Structures of b and a Product Ions from the Fragmentation of Argentinated Peptides

Vicky W.-M. Lee,[†,‡] Hongbo Li,[†,§] Tai-Chu Lau,[‡] and K. W. Michael Siu*[,†,§,||]

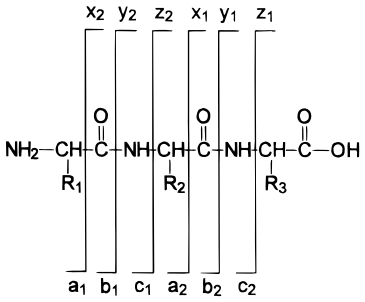
Contribution from the Institute for National Measurement Standards, National Research Council of Canada, Montreal Road, Ottawa, Ontario, Canada K1A 0R6, Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, and Ottawa-Carleton Chemistry Institute, Carleton University, Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

Received March 12, 1998

Abstract: Argentinated (silver-containing) oligopeptides fragment under low-energy collision conditions to yield abundant argentinated product ions. The structures of the $[b_2 - H + Ag]^+$ and $[a_2 - H + Ag]^+$ ions have been determined by means of tandem mass spectrometry and confirmed by comparison with synthesized derivatives of the candidate $[b_2 - H + Ag]^+$ ion structure. The $[b_2 - H + Ag]^+$ ion was found to be an N-argentinated oxazolone, which could subsequently form the $[a_2 - H + Ag]^+$ ion and other product ions after collision activation. Tripeptides containing proline as their second residue were observed to form a relatively less abundant $[b_2 - H + Ag]^+$ ion, which was postulated to be an argentinated ketene.

Introduction

The bio-inorganic chemistry of the silver ion is rich and fascinating. The Ag^+ ion has long been used as a bactericide in the form of eye drops for the newborns.[1,2] Some of its complexes display remarkable antimicrobial activities.[3,4] The metallothioneins, a class of small proteins believed to be responsible for heavy-metal detoxification in mammals, exhibit very high affinity for Ag^+.[5−7] Binding of the silver ion to other proteins and peptides is also observed. Argentinated (silver-containing) oligopeptides fragment upon low-energy collision-induced dissociation (CID) to give abundant argentinated b, a, y, and b + OH ions that are indicative of the peptide sequence.[8,9] The protonated equivalents of b, a, and y ions are common fragment ions of protonated peptides,[10−12] while the alkali-metal-containing equivalents of b + OH and a ions are dominant product ions of alkali-metal-containing peptides.[13,14]



Owing to its diversity, the fragmentation of argentinated peptides, once understood, may potentially provide a higher information content than that of protonated or alkali-metal-containing peptides.

Much effort has been spent on understanding fragmentation mechanisms and the resulting product ion structures of protonated peptides, which leads to a relative wealth of information.[10−12,15−42] Recent efforts from Wysocki's,[15−18]

† National Research Council of Canada.
‡ City University of Hong Kong.
§ Carleton University.
|| Present address: Department of Chemistry, York University, 4700 Keele St., Toronto, Ontario, Canada M3J 1P3. phone: (416)650-8021. Fax: (416)736-5936. E-mail: kwmsiu@yorku.ca.

(1) Petering, H. G. *Pharmac. Ther. A* **1976**, *1*, 127−130.
(2) Wigley, R. A.; Brooks, R. R. In *Noble Metals and Biological Systems*; Brooks, R. R., Ed.; CRC Press: Boca Raton, FL, 1992; pp 277−297.
(3) Nomiya, K.; Kondoh, Y.; Nagano, H.; Oda, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1679−1680.
(4) Nomiya, K.; Onoue, K. I.; Kondoh, Y.; Kasuga, N. C.; Nagano, H.; Oda, M.; Sakuma, S. *Polyhedron* **1995**, *14*, 1359−1367.
(5) Narula, S. S.; Mehra, R. K.; Winge, D. R.; Armitage, I. M. *J. Am. Chem. Soc.* **1991**, *113*, 9354−9358.
(6) Stillman, M. J.; Presta, A.; Gui, Z.; Jiang, D.-T. In *Metal-Based Drugs*; Gielen, M., Ed.; Freund: London, 1994; Vol. 1, pp 375−393.
(7) Gui, Z.; Green, A. R.; Kasrai, M.; Bancroft, G. M.; Stillman, M. J. *Inorg. Chem.* **1996**, *35*, 6520−6529.
(8) Tang, X.; Ens, W.; Standing, K. G.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791−1799.
(9) Li, H.; Siu, K. W. M.; Guevremont, R.; Le Blanc, J. C. Y. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 781−792.
(10) Hunt, D. F.; Yates, J. R., III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233−6237.
(11) Johnson, R. S.; Martin, S. A.; Bieman, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *86*, 137−154.
(12) Papayannopoulos, I. A. *Mass Spectrom. Rev.* **1995**, *14*, 49−73.
(13) Teesch, L. M.; Adams, J. *J. Am. Soc. Chem.* **1991**, *113*, 812−820.
(14) Teesch, L. M.; Orlando, R. C.; Adams, J. *J. Am. Soc. Chem.* **1991**, *113*, 3668−3675.
(15) McCormack, A. L.; Somogyi, Á.; Dongré, A. R.; Wysocki, V. H. *Anal. Chem.* **1993**, *65*, 2859−2872.
(16) Jones, J. L.; Dongré, A. R.; Somogyi, Á.; Wysocki, V. H. *J. Am. Soc. Chem.* **1994**, *116*, 8368−8369.
(17) Dongré, A. R.; Somogyi, Á.; Wysocki, V. H. *J. Mass Spectrom.* **1996**, *31*, 339−350.
(18) Dongré, A. R.; Jones, J. L.; Somogyi, Á.; Wysocki, V. H. *J. Am. Soc. Chem.* **1996**, *118*, 8365−8374.
(19) Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 249−257.
(20) Ballard, K. D.; Gaskell, S. J. *Int. J. Mass Spectrom. Ion Processes* **1991**, *111*, 173−189.
(21) Burlet, O.; Orkiszewski, R. S.; Ballard, K. D.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 658−662.
(22) Burlet, O.; Yang, C.-Y.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 337−344.

