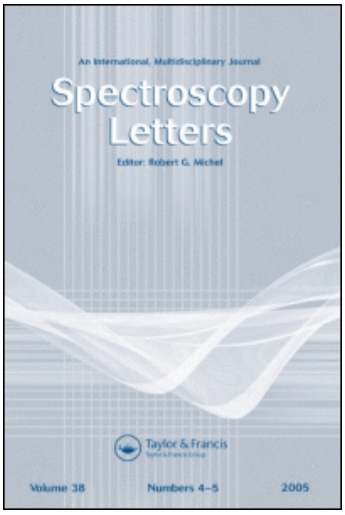

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Analysis of a Mastoparan B Isolated from the Hornet (*Vespa basalis*) Venom by Fast Atom Bombardment Mass Spectrometry with B/E Linked Scan

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ANALYSIS OF A MASTOPARAN B ISOLATED FROM THE HORNET (VESPA BASALIS) VENOM BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY WITH B/E LINKED SCAN

KEY WORDS: FAB-MS, B/E linked scan, Peptide, Toxin

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ABSTRACT

Positive and negative FAB-MS as well as B/E linked scan are used for the determination of the amino acid sequence of mastoparan B, a toxin isolated from the hornet (Vespa Basalis) venom. It is demonstrated that useful sequence information can be deduced from the FAB-MS and B/E-CAD spectra. In conjunction with the information from Edman degradation method, complete deduction of the entire amino acid sequence is presented.

INTRODUCTION

The hornet (Vespa Basalis) is one of the most dangerous species of wasps found in Taiwan. The incidence of sting death caused by this hornet is high and is probably because of its aggressiveness and highly toxic venom. In a previous study, a lethal protein was purified from freshly collected venom and was estimated to have a molecular mass of about 32 kDa [1]. Another toxin, a tetradecapeptide amide designated as mastoparan B, was purified later and its molecular weight was measured to be 1161 Da. The peptide possessed a potent hemolytic activity which synergized with the above lethal protein, suggesting the possible involvement of mastoparan B in the lethal effect of Vespa Basalis venom. Initial sequencing results of the mastoparan B using Edman degradation method were unsatisfactory because of the difficulty of ascertaining the amino acid from position 11 to 14 [2].

Fast atom bombardment (FAB) mass spectrometry since its introduction by Barber [3], has been the most appropriate technique for the analysis of peptides because of its simplicity, reproducibility, adaptability, and extended mass range. However, the use of a liquid matrix in FAB often complicates the mass spectrum. In addition, structural analysis with FAB suffers the drawback of not enough fragmentations. To overcome these difficulties, much effort has been put into the development of methods using tandem mass spectrometry (MS/MS) [4,5]. The ideal MS/MS approach requires true tandem mass spectrometers such as multi-

sector, multiquadrupole, or hybrid instruments. In situations where true tandem mass spectrometers are not available, an alternative mode of MS/MS can nevertheless be accomplished by linked scanning the E(electric) and B (magnetic) field at constant B/E using a conventional double-focusing instrument of forward (E, B) geometry. The mass spectrum consists mainly of daughter ions from a selected ion in a normal FAB spectrum under collision-activated dissociation (CAD) with a neutral gas in the first field-free region.

At present, there are few representative reports in the literature describing the use of FAB-MS and B/E-CAD for sequencing the peptides with a forward geometry instrument [6-10]. This study was undertaken to explore the possibility of using FAB-MS and B/E-CAD to determine the amino acid sequence of the mastoparan B. The limitations of precursor ions originating from a narrow mass range and the appearance of artefact peaks with B/E linked scan was evaluated by comparing the mass analysis results with those from Edman degradation methods. Good correlations between these two results suggest that FAB-MS and B/E-CAD techniques are useful in determining the amino acid sequence of biologically active peptides.

EXPERIMENTAL

Isolation and purification of the mastoparan B were described elsewhere [1,2]. A 1-μl aliquot of the sample solution (ca. 5-μg mastoparan B dissolved in 5-μg water) was mixed with 1-μL of thioglycerol matrix on the FAB probe tip.

