RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. **12**, 849–851 (1998)

The b_1 Ion Derived from Methionine is a Stable Species

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Unimolecular fragmentation of the MH^+ ions of a number of methionine derivatives such as H-Met-OMe, H-Met-β-naphthylamide, H-Met-Gly-OH, H-Met-Ala-OH and H-Met-Phe-OH results in formation of the methionine b_1 ion (*m/z* 132) in significant yields. Since α-aminoacylium ions are generally unstable and not observed, it is proposed that the stable form of the b_1 ion from these methionine derivatives is methyl-cationated α-amino-γ-thiobutyrolactone. Under low-energy collision-induced dissociation conditions this b_1 ion readily loses CO to form the a_1 ion, $CH_3SCH_2CH_2CH{=}NH_2^+$. © 1998 John Wiley & Sons, Ltd.

Received 24 March 1998; Revised 27 April 1998; Accepted 28 April 1998

There is substantial evidence[1–5] that α-aminoacylium ions, $RCH(NH_2)CO^+$, are unstable species and exothermically eliminate CO to form the immonium ion $RCH{=}NH_2^+$. Consequently, b_1 ions normally are not observed in the fragmentation of protonated peptides. The higher b_n ions, which nominally have an acylium structure, are frequently abundant in the fast atom bombardment (FAB) mass spectra of peptides as well as in the collision-induced dissociation (CID) mass spectra of protonated peptides.[6–12] The stability of these b_n ions has been attributed to cyclization to a protonated oxazolone structure;[3,13] such cyclization is not possible for the b_1 ion, although acetylation at the N-terminus does lead to fragmentation at the first peptide amide bond and formation of stable b ions which also have a protonated oxazolone structure.[3]

However, there are exceptions to the general rule that b_1 ions are not stable. A recent communication from this laboratory[14] showed that protonated lysine derivatives H-Lys-XH^+ fragmented to a significant extent by loss of HX to yield an ion of *m/z* 129 which is the b_1 ion derived from lysine. Evidence was presented that this *m/z* 129 ion did not have an α-aminoacylium ion structure but rather that cyclization had occurred to form protonated α-amino-ε-caprolactam. This species was formed by interaction of the side-chain amino group with the carbonyl function as HX departed. In the present communication we show that the b_1 ion formed by loss of HX from protonated methionine derivatives, H-Met-XH^+, also is stable and propose that this stability arises by interaction of the CH_3S group of the side-chain with the carbonyl function as HX departs; the stable form of the b_1 ion thus represents methyl-cationated α-amino-γ-thiobutyrolactone.

EXPERIMENTAL

All experimental work was carried out using a ZAB-2FQ hybrid BEqQ mass spectrometer (VG Analytical, Manchester, UK) which has been described in detail previously.[15] Briefly, this instrument is a reversed-geometry (BE) double-focusing mass spectrometer that is followed by a deceleration lens system, an RF-only quadrupole collision cell (q) and a quadrupole mass analyzer (Q). The ions studied were produced by FAB using an argon atom beam of 7–8 keV energy with the appropriate sample dissolved in glycerol.

To obtain the relative abundances 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 kinetic energy and introduced into the RF-only quadrupole cell q in the absence of collision gas. Low-energy CID studies were carried out in the same fashion but with the addition of N_2 at an indicated pressure of *ca.* 1×10^{-7} Torr (1 Torr = 133.3 Pa) to the quadrupole collision cell. In both the unimolecular and CID studies the ionic fragments were analyzed by scanning the final quadrupole Q with, typically, 20–30 two second scans being accumulated on a multichannel analyzer.

The compounds used were obtained from Sigma Chemical or BACHEM Biosciences and were used as received.

RESULTS AND DISCUSSION

The results obtained for the unimolecular (metastable ion) fragmentation of protonated methionine and a number of methionine derivatives are presented in Table 1. Although we[4] do not see formation of the b_1 ion ($[MH - H_2O]^+$) in the fragmentation of protonated methionine, Kulik and Heerma[16] reported observation of $[MH - H_2O]^+$ with an abundance 2% of the $[MH - NH_3]^+$ ion signal in the mass-analyzed ion kinetic energy (MIKES) spectrum of protonated methionine. A weak metastable ion signal is observed at *m/z* 132 for the b_1 ion resulting from the elimination of CH_3OH from protonated methionine methyl ester; as for protonated methionine, the major fragmentation reactions remain loss of NH_3 and formation of the a_1 ion $CH_3SCH_2CH_2CH{=}NH_2^+$ (*m/z* 104).

