Elucidation of Fragmentation Pathways for the Collision-Induced Dissociation of the Binary Ag(I) Complex with Phenylalanine

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During our ongoing investigation of the formation and reactivity of gas-phase complex ions composed of Ag(I) and various α-amino acids, we discovered that the mass-to-charge ratio for the major collision-induced dissociation (CID) product generated from a binary Ag^+ complex with phenylalanine was consistent with the formation of an Ag^+ complex with an aldehyde. In this study we investigated and compared the fragmentation pathways for complexes of Ag^+ with phenylalanine, phenylalanine with exchangeable protium replaced with deuterium, phenylalanine with the carboxylic acid group labeled with ^{13}C, and phenylalanine with the benzylic group labeled with deuterium. The reaction pathways were determined using multidimensional dissociation steps in an ion-trap mass spectrometer. The dissociation experiments provide clear evidence for the formation of several novel product species, including the Ag^+ complex with phenylacetaldehyde, as well as the formation of an Ag^+ complex with either a benzyl carbene or styrene. These dissociation products are markedly different from those observed following the fragmentation of other transition and alkali metal adducts of phenylalanine. On the basis of the dissociation of the various isotope-exchanged and -labeled versions of phenylalanine, we propose several reaction pathways that implicate the formation of an Ag^+ complex with an aziridinone (α-lactam), for which a peak at the correct mass-to-charge ratio was observed in the MS/MS spectrum of the $(M + Ag)^+$ ion. A comparison of the apparent reactivity toward water and methanol in the ion-trap mass spectrometer of the Ag^+-containing product ions to Ag^+ complexes with various low-mass organic molecules provided further evidence to support the proposed formation of the aldehyde and styrene complexes with Ag^+ ions. For instance, the apparent reactivity of the Ag^+/aldehyde product ion generated from the CID of the $(M + Ag)^+$ ion is identical to that observed for a complex produced by the electrospray ionization of a solution containing Ag^+ ions and neat phenylacetaldehyde. Similar results were obtained for a dissociation product ion assumed to be a complex composed of Ag^+ ions and styrene.

Introduction

Covalent and noncovalent interactions between metal ions and organic molecules are of fundamental interest in areas ranging from the biological function of metalloproteins and enzymes to coordination chemistry and catalysis. The interactions between molecules bearing electron-donating groups such as amines,[1−9] carbonyls and alcohols,[10−16] crown ethers,[17−26] glymes,[22,27,28] cryptands,[29−31] and calixarenes[32−35] and alkali or transition metal ions have been investigated using a variety of mass spectrometric techniques. In addition, the collision-induced dissociation (CID) of metal-ion adducts of biomolecules such as amino acids,[36−48] peptides,[49−61] and oligosaccharides[62−64] has been scrutinized due to interests in measuring metal-ion affinities and identifying metal-ion binding sites, and because the metal-ion adducts of these molecules often exhibit dissociation pathways that are distinct from those of their protonated analogues. In particular, Gross and co-workers have discussed both the influence of amino acid side-chain groups containing aromatic rings on the binding of metal ions to peptides and the fragmentation pathways observed in dissociation experiments.[61]

Recent investigations by Sui and co-workers[65−69] have probed the production of Ag(I) complexes with amino acids and peptides and shown that the CID of Ag(I)-cationized (argentinated) peptides may be a valuable tool for the determination of peptide primary sequence by mass spectrometry in the burgeoning field of proteomics. In an effort to better understand the activation and dissociation of Ag(I) complexes with biologically relevant molecules, we have initiated a comprehensive investigation of the gas-phase reactions of Ag(I)-cationized amino acids and model peptides. Recently, we reported experiments in which binary Ag^+(I) complexes with certain α-amino acids reacted upon isolation and storage in an ion-trap mass spectrometer to form water and methanol adduct ions.[70] Our general observation with respect to reactivity and amino acid composition was that amino acids with aliphatic groups at the α-carbon position such as alanine, *tert*-leucine, valine, and α-aminocyclohexanepropionic acid formed adduct ions, while those with aromatic side groups such as phenylalanine, tyrosine, and tryptophan did not. By way of a preliminary molecular-modeling investigation, we found that the aromatic ring in phenylalanine coordinates the Ag ion along with the carbonyl oxygen and the amine nitrogen, and this three-coordinate type of geometry inhibits the uptake of H_2O and MeOH in the gas phase. In a study that preceded ours, Dunbar, Wesdemiotis, and co-workers showed that the Na^+ and K^+ complexes with phenylalanine (along with tyrosine and tryptophan) adopt a similar geometry,[71] indicating that the conformation adopted by the Ag^+ complex is not unique. In our study, however, we found that the alkali-metal complexes with the amino acids, regardless