The mass spectrometric analysis was carried out with a two-sector magnetic deflection mass spectrometer (JEOL HX-110, EB geometry) operated either in the normal scan or in the B/E linked scan mode. Ionization was achieved by bombardment with Xe atoms of ca. 6 KeV impact energy. The accelerating voltage of the mass spectrometer was operated at 10 KV. $(CsI)_nCs^+$ cluster ions were used for mass calibration. High resolution measurements for ions in the normal FAB spectrum were performed at the resolving power of 10,000; otherwise the resolving power was set at 3,000. The normal FAB spectrum was collected in 10 sec.

In the B/E linked scan mode, the CAD spectra were obtained by admitting the helium into the collision cell (first field-free region) at the pressure (ca. $10^{-4}\sim 10^{-5}$ torr)that the precursor ion was reduced to one-half of its initial intensity. The linked scan at constant B/E ratio was generated by the JEOL JMA-DA5000 data system. The single scan raw data profile was recorded in 1 min.

RESULTS AND DISCUSSION

The positive and negative ion FAB spectra of the mastoparan B are shown in Fig. 1 and 2, respectively. It is apparent that prominent peaks are in both the molecular ion region and the low-mass region. The formation of dominant cationized ions of $(M+Na)^+$ = m/z = 1634 and $(M+K)^+$ = m/z = 1650 by deliberately adding a small amount of 0.1 N $NaNO_3$ and KNO_3 solution to the sample provides confirmatory evidence in the molecular mass determinations. The protonated molecular ion was

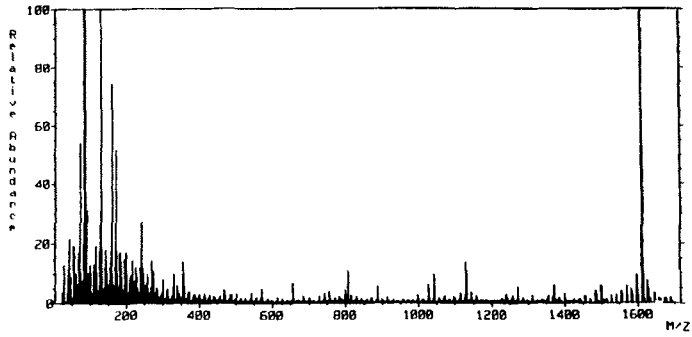


FIG. 1 Positive Ion FAB Mass Spectrum of Mastoparan B

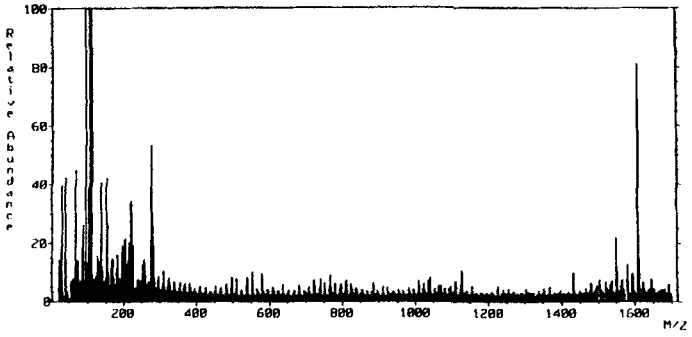
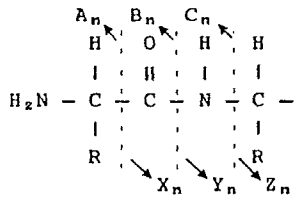


FIG. 2 Negative Ion FAB Mass Spectrum of Mastoparan B

further ascertained to be of m/z = 1612.01 using high mass resolution measurements and the corresponding elemental formula indicates that both terminuses are of amide group.