S0002-7863(98)00824-5 CCC: $15.00
Published on Web 07/08/1998

Gaskell's,[19−27] Boyd's,[28−30] and other's[31−36] groups have established the "mobile proton theory", namely that the "external" proton on a protonated peptide—presumably first attached to the N-terminus or to the basic site on a side chain—is easily transferred to amide nitrogen atoms on the peptide backbone upon collision activation, thus producing a heterogeneous population of precursor ion structures. (This is to be contrasted with an alternative view in which the ionization process produces a heterogeneous population of ionized structures whose protonation sites are fixed.) Protonation of an amide nitrogen weakens the amide bond, which then fragments to yield either the b or the y″ (y + 2H) ion in a charge-directed cleavage.[10−12,15] Subsequent elimination of CO from the b ion produces the a ion.[10−12] It is generally believed that the y″ ion has the structure of a protonated peptide or amino acid, the a ion an immonium ion, and until recently the b ion an acylium ion.[10−12]

Recent studies of Harrison's,[37−39] Wesdemiotis',[40] and Hunt's groups[41] showed that the b ion is not an acylium ion but a protonated oxazolone, with the acylium ion structure being that of the activated complex.[37] Employing neutral fragment reionization, Wesdemiotis and co-workers[40,42] were able to demonstrate that the C-terminal neutral fragment formed with protonated oxazolone (the b ion) is a truncated peptide or amino acid; however, the N-terminal neutral fragment formed with the y″ ion is either an aziridinone or a diketopiperazine, but not an oxazolone unless the peptide is N-acylated. These studies have painted an interesting dichotomy in fragmentation depending on which terminus the charge is located.

On the basis of results of tandem mass spectrometry and ZINDO calculations, Li et al.[9] showed that, in argentinated peptides, the silver ion is chelated to a number of sites, including the amide nitrogen, the amide oxygen, and the methionine sulfur atoms. Some of these bonds are formed as a consequence of self-solvation in the gas phase after solvent removal and do not exist in solution prior to electrospray introduction. Low-energy collision-induced dissociation of the $[M + Ag]^+$ ion of argentinated peptides yields a variety of silver-containing ions, including $[b_n - H + Ag]^+$, $[a_n - H + Ag]^+$, $[b_n + OH + Ag]^+$, and $[y_n + H + Ag]^+$. The observation of fragmentation along the peptide backbone parallels that in protonated peptides and strongly suggests a heterogeneous population of argentinated precursor ions. This heterogeneity could arise as a consequence of collision activation or of ionization plus desolvation, i.e., from silver ion migration to form a mixture of precursor structures or from a distribution of fixed, silver-containing structures. The fact that the silver ion is chelated to a number of sites would appear, at least superficially, to decrease its likelihood of being relatively mobile.

In this article, we report the results of our study on the structures of the $[b_n - H + Ag]^+$ and $[a_n - H + Ag]^+$ product ions, and the mechanisms of their formation, from the $[M + Ag]^+$ precursor of oligopeptides. The 2-phenyl derivatives of the candidate structure of the b ions, an oxazolone, were synthesized,[43] argentinated, and probed by means of tandem mass spectrometry along with argentinated oligopeptides and some of their *N*-acetyl derivatives. Energy-resolved CID was performed on a representative tripeptide to yield the fragment intensity versus collision energy relationship.

Experimental Section

Instrumental. Experiments were performed on a triple quadrupole mass spectrometer equipped with electrospray introduction, PE SCIEX API 300 (Concord, Ontario). Samples were typically 0.1 mM oligopeptides (Sigma, St. Louis, MO) in 50/50 methanol/water containing 0.2 mM silver nitrate (Aldrich, St. Louis, MO). These were continuously infused with a syringe pump (Harvard Apparatus, Model 22, South Natick, MA) at a typical flow rate of 2 μL/min into the electrospray probe. The optimum probe position was established from time to time but was typically with the tip about 2 cm from the interface plate and with the spray off-axis from the orifice. Mass spectra were acquired in the positive ion detection mode with unit mass resolution at a step size of 0.1 *m*/*z* unit and at a dwell time of 10 ms/step. Typically, 10 scans were summed to produce a mass spectrum. Tandem mass spectrometry was performed with a nitrogen pressure of 2.5 mTorr in q2 and at a collision energy at the center-of-mass frame (E_{cm}) typically of 1−2 eV. Apparent MS^3 was achieved by raising the orifice bias potential to induce fragmentation in the lens region, isolating the product ion of interest with Q1, inducing further fragmentation in q2, and mass-analyzing the second generation product ions with Q3.

Synthesis of 2-Phenyl Oxazolones. 2-Phenyl-5-oxazolone was synthesized according to an established procedure.[43] A mixture of *N*,*N*′-dicyclohexylcarbodiimide and hippuric acid in 10 mL of $CHCl_3$ was stirred magnetically overnight to give a precipitate of dicyclohexylurea while the filtrate gave 55% 2-phenyl-5-oxazolone as yellow plates. Positive ion electrospray mass spectrometric analysis showed *m*/*z* 162 $\{MH\}^+$. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, relative to Me_4Si) revealed δ 4.39 (s, 2H, NCH_2CO), 7.45 (t, 2H, *meta*-H), 7.55 (t, 1H, *para*-H), 7.95 (d, 2H, *ortho*-H). ^{13}C NMR (400 MHz, $CDCl_3$, 298 K): δ 54.9 (s, 1C, CH_2), 125.8 (s, 1C, aryl carbon with CNO), 127.0 (s, 2C, *ortho*-C), 128.7 (s, 2C, *meta*-C), 132.7 (s, 1C, *para*-H), 163.4 (s, 1C, N═C—O), 175.9 (S, 1C, carbonyl carbon). Synthesis of 2-phenyl-4-methyl-5-oxazolone from *N*-benzoylalanine and *N*,*N*′-dicyclohexylcarbodiimide was similar.

Modeling. Molecular modeling was performed by means of AM1 and ZINDO, HyperChem (Hypercube, Inc., Guelph, Ontario). The structures were found by means of an iterative process where the geometry of the ion under consideration was optimized via a minimization of the ion's total energy.

(23) Burlet, O.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 461−469.
(24) Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 477−481.
(25) Cox, K. A.; Gaskell, S. J.; Morris, M.; Whiting, A. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 522−531.
(26) Summerfield, S. G.; Bolgar, M. S.; Gaskell, S. J. *J. Mass Spectrom.* **1997**, *32*, 225−231.
(27) Summerfield, S. G.; Whiting, A.; Gaskell, S. J. *Int. J. Mass Spectrom. Ion Processes* **1997**, *162*, 149−161.
(28) Alexander, A. J.; Boyd, R. K. *Int. J. Mass Spectrom. Ion Processes* **1989**, *90*, 211−240.
(29) Alexander, A. J.; Thibault, P.; Boyd, R. K. *Int. J. Mass Spectrom. Ion Processes* **1990**, *98*, 107−134.
(30) Tang, X.-J.; Thibault, P.; Boyd, R. K. *Anal. Chem.* **1993**, *65*, 2824−2834.
(31) Yeh, R. W.; Grimley, J. M.; Bursey, M. M. *Biol. Mass Spectrom.* **1991**, *20*, 443−450.
(32) Kenny, P. T. M.; Nomoto, K.; Orlando, R. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 95−97.
(33) Schwartz, B. L.; McClain, R. D.; Erickson, B. W.; Bursey, M. M. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 339−342.
(34) Morgan, D. G.; Bursey, M. M. *Biol. Mass Spectrom.* **1993**, *22*, 502−510.
(35) Fabris, D.; Kelly, M.; Murphy, C.; Wu, Z.; Fenselau, C. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 652−661.
(36) Johnson, R. S.; Krylov, D.; Walsh, K. A. *J. Mass Spectrom.* **1995**, *30*, 386-387.
(37) Yalcin, T.; Khouw, C.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 1165−1174.
(38) Yalcin, T.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 233−242.
(39) Ambihapathy, K.; Yalcin, T.; Leung, H.-W.; Harrison, A. G. *J. Mass Spectrom.* **1997**, *32*, 209−215.
(40) Nold, M. J.; Wesdemiotis, C.; Yalcin, T.; Harrison, A. G. *Int. J. Mass Spectrom. Ion Processes* **1997**, *164*, 137−153.
(41) Arnott, D.; Kottmeier, D.; Yates, N.; Shabanowitz, J.; Hunt, D. F. *Proceedings of the 42nd ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, IL, 1994; p 470.
(42) Cordero, M. M.; Houser, J. J.; Wesdemiotis, C. *Anal. Chem.* **1993**, *65*, 1594−1601.
(43) Grahl-Nielsen, O.; Solheim, E. *Anal. Chem.* **1975**, *47*, 333−335.

