigned peaks in peptide collision-induced dissociation (CID) spectra. For example, Biemann and coworkers [6] identified several sequence requirements for the formation of b_2-NH_3 ions, c_n ions, and internal fragment ions. In related research, Ackermann et al. [7] recently identified a lysine-specific fragmentation in the normal liquid secondary ionization mass spectra of peptides. Although this ion is usually of low relative abundance in CID spectra, its potential use for differentiating lysine from glutamine was reported. To maximize the accuracy and confidence of sequence assignments of unknown peptides, it is important that all significant peaks in a spectrum be interpreted in terms of the structure of the peptide. This study reports the characterization of an ion observed in the high-energy CID spectra of some small peptides.

A relatively abundant fragment ion of m/z 99 was observed in the CID spectrum of the peptide pentaalanine (Ala_5). Although oligoalanines have been studied extensively under low-energy collision conditions [8, 9], this type of ion has not been previously reported. On further investigation, an analogous ion, located 16 u lower than the a_2 ion, was observed in the CID spectra of several other peptides. A study was therefore initiated to characterize the structure and origin of this unknown ion type. Accurate mass measurements and precursor ion scans were used to study this ion, which is designated a_2-16 (using the conventional nomenclature of Roepstorff and Fohlman [10], as modified by Biemann [11]).

Experimental

All measurements were carried out on a JEOL (Tokyo, Japan) HX110/HX110 four-sector tandem mass spectrometer of EBEB configuration operating with an accelerating voltage of 10 kV. Precursor ions were generated in a standard LSIMS ion source using a JEOL cesium ion gun operated at 20 kV (10-keV cesium ion beam energy). Glycerol was used as the matrix for all experiments reported here.

CID spectra were recorded by focusing the ^{12}C species of the $[M + H]^+$ cluster into the collision cell

Address reprint requests to Kelly R. Thornburg, Department of Pharmacology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

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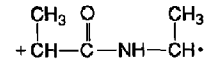
(third field-free region) and attenuating the intensity to approximately 30% with helium. The collision cell was floated to 3 kV for the high-energy CID experiments and 9.9 kV for the low-energy CID experiments. The mass resolution of both mass spectrometers was approximately 1000. The JEOL MS-MP7000 data system was used to record the CID spectra, which are average profile data of one to five scans. Product ion spectra were acquired at a rate corresponding to scanning MS-II from m/z 1–6000 in 90 s with 100-Hz filtering.

Precursor ion scans were performed with MS-I only, using the JEOL data system to generate the B^2/E scans and acquire the data. Helium was used as the collision gas in the first field-free region. Exact mass measurements were obtained by operating MS-I at 10,000 resolution in the peak-matching mode. Glycerol matrix ions were used for reference.

All peptides used in this study were obtained from commercial sources and were used without further purification. Peptides AAAAA, GLLG, LLVY, VLS, IVV, TYSK, VLSEG, MGMM, DSDPR, MEHFRWG, and WMDF were purchased from Bachem Bioscience (Philadelphia, PA); LLLY-methyl ester, YGGFM, YKT, TKY, FGPETP-NH_2, MAS, MRF, SGAGAG, MDF-NH_2, ETTTDK, and GWMDF-NH_2 were purchased from Research Plus (Bayonne, NJ); AAA, YGGFL, GGG, and GGGG were purchased from Sigma Chemical Co. (St. Louis, MO); and LGG, and III were purchased from Chemical Dynamics (South Plainfield, NJ).

Results and Discussion

The high- and low-energy CID spectra of Ala_5 are presented in Figure 1. The absence of the a_2-16 ion at m/z 99 in the low-energy spectrum indicates that it is formed through a high-energy process. A proposed general structure of this ion, based on the observed relationship to the a_2 ion, is given by **1**.



1

An exact mass measurement of m/z 99 found in the normal liquid secondary ionization mass spectrum of Ala_5 confirmed the elemental composition of **1**. A mass of 99.0687 u was measured for this peak, which agrees well with a calculated mass of 99.0684 u for the proposed elemental formula C_5H_9NO. Exact mass measurements of a_2-16 ions for other peptides were unsuccessful owing to the low abundance of those ions in the liquid secondary ionization mass spectra.