The types of positive and negative ions most useful for deriving the peptide sequence information are summarized in Table 1. The nomenclature used here is that proposed by Roepstoff and Fohlman [11]. The

TABLE 1 Type of ions in Positive and Negative Ions Peptide Spectra



Positive	Negative
$[M + K]^+$	--
$[M + Na]^+$	--
$[M + H]^+$	$[M - H]^-$
$[M + H - H_2O]^+$	$[M - H - H_2O]^-$
$[M + H - NH_3]^+$	$[M - H - NH_3]^-$
$[Y_n + 2H]^+$	$[Y_n]^-$
$[Z_n + 2H]^+$	$[Z_n]^-$
$[A_n]^+$	--
$[B_n]^+$	$[B_n]^-$
$[C_n + 2H]^+$	$[C_n]^-$

[a] The nomenclature is that of Roepstorff and Fohlman.[11]

characteristics of the normal FAB mass spectrum that the lack of uninterrupted series of ions of one type and the difficulty of interpreting the mass spectrum in the low-mass region due to the matrix interferences are evident in Fig. 1 and 2. The attributes of the CAD mass spectrum of peptide exhibiting extensive series of the same ion type and reduced matrix interference effects via the appropriate selection of the precursor ion are exploited to overcome the above shortcomings. The illustrative examples are the B/E-CAD spectrum of the $(M+H)^+$ = m/z = 1612 protonated molecular ion (Fig. 3) and the ion with m/z = 1030 (Fig. 4).

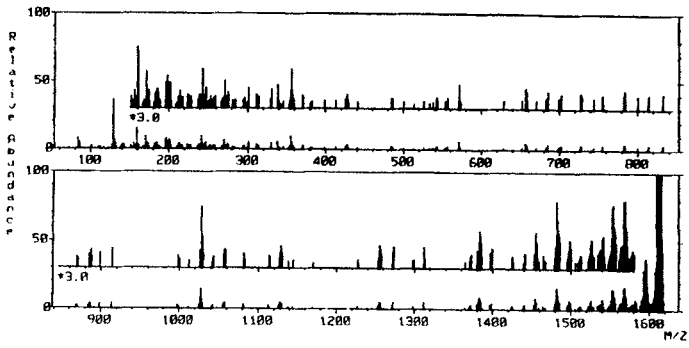


FIG. 3 B/E-CAD Spectrum of Protonated Molecular Ion with m/z = 1612

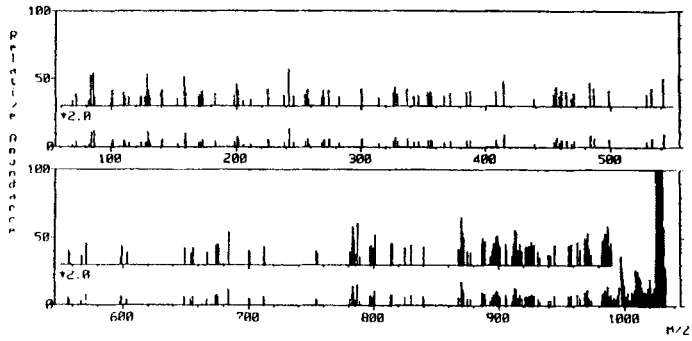


FIG. 4 B/E-CAD Spectrum of Ion with m/z = 1030

From Table 1, six different fragmentation processes are involved in the formation of sequence ions. N-terminus sequence ions formed by cleavage of the -CHR-CO-, -CO-NH-, and -NH-CHR- bonds are designated as A, B, and C, respectively. The corresponding C-terminus sequence ions are denoted as X, Y, and Z. However, not all sequence ion series appear in the mass spectra. In addition, abundant loss of $-CH_3$, $-NH_3$, or $-H_2O$ moiety