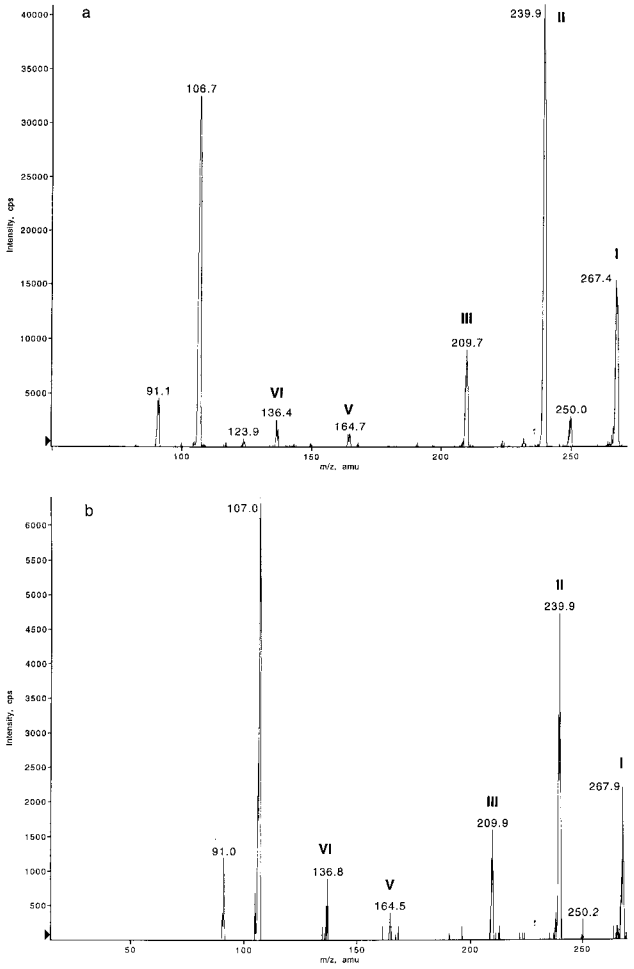


Figure 1. Product ion spectra at $E_{cm} = 2$ eV: (a) $[M + Ag]^+$, M = 2-phenyl-5-oxazolone; (b) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = *N*-benzoylglycine. See Scheme 1 for ion structures; X = phenyl and R = H.

Results and Discussion

Since the b ion of protonated peptides is found to be a protonated oxazolone,[37−41] and given the fact that b, a, and y ions are abundant product ions observed in the CID of protonated[10−12] and argentinated peptides,[8,9] a logical starting candidate structure for the $[b - H + Ag]^+$ ion is the argentinated oxazolone. Figure 1a shows the product ion spectrum of the $[M + {}^{107}Ag]^+$ ion from electrospraying a solution containing 2-phenyl-5-oxazolone and Ag^+ (all product ion spectra involving Ag^+ will be those of the ^{107}Ag isotope; no subsequent isotope identification will be made from now on). Figure 1b shows, as a comparison, the product ions of the $[b_2 - H + Ag]^+$ ion generated from fragmentation of $[M + Ag]^+$ in the lens region, where M is *N*-benzoylglycine. The $[b_2 - H + Ag]^+$ ion is formed via cleavage of the carbon/hydroxyl bond in the carboxylic group of glycine, but since the N-benzoyl group acts as though it were an N-terminal amino acid residue, the b_2 (as opposed to b_1) description is technically less confusing. The close resemblance of the two spectra is self-evident and strongly suggests that the $[b_2 - H + Ag]^+$ ion of argentinated *N*-benzoylglycine is argentinated 2-phenyl-5-oxazolone. Similarly, a comparison of the product ion spectrum of the $[M + Ag]^+$ ion of 2-phenyl-4-methyl-5-oxazolone and Ag^+ (Figure 2a) and that of the $[b_2 - H + Ag]^+$ from argentinated *N*-benzoylalanine (Figure 2b) shows that the $[b_2 - H + Ag]^+$ ion of *N*-benzoylalanine is argentinated 2-phenyl-4-methyl-5-oxazolone. As