A precursor ion (B^2/E) scan was performed (Figure 2) to identify the origin of the m/z 99 ion. The major peak in this spectrum was m/z 116, which corresponds to the a_2 + 1 ion for this peptide. Precursor ion spectra for a_2-16 ions were acquired for other peptides as well. In all cases, the major ion observed was the

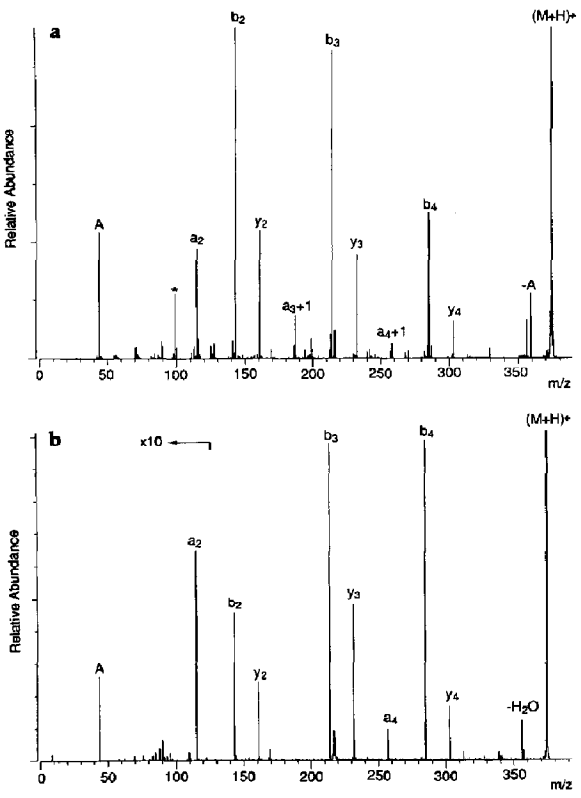


Figure 1. CID spectra of Ala_5 with (**a**) 7-keV and (**b**) 100-eV collision energy. The a_2-16 ion, observed at m/z 99 in the high-energy spectrum (*), is not observed in the low-energy spectrum.

a_2 + 1 ion. A list of peptides that were examined for the presence of a_2-16 ions is presented in Table 1. Also indicated are the peptides for which precursor ion data were acquired. The minor peak at m/z 127 in the precursor ion spectrum of m/z 99 is also of interest

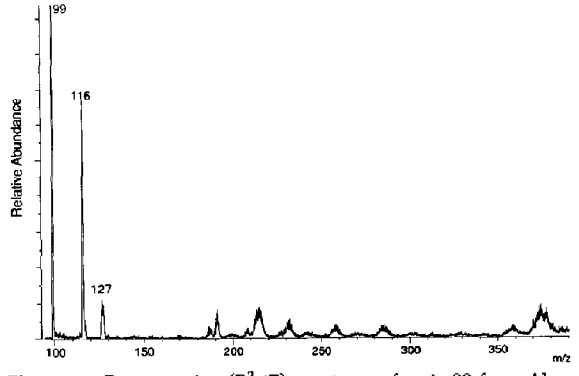


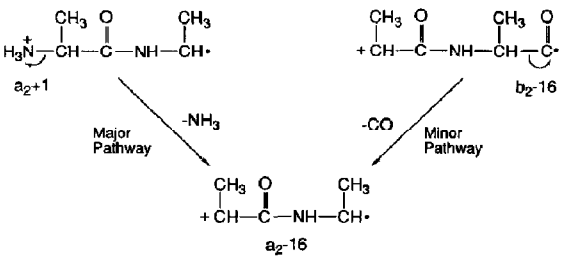
Figure 2. Precursor ion (B^2/E) spectrum of m/z 99 from Ala_5. The most abundant precursor observed at m/z 116 corresponds to the a_2 + 1 ion for Ala_5.