TABLE 2 Amino Acid Sequence-determining Ions

Amino Acid		H_2N- Leu- 1	Lys- 2	Leu- 3	Lys- 4	Ser- 5	Ile- 6	Val- 7	Ser- 8	Trp- 9	Ala- 10	Lys- 11	Lys- 12	Val- 13	Leu-NH_2 14
Y-sequence ions	m/z	129	228	356	484	555	742	829	928	1041	1128	1256	1369	1497	1610
	F1[a]	104*	5*	6*	-	-	-	-	-	-	-	-	4	6	100
	F2[b]	16	19	-	-	10	-	-	-	8	10	-	-	-	81
	F3[c]	6*	3	4	-	3	3	-	-	3	5*	4	3	4*	100*
	F4[d]	10	-	4	7	4	-	-	6	-	-	-	-	-	-
Z-sequence ions	m/z	114	213	341	469	540	727	814	913	1026	1113	1241	1354	1482	1595
	F1[a]	9*	9*	-	-	-	-	-	-	-	-	-	-	-	10
	F2[b]	17	14	-	-	8	-	-	-	-	-	-	-	-	9
	F3[c]	-	4	-	-	-	4	4	5	4*	3	-	-	14*	32*
	F4[d]	-	-	-	5	10	-	8	12	118	-	-	-	-	-
B-sequence ions	m/z	114	242	355	483	570	684	783	870	1056	1127	1255	1383	1482	1595
	F1[a]	9	27	14	-	5	-	-	-	-	-	-	-	-	-
	F2[b]	17	-	-	-	-	-	-	-	-	-	-	-	-	-
	F3[c]	-	9*	6	3	6	-	5	-	5	4	4*	7*	14*	32*
	F4[d]	-	14	5	4	6	8	14	14	-	-	-	-	-	-
C-sequence ions	m/z	129	257	370	498	585	699	798	885	1071	1142	1270	1398	1497	1610
	F1[a]	104*	10*	-	-	-	-	4	6	-	-	4	-	6	100
	F2[b]	16	14	-	8	-	-	-	-	-	-	-	-	-	81
	F3[c]	6*	-	-	-	-	3	-	3	-	-	-	5	4*	57*
	F4[d]	10	3	5	6	-	6	6	10	-	-	-	-	-	-

[a] : from positive ion FAB mass spectrum ;
[b] : from negative ion FAB mass spectrum ;
[c] : B/E-CAD spectrum of protonated molecular ion with m/z = 1612 ;
[d] : B/E-CAD spectrum of ion with m/z = 1030 ;
* : Both sequence ion and 2H-added sequence ion are observed.

from the parent ion are also observed. To better understand the fragmentation characteristics, Table 2 lists the dominant sequence ion series from the normal FAB (Fig. 1 and 2) and B/E-CAD (Fig. 3 and 4) spectra. The amino acid number is arbitrarily designated by denoting the Leu in the Leu-Lys- terminus as number 1. Numbers specified in the "m/z" rows are the expected m/z values for the corresponding sequence ions. Numbers specified in the rows from F1 to F4 are the relative abundances of the ions read from Fig. 1 to Fig. 4. A dash line indicates the absence of the ions. Detailed

analysis of Table 2 reveals several findings. Y- and B-sequence ions are the prominent ions under the B/E-CAD process; whereas the C-sequence ions are the prominent ions under normal FAB process. Hence, these observations suggest that the energetics of the FAB-induced versus CAD-induced fragmentation of the precursor ions of the mastoparan B are different. The same phenomena have been observed by Dass and Desiderio while analyzing the ME peptides [12]. Addition of two H atoms to the C- and Y-sequence ions are also observed as Biemann and Martin's report [7]. Z- and B-sequence ions also have two H atoms added. However, it is noted that low-mass ions in the normal positive FAB spectra and high-mass ions in the B/E-CAD spectra all consist of both the sequence ion and the 2H-added sequence ion. This might be because that in B/E linked scan the precursor ions are originating from a narrow mass range rather than from a single m/z value as in a true tandem mass spectrometer. Some artefact peaks might be responsible for those 2H-added sequence ions. Because different portions of the Y- and B-sequence ions are overlapped, the complete sequence can be read as NH_2-Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2. The differentiations of Gln/Lys and Leu/Ile are clarified with the partial information from the amino acid analysis and the Edman degradation method.

CONCLUSION

The amino acid sequence of a mastoparan B toxin isolated from the hornet (Vespa Basalis) venom has been determined using FAB and B/E linked scan mass spectrometry. Poor mass selection of the precursor ions and the appearance of the artefact peaks in the B/E-CAD

mass spectra can be overcome by careful spectrum analysis. The Y- and B-sequence ions provide complementary information and must be considered in conjunction with the information from Edman degradation method for the complete deduction of the entire amino acid sequence.

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