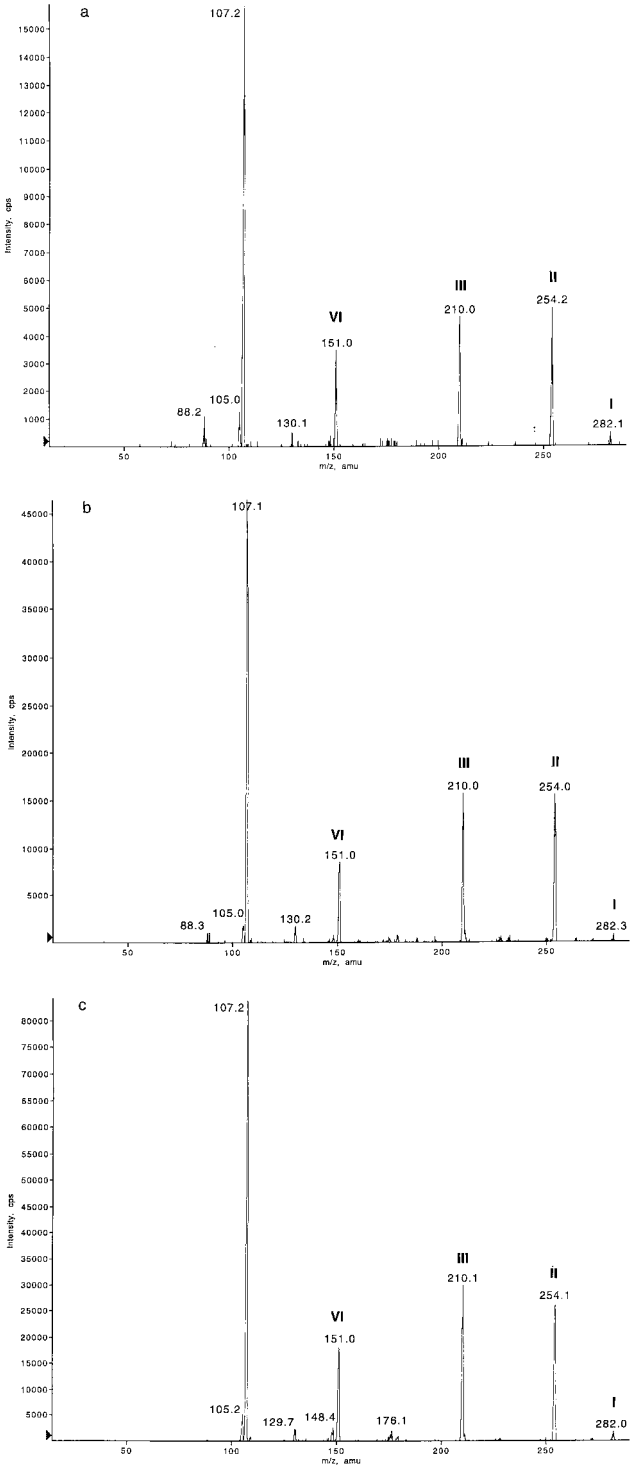
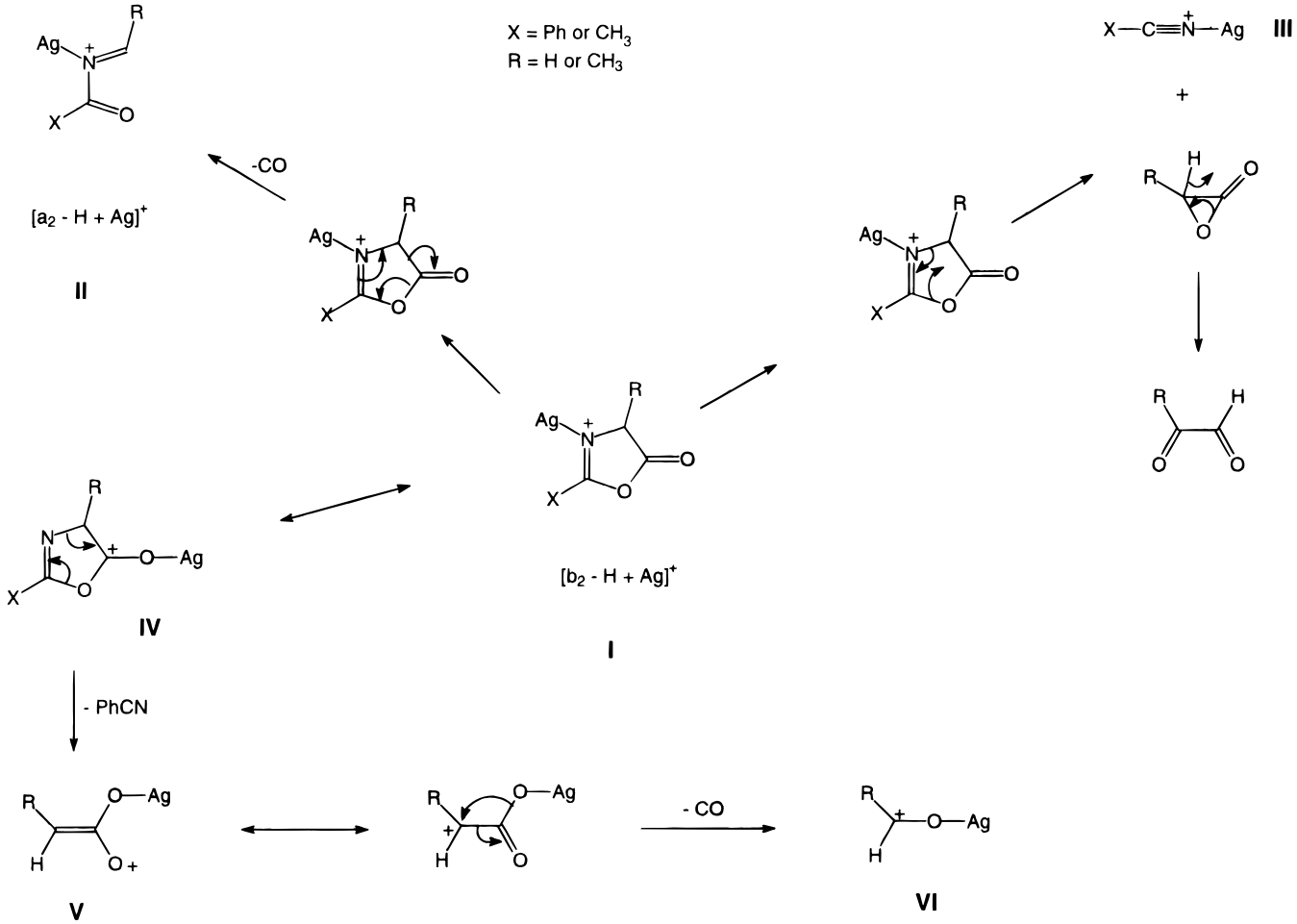


Figure 2. Product ion spectra at $E_{cm} = 2$ eV: (a) $[M + Ag]^+$, M = 2-phenyl-4-methyl-5-oxazolone; (b) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = *N*-benzoylalanine; (c) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = *N*-benzoylalanylalanine. See Scheme 1 for ion structures; X = phenyl and R = CH_3.

expected, the product ion spectrum of $[b_2 - H + Ag]^+$ of argentinated *N*-benzoylalanylalanine (Figure 2c) is virtually identical to that of argentinated *N*-benzoylalanine shown in Figure 2b since in both cases the ion is argentinated 2-phenyl-4-methyl-5-oxazolone. The softest basic site on oxazolone is the nitrogen atom;[44] furthermore, the silver(I) ion is one of the softest Lewis acids known.[44] This means the most likely

(44) Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; John Wiley and Sons: London, 1976; pp 34−85.

Scheme 1



argentination site on oxazolone is the nitrogen atom. Scheme 1 depicts proposed fragmentation reactions leading to the major product ions for X = Ph (phenyl): *m*/*z* 240/254 (**II**, R = H/R = CH_3), *m*/*z* 210 (**III**), *m*/*z* 165/179 (**V**), and *m*/*z* 137/151 (**VI**); the *m*/*z* 107 ion is Ag^+. It is noteworthy that the major product ions of argentinated oxazolones are very different from those of protonated oxazolones. For protonated 2-phenyl-5-oxazolone (*m*/*z* 162), the two major product ions are *m*/*z* 134 ($[M - CO]^+$) and *m*/*z* 105 ($[PhCO]^+$) [ref 37 and our observations], while for protonated 2-phenyl-4-methyl-5-oxazolone, the analogous ions observed are *m*/*z* 148 ($[M - CO]^+$) and *m*/*z* 105 ($[PhCO]^+$) (spectra not shown). Evidently, the central ion plays a critical role in determining the fragmentation patterns.