Table 1. Peptides studied for the presence of a_2-16 ions

Peptide sequence	Predicted mass, a_2-16 ion	a_2-16 /a_2[a]	a_2 Relative abundance[b]
AAA	99	1.11	0.30
AAAAA	99[c]	0.61	0.33
GGG	71	0.57	0.11
GGGG	71	0.52	0.17
LGG	127	3.50	0.02
GLLG	127[c]	0.12	0.53
LLVY	183	0.31	0.29
LLLY-methyl ester	183	0.46	0.22
YGGFL	177[d]	—	—
YGGFM	177[d]	—	—
III	183	0.14	0.36
VLS	169	0.17	0.39
IVV	169[c]	0.23	0.35
MRF	244[e]	NO	0.06
MAS	159[c]	0.14	0.30
YKT	248[d]	—	—
TKY	186[e]	NO	NO
TYSK	221	0.06	0.96
FGPETP-NH_2	161	0.75	0.07
MDF-NH_2	203[e]	NO	0.37
ETTTDK	187[e]	NO	0.17
VLSEG	169	0.28	0.54
SGAGAG	101[d]	—	—
MGMM	145	0.25	0.09
DSDPR	159[d]	—	—
MEHFRWG	216[e]	NO	NO
GWMDF-NH_2	199[e]	NO	NO
WMDF	273[e]	NO	0.07

[a] a_2-16 /a_2 Ratio calculated based on peak height.
[b] Abundance relative to the most abundant fragment ion.
[c] Precursor ion data also acquired for this peptide.
[d] Predicted mass is isobaric with another predicted fragment ion, causing ambiguous peak assignment; therefore, no abundance ratios were calculated.
[e] Ion not observed.

because the analogous peak (11 u above the $a_2 + 1$ peak) is observed with the other peptides for which parent ion scans were performed. The peak at m/z 127 corresponds to a b_2-16 ion type and is also observed with very low abundance in the high-energy CID spectrum of Ala_5 (see Figure 1a). Although the structure and origin of the b_2-16 ion is currently under investigation in this laboratory, these results suggest that it can fragment further to yield the a_2-16 ion. On the basis of the reported structure of $a_n + 1$ ions [5], and our results indicating pathways for formation of m/z 99 from the $a_2 + 1$ and b_2-16 ions, a mechanism for the formation of the a_2-16 ion is proposed. Scheme I shows the proposed major and minor fragmentation mechanisms that involve the loss of ammonia from the $a_2 + 1$ radical cation and the loss of CO from the proposed b_2-16 radical cation, respectively. The formation of a_2-16 ions may, alternatively, involve cyclization of the precursor $a_2 + 1$ (or b_2-16) ion to yield a cyclic radical cation structure. The structures shown in Scheme I are intended only to show ion compositions; the actual structures of the ions are unknown.



Scheme I

To confirm the $a_2 + 1$ ion as the precursor for the a_2-16 ion, high-energy CID spectra for m/z 115 and 116 from Ala_5 were acquired. These masses correspond to the a_2 and $a_2 + 1$ ions for this peptide, respectively, and were present in the normal liquid secondary ionization mass spectrum. The results, presented in Figure 3, indicate that m/z 99 is the major fragment ion in the $a_2 + 1$ spectrum, whereas the a_2 ion produces only a very small m/z 99. Similar results were also obtained for the tripeptide IVV (data not shown). It is interesting to note that the d_2 ion, predicted to be formed from the $a_2 + 1$ ion of the peptide IVV, was not observed. These data support the major fragmentation mechanism shown in Scheme I.

Further experimental evidence for the proposed major fragmentation pathway is presented in a recently published report by Kenny et al. [12]. The CID spectra of the peptide GGGAA (with and without deuterium labels) presented in this report show the presence of the a_2-16 ion. In the spectrum of the unlabeled peptide, the a_2 ion is present at m/z 87, and the a_2-16 ion is present at m/z 71. A CID spectrum of the same peptide in which all exchangeable hydrogens have been replaced with deuterium shows the mass of the a_2 ion increased by 3 u as expected, whereas the a_2-16 ion appears at m/z 72. This indicates that the a_2-16 ion contains only one deuterium label; presumably, the two deuteriums attached to the terminal nitrogen have been lost. A CID spectrum is also presented for the peptide in which all α-carbon hydrogens have been replaced by deuterium. The a_2 ion for this peptide is observed at m/z 91 (this is also the mass of the y_2 ion for the peptide), whereas the a_2-16 ion appears at m/z 75. All four deuteriums from the a_2 ion are retained in the a_2-16 ion, which is predicted by Scheme I. Thus, these data support the mechanism proposed here for the formation and structure of the a_2-16 ions.