However, for methionine β-naphthylamide, H-Met-Gly-OH and H-Met-Ala-OH unimolecular fragmentation of MH^+ yields the b_1 ion at *m/z* 132 at intensities ranging from 26% to 38% of the total metastable ion signal. For

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Contract/grant sponsor: Natural Sciences and Engineering Research Council of Canada.

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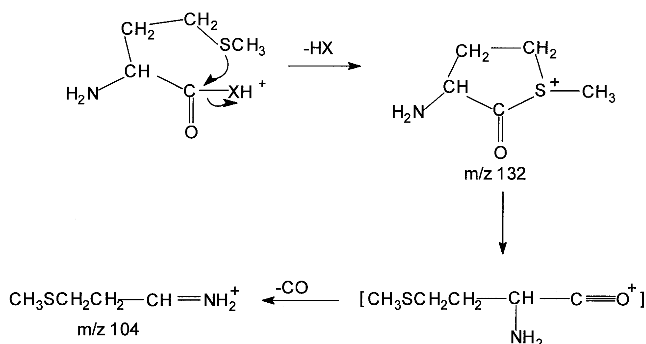
Table 1. Metastable ion fragmentation of protonated methionine derivatives

Derivative	a_1	b_1	% of fragment ion abundance $-NH_3$	$-CH_3SH$	Other (%)
H-Met-OH[a]	23.8		76.2		
H-Met-OMe	32.6	4.0	61.4	2.0	
H-Met-β-Napth	74.0	26.0			
H-Met-Gly-OH	47.6	38.1	6.7	3.8	$-CO(3.8)$
H-Met-Ala-OH	61.8	25.7	5.4	4.2	$-CO(3.0)$
H-Met-Phe-OH	58.3	10.1		5.0	$-H_2O(12.2)$, $y''_1(14.4)$

[a] From Ref. 4

protonated H-Met-Phe-OH the b_1 ion accounts for 10% of the total metastable ion signal. In low energy CID studies the b_1 ion was observed at low collision energies but the relative intensity decreased rapidly with increasing collision energy and there was a corresponding increase in the ion signal for the a_1 ion. Clearly, the b_1 ion is of only modest stability and readily fragments by elimination of CO to form the a_1 ion.

In this respect the methionine b_1 ion differs from the lysine b_1 ion which dominates many CID mass spectra of peptides containing the lysine residue.[14,17–19] We did not observe the *m/z* 132 ion from fragmentation of dipeptides containing methionine in any position other than the N-terminus or in tripeptides containing methionine. This result is in agreement with the observations of Kulik and Heerma[20,21] on the fragmentation of a limited number of di- and tri-peptides containing methionine. In the case of lysine the stability of the b_1 ion was attributed[14] to cyclization to a protonated caprolactam structure by attack of the side-chain amino function on the carbonyl function of $H\text{-}Lys\text{-}XH^+$ as HX was lost. A similar participation of the terminal thiomethoxy group, as outlined in Scheme 1, can adequately rationalize the observed stability of the b_1 ion derived from protonated methionine derivatives. However, it is apparent from the CID studies that the methylated aminothiobutyrolactone formed is of limited stability and readily loses CO, presumably involving the transient formation of the α-aminoacylium ion.



The data of Table 1 show that formation of the a_1 ion is a more prevalent metastable ion fragmentation reaction than formation of the b_1 ion. In MIKES studies of the fragmentation of protonated methionine β-naphthylamide, the metastable peak for formation of the b_1 ion was Gaussian in shape while that for formation of the a_1 ion was flat-topped. The latter observation is in agreement with earlier observations[2–4] that formation of a_1 ions on metastable ion fragmentation of protonated amino acid derivatives occurs with release of kinetic energy. We propose that formation of the a_1 ion occurs through the intermediate formation of the unstable acyclic acylium ion which immediately eliminates CO. It thus appears that formation of the methyl-cationated α-amino-γ-thiobutyrolactone and the unstable acylium structure are competitive in the time window sampled by metastable ion measurements. One would expect the cyclic thiobutyrolactone to be more stable than the acyclic acylium ion, i.e. have a lower critical energy for its formation. However, formation of the acyclic acylium ion should be favored entropically since formation of the thiobutyrolactone involves a highly ordered transition state. Hence, in the metastable ion time window, fragmentation by both pathways becomes competitive, leading to metastable ion signals for formation of both the b_1 and a_1 ions.

It is worthwhile noting that the methionine b_1 ion is isobaric with protonated leucine or isoleucine. However, the evidence to date indicates that the methionine b_1 ion is not formed in significant yield in the fragmentation of larger protonated peptides with the result that faulty attribution of the *m/z* 132 ion signal is unlikely.

Acknowledgement

The authors are indebted to the Natural Sciences and Engineering Research Council of Canada for financial support.

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