Although attempts in synthesizing and isolating 2-methyl-5-oxazolones were not successful and, therefore, a direct proof is lacking, the similarity between the fragmentation patterns of argentinated 2-phenyl-5-oxazolones and those of the $[b_2 - H + Ag]^+$ ions of argentinated *N*-acetyl peptides leaves little doubt that these ions are argentinated 2-methyl-5-oxazolones. Figure 3 shows the product ion spectra of the $[b_2 - H + Ag]^+$ ions of (a) argentinated *N*-acetylglycylglycine and (b) argentinated *N*-acetylalanylglycine. The product ions seen and the fragmentation reactions are depicted in Scheme 1 as well for X = CH_3: *m*/*z* 178/192 (**II**, R = H/R = CH_3), *m*/*z* 148 (**III**), *m*/*z* 137/151 (**VI**), and *m*/*z* 107 (Ag^+).

Moving one step further, the similarity between the fragmentation patterns of the $[b_2 - H + Ag]^+$ ions of argentinated peptides, the $[b_2 - H + Ag]^+$ ions of argentinated *N*-acetyl peptides, and argentinated 2-phenyl-5-oxazolones leads to the conclusion that the $[b_2 - H + Ag]^+$ ions of argentinated (non-N-derivatized) peptides are also argentinated oxazolones. Figure 4 shows the product ion spectrum of the $[b_2 - H + Ag]^+$ ion of argentinated alanylalanylglycine, while Scheme 2 displays the proposed product ion structures and fragmentation pathways. Assignment of the major product ions: *m*/*z* 221, **VIII**, $R_1 = R_2 = CH_3$, $[a_2 - H + Ag]^+$; *m*/*z* 206, **IX**; *m*/*z* 178**, X**, an internal ion; *m*/*z* 150, **XI**, an internal immonium ion; *m*/*z* 134, **XII**, $[HCNAg]^+$; and *m*/*z* 107, Ag^+ was aided by H/D exchange by repeating the experiment in D_2O/CH_3OD. The analogous ions observed were *m*/*z* 251, d_2-$[b_2 - H + Ag]^+$; *m*/*z* 223, d_2-$[a_2 - H + Ag]^+$; *m*/*z* 207, d_1-(**IX**); *m*/*z* 179, d_1-(**X**); *m*/*z* 151, d_1-(**XI**); *m*/*z* 135, d_1-(**XII**), $[DCNAg]^+$; and *m*/*z* 107, Ag^+. The lineage of product ions was developed using energy-resolved CID as well as confirmatory precursor or product ion scans. Energy-resolved CID yielded breakdown curves of the various product ion intensities versus collision energy. Under a nitrogen pressure of 2 mTorr in our q2, multiple collisions occur, thus a product ion seen may be the result of sequential fragmentation reactions. A suspected precursor/product relationship may then be confirmed or rejected by raising OR to induce fragmentation and formation of the precursor ion in the lens region, mass-selecting the precursor ion with Q1, fragmenting the ion in q2, and mass-analyzing the product ions with Q3. Figure 5 shows the breakdown curves of the energy-resolved CID experiment of the $[b_2 - H + Ag]^+$ ion of argentinated alanylalanylalanine. Abundances of product ions of smaller *m*/*z* values generally peak at higher collision energies. These observations emphasize the need to select the appropriate collision energy for optimizing the abundances of the ions of interest.

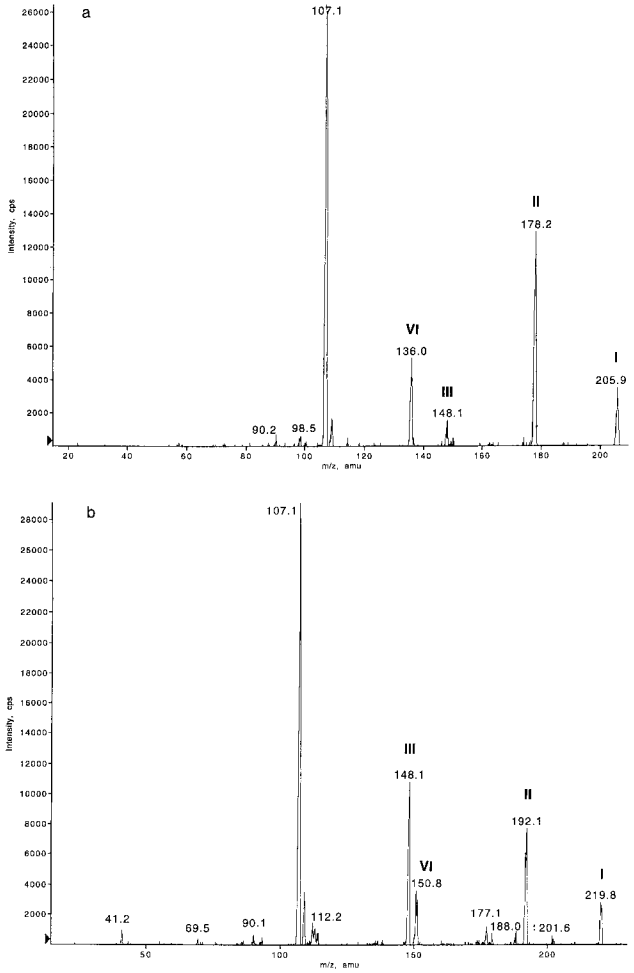


Figure 3. Product ion spectra at $E_{cm} = 2$ eV: (a) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = *N*-acetylglycylglycine; (b) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = *N*-acetylalanylglycine. See Scheme 1 for ion structures; X = CH_3, R = H for (a) and R = CH_3 for (b).

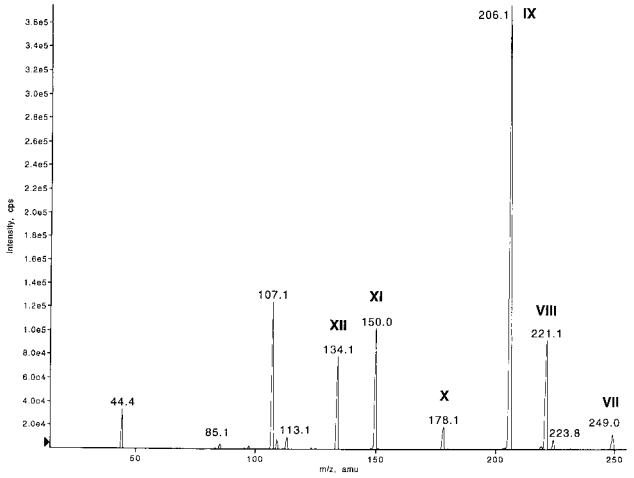


Figure 4. Product ion spectrum at $E_{cm} = 2$ eV: $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = alanylalanylalanine. See Scheme 2 for ion structures; $R_1 = R_2 = CH_3$.