The data in Table 1 indicate that some of the pre-

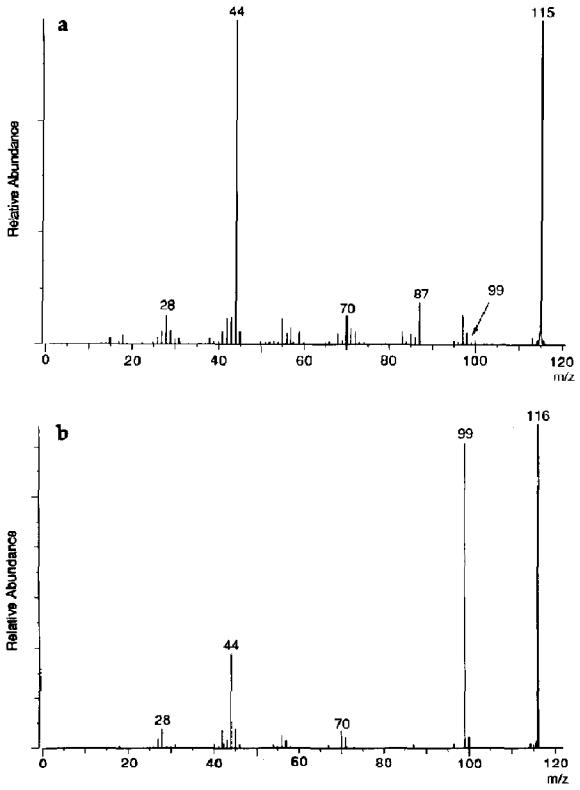


Figure 3. (a) CID mass spectrum of m/z 115 (a_2) from Ala_5; *arrow* indicates the very weak peak at m/z 99. (b) CID mass spectrum of m/z 116 (a_2 + 1) from Ala_5; an abundant fragment is observed at m/z 99, corresponding to the a_2-16 ion.

dicted a_2-16 ions were not observed. There are several possible reasons for this. The loss of ammonia from the a_2 + 1 implies that the site of protonation is the terminal nitrogen atom (this conclusion is supported by the deuterium-labeling data shown by Kenny et al. [12]). The introduction of a basic amino acid (i.e., K or R) at either of the two N-terminal sites could alter the site of protonation and result in the stabilization of the a_2 + 1 ion or favor another type of fragmentation. Another possible explanation for the absence of the a_2-16 ion is the existence of a basic residue at or near the C-terminus of the peptide. This would favor the formation of C-terminal ions over N-terminal ions. The effect of the site of protonation and the presence of basic residues at the C-terminus would be expected to be equally important in the formation of an a_2-16 ion from a b_2-16 ion.

Conclusions

The data reported here show that a_2-16 ions appear in the high-energy CID spectra of some small peptides. This ion type was characterized for the model peptide Ala_5 and is formed by the loss of NH_3 from the N-terminus of the a_2 + 1 ion. A secondary mechanism for the formation of the a_2-16 ion appears to be the loss of CO from a b_2-16 ion. The formation of the a_2-16 ion is suppressed by the location of basic amino acid residues at either the N-terminal or C-terminal portion of the peptide. Although the a_2-16 ion provides no information on the sequence of the two N-terminal residues, it can be used to confirm the identity of the two terminal residues in unknown peptides. It is important to continue to identify unexplained peaks in the CID spectra of peptides to facilitate the development of computer-based interpretation algorithms and the identification of novel amino acids and posttranslational modifications.

Acknowledgments

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