The identities of the product ions in Scheme 2 were confirmed by the CID of the $[b_2 - H + Ag]^+$ ion of other argentinated tripeptides. For example, Figure 6a shows the product ion spectrum for argentinated alanylleucylglycine: *m*/*z* 291, **VII**, $R_1 = CH_3$, R_2 = isobutyl; *m*/*z* 263, **VIII**, $R_1 = CH_3$, R_2 = isobutyl; *m*/*z* 248, **IX**, R_2 = isobutyl; *m*/*z* 221, **VIII**, $R_1 = R_2 = CH_3$; *m*/*z* 192, **XI**, R_2 = isobutyl; *m*/*z* 178, **X**, $R_2 = CH_3$; *m*/*z* 150, **XI**, $R_2 = CH_3$; *m*/*z* 134, **XII**; and *m*/*z* 107, Ag^+. The observation of product ions with $R_2 = CH_3$ (*m*/*z* 221, 178, and 150) is in accordance with a hypothesis in which they are formed from product ions with R_2 = isobutyl after cleavage of the side chain and loss of propene.[45] Fragmentation of the side chain to yield apparent methyl substitution was confirmed in the product ion spectrum of the $[b_2 - H + Ag]^+$ ion of argentinated leucylleucylleucine shown in Figure 6b. Most product ions are identical to those in Figure 6a, many as a result of fragmentation of the isobutyl side chain to yield methyl; *m*/*z* 333 is **VII** with $R_1 = R_2$ = isobutyl, and *m*/*z* 305 is **VIII** again with $R_1 = R_2$ = isobutyl.

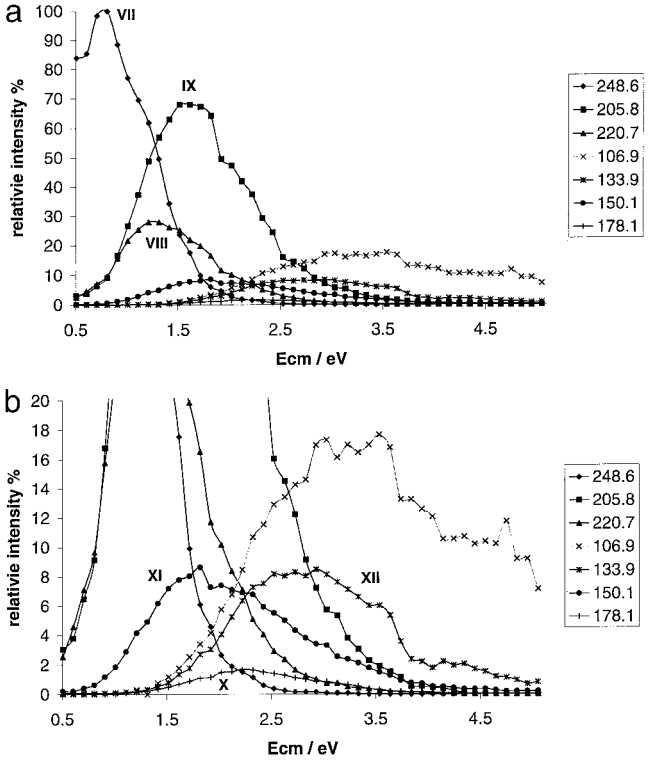


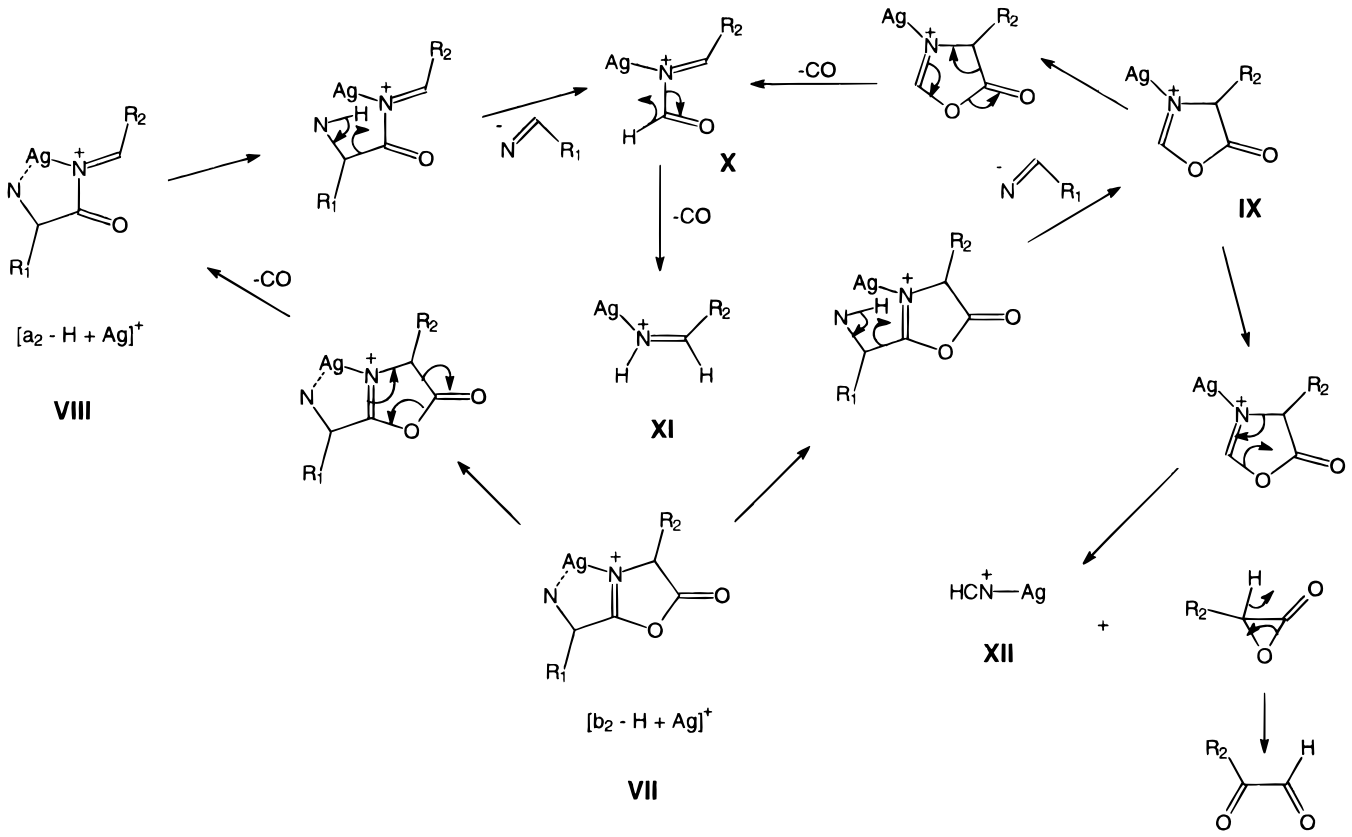
Figure 5. Energy-resolved CID: $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = alanylalanylalanine. See Scheme 2 for ion structures; $R_1 = R_2 = CH_3$.

A comparison of Schemes 1 and 2 reveals differences in fragmentation, between argentinated 2-substituted oxazolones (**I**) and oxazolones of oligopeptides (**VII**). In Scheme 1, it is proposed that the N-argentinated oxazolone (**I**) is interconverting with the less energetically favorable O-argentinated isomer (**IV**), which fragments to yield **V** and **VI**, both O-argentinated product ions. This is to be contrasted with **VII** in Scheme 2 in which Ag^+ is chelated to both nitrogen atoms (ZINDO calculations reveal that the Ag^+ is not in plane with the ring structure and is slightly closer to the N of the second residue (2.220 D) than to that of the first (2.281 D)); hence, it is not available for complexing with the carbonyl oxygen. However, loss of the N-terminal fragment (HN═CH─R_1) provides additional fragmentation routes to yield **IX** and **X**, and their fragmentation products, **XI** and **XII**.

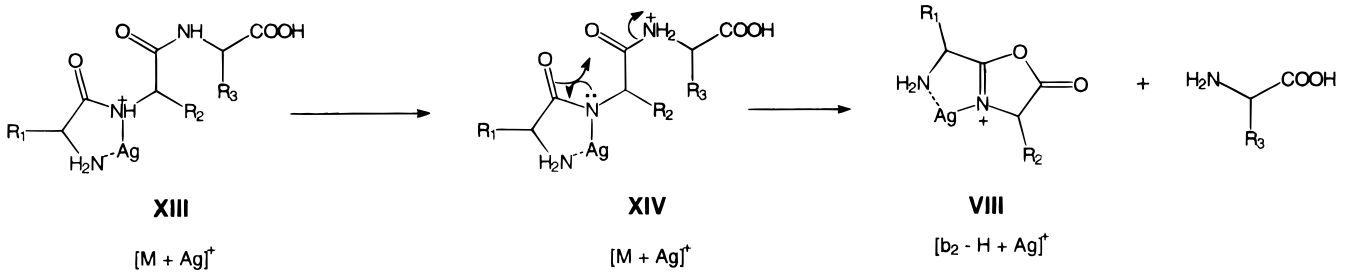
The protonated oxazolone structure of the b ion is believed to form as a result of charge-induced cyclization of protonated oligopeptide followed by elimination of a C-terminal amino acid or peptide.[26,37−41] Yalcin et al.[37−39] and Nold et al.[40] favored protonation of the amide nitrogen of the amide bond to cleave

(45) McLafferty, F. W.; Tureček, F. *Interpretation of Mass Spectra*, 4th ed.; University Science: Mill Valley, CA, 1993.

Scheme 2

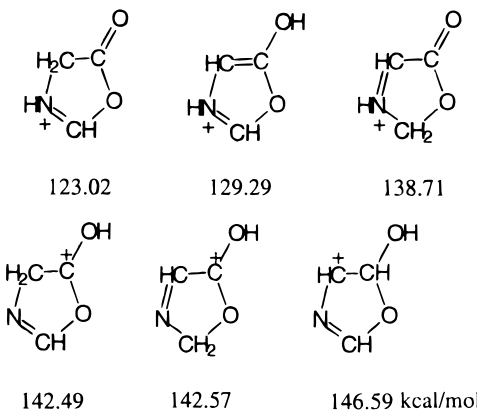


Scheme 3

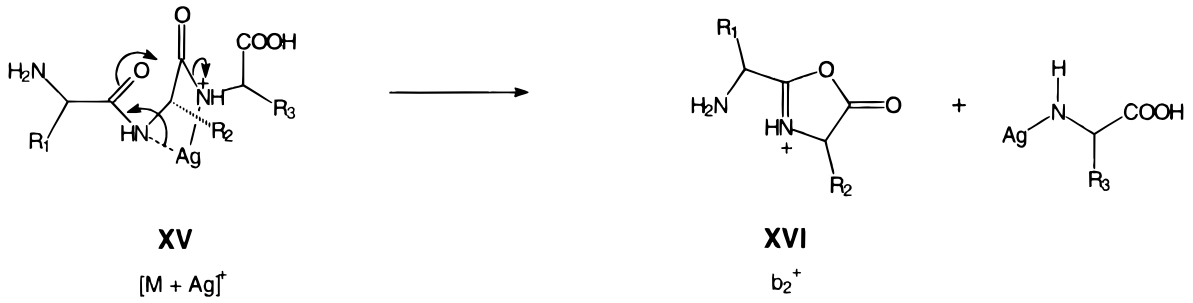


whereas Summerfield et al.[26] and Arnott et al.[41] opted for protonation of the amide oxygen. This second option is in line with the known higher stability of protonation at the amide oxygen than that at the amide nitrogen[10−12,15−18] as well as elegantly allowing the formation of both the b and the y″ product ions from the same precursor ion depending on details of the ensuing fragmentation.[26] It is, however, unlikely to be correct, as it produces an O-protonated oxazolone, which presumably will convert to the N-protonated isomer,[46] but more importantly, a neutral oxazolone fragment in the formation of the y ion, which is not in accordance with recent observations. The neutral fragment that accompanied the formation of the y″ ion from protonated oligopeptides was found to be either an aziridinone or a diketopiperazine, and not an oxazolone.[40,42] For argentinated oligopeptides, the lack of O-argentinated product ions (vide supra) and silver's preference for N- over O-complexation[44] make argentination of the amide oxygen arguably even less likely. Scheme 3 shows the proposed mechanism for the formation of the $[b_2 - H + Ag]^+$ ion from an argentinated tripeptide whose Ag^+ is originally chelated to N atoms (**XIII**). Collision activation of **XIII** results in transfer of the proton at the charge site to the amide nitrogen of the ensuing residue to form **XIV** and subsequent cyclization to form argentinated oxazolone (**VIII**) plus an amino acid. The transfer and loss of an exchangeable proton is in accordance with the observation of d_2-$[b_2 - H + Ag]^+$ (vide supra). A variation of the aforementioned fragmentation is depicted in Scheme 4, in which chelation of Ag^+ to the last two amide nitrogen atoms (**XV**) and cyclization result in the loss of Ag^+ and the formation of

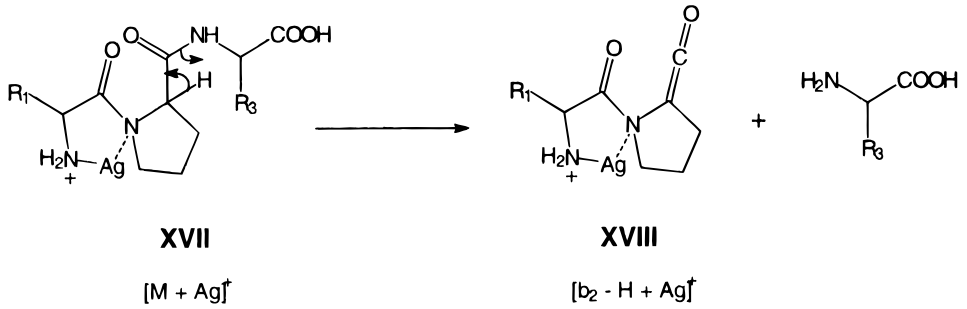
(46) Results of AM1 calculations, in increasing $\Delta_f H$, are



Scheme 4



Scheme 5



protonated oxazolone, b_2^+ (**XVI**), a minor product ion observed in the CID of the $[M + Ag]^+$ ion of argentinated tripeptides.

The proposed mechanism for the formation of the $[b_2 - H + Ag]^+$ ion depicted in Scheme 3 requires that the amide hydrogen of the second residue be transferred to the third residue of the tripeptide, which means that it cannot be operative in any tripeptide that contains proline as the second residue. Indeed, the $[b_2 - H + Ag]^+$ product ions of alanylprolylglycine (APG) and glycylprolylalanine (GPA) were found to be comparatively less abundant than the $[b_2 - H + Ag]^+$ product ions of non-proline-containing tripeptides under identical experimental conditions (Table 1). Scheme 5 depicts a proposed mechanism for the formation of the $[b_2 - H + Ag]^+$ ions of tripeptides that contain proline as the second residue. Similar to **XIII**, **XVII** has its Ag^+ bound to N atoms of the first and second residue. However, the hydrogen that is transferred to the neutral fragment is a −CH− hydrogen on the α-carbon of the proline residue since it lacks an −NH− hydrogen. This mechanism is identical to the one proposed by Teesch et al.[14] for alkali-metal-containing oligopeptides. H/D exchange showed that the $[b_2 - H + Ag]^+$ ion formed is d_2, in accordance with transfer of a −C−H instead of an −N−H hydrogen to the neutral fragment. The $[b_2 - H + Ag]^+$ ion involving proline, **XVIII**, is postulated here to have an argentinated ketene structure, the acylium equivalent of metalated peptide fragments; the acylium ion was hypothesized to be the structure of the activated complex of the b ion prior to association into the a ion and CO.[37,38] The low abundances of the $[b_2 - H + Ag]^+$ product ions of APG and GPA, relative to those of the $[b_2 - H + Ag]^+$ ions of non-proline-containing tripeptides (Table 1), are in accordance with the hypothesis that the acylium, relative to the oxazolone structure, is of higher energy content, and hence the former $[b_2 - H + Ag]^+$ ions have comparatively low abundances.

Conclusions

The $[b_2 - H + Ag]^+$ product ion of argentinated oligopeptides is found to be an N-argentinated oxazolone. This product ion can further fragment to yield the $[a_2 - H + Ag]^+$ and other

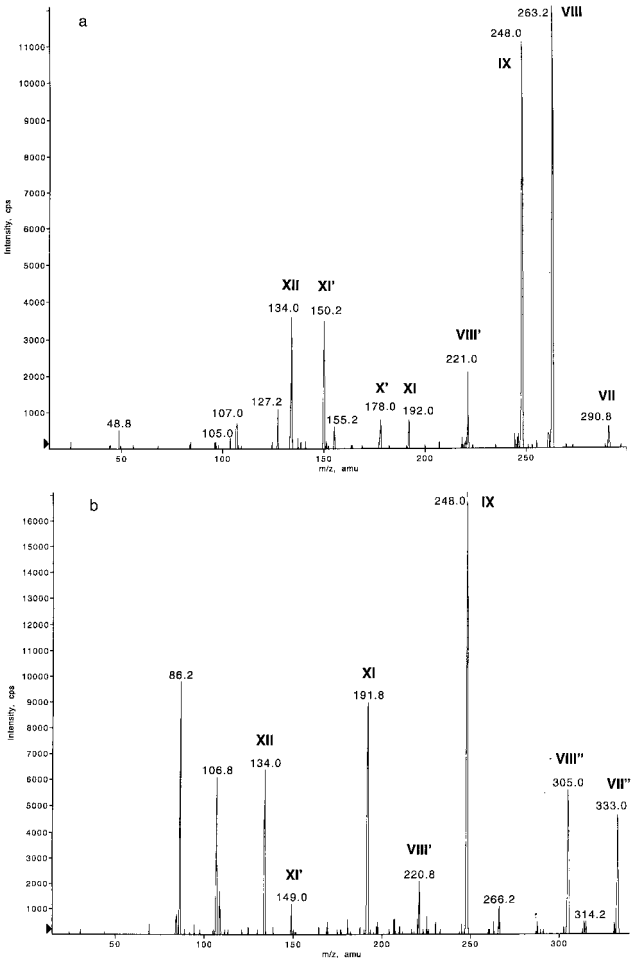


Figure 6. Product ion spectra at $E_{cm} = 2$ eV. (a) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = alanylleucylglycine. See Scheme 2 for ion structures, $R_1 = CH_3$ and R_2 = isobutyl. **VIII′**, **X′** and **XI′** have their $R_2 = CH_3$ after elimination of propene. (b) $[b_2 - H + Ag]^+$ ion of $[M + Ag]^+$, M = leucylleucylleucine. **VII″** and **VIII″** have their $R_1 = R_2$ = isobutyl. See (a) for the identification of the other ions.

Table 1. Percent of $[b_2 - H + Ag]^+$ abundance of the base peak $[M + Ag]^+$ ($E_{cm} = 1.2$ eV)

peptide	% abundance
AGG	29
ALG	37
APG	9
GGA	32
GAA	94
GLA	73
GPA	4

product ions. Reaction schemes leading to these ions have been proposed. The $[b_2 - H + Ag]^+$ ion is postulated to form via proton displacement and migration, cyclization, and elimination of the C-terminal fragment as a neutral amino acid or peptide. Tripeptides having the second residue as proline form their relatively less abundant $[b_2 - H + Ag]^+$ ion via transfer of the −CH− hydrogen α to the cleaved amide bond. The $[b_2 - H + Ag]^+$ ion of these peptides is hypothesized to be an argentinated ketene.

Acknowledgment. Financial support to V.W.M.L. and T.C.L. from the RGC of Hong Kong are gratefully acknowledged. H.L. thanks Carleton University for a studentship.

JA9808245