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of the functional group at the α-carbon position, did not form H_2O and MeOH adduct species when compared to the Ag^+ complexes under identical experimental conditions.[70] This suggests that the presence of Ag^+ is central to the reactivity that was observed, and that these gas-phase association reactions may probe general features of gas-phase conformation and electronic structure when combined with high-level ab initio calculations. A comparison of the H_2O and MeOH adduct formation exhibited by phenylalanine, 4-fluorophenylalanine, 4-nitrophenylalanine, and α-aminocyclohexanepropionic acid demonstrated that the π-electron density within the ring functional group significantly influences the reactivity of the Ag^+/amino acid complexes.[72] In general, decreased delocalized electron density leads to greater reactivity or, more appropriately, greater apparent reaction rates.

In our earlier report, we showed briefly that the CID of the Ag^+ complexes with phenyalanine, tyrosine, and tryptophan, causing a change in conformation and/or loss of coordinating functional groups, in turn led to the formation of H_2O and MeOH adducts to the Ag-cationized product ions.[70] Because of the profound influence of the π-system$-Ag^+$ interaction on the reactivity observed in the gas phase, we are also investigating the dissociation pathways for a range of aromatic and aliphatic amino acid/Ag^+ complexes when fragmentation is induced by collisions with the He buffer gas in an ion-trap mass spectrometer. The fragmentation reactions for amino acids cationized by the attachment of mono-, di-, and trivalent metal ions such as Co^+, Fe^+, Cu^+, Ni^+, Ni^{2+}, Cu^{2+}, and Al^{3+} have been investigated.[36−44] To date, however, less attention has been paid to the fragmentation of Ag(I)-cationized amino acids.

In this report, we elaborate on the CID pathways discerned for the binary Ag^+ complex with phenylalanine. A description of the CID of the Ag^+ complexes with a range of α-amino acids will be the subject of a forthcoming report. Our focus on the phenylalanine complex is due to our observation that the mass-to-charge ratio (*m*/*z*) of the most prominent dissociation product was consistent with the formation of an Ag^+/aldehyde complex, indicating the loss of the amine functional group. This product ion is quite distinct from those obtained in the dissociation of protonated phenylalanine or phenylalanine cationized by the attachment of alkali or other transition-group ions. For instance, the major dissociation product for the $(M + H)^+$ ion derived from phenylalanine is an immonium ion formed via the loss of H_2O and CO. As described here, we used a comparison of the CID spectra obtained from the binary complexes of Ag^+ with phenylalanine, phenylalanine with exchangeable protium replaced with deuterium, phenylalanine with the carboxylic acid group labeled with ^{13}C (*carboxy*-^{13}C phenylalanine), and phenylalanine with the benzylic group labeled with deuterium (β,β-d_2-phenylalanine) to elucidate the fragmentation pathways for the CID of the $(M + Ag)^+$ ion. On the basis of these experiments, we propose that the generation of the Ag^+/aldehyde product ion may involve the formation of an α-lactam (or aziridinone) intermediate. Because we have found that the tendency for Ag(I) complexes to form H_2O and MeOH adduct species upon storage in the ion trap is sensitive to the stoichiometry and structure of the Ag^+/molecule complex, we also investigated the possibility that the ion/molecule chemistry in the ion trap might provide additional evidence to support the proposed product-ion compositions.

Experimental Section

Phenylalanine, *carboxy*-^{13}C phenylalanine (99 at. % ^{13}C), β,β-d_2-phenylalanine (98 at. % D), phenylacetaldehyde, benzaldehyde, acetophenone, styrene, ethyl benzene, *p*-xylene, and silver nitrate ($AgNO_3$) were purchased from Aldrich Chemical (St. Louis, MO) and used as received. One millimolar solutions of each organic compound were prepared by dissolving the appropriate amount of solid material in a 1:1 (v:v) mixture of HPLC-grade MeOH and deionized H_2O. The 1 mmol solution of $AgNO_3$ was prepared in deionized H_2O. To produce amino acids in which the exchangeable amine and carboxyl protons were replaced by deuterons, 1 mmol solutions of the respective phenylalanine analogues were prepared in a 1:1 (v:v) mixture of D_2O and CD_3OD (also purchased from Aldrich Chemical) and allowed to incubate for >2 h.

ESI mass spectra were collected using a Finnigan LCQ-Deca ion-trap mass spectrometer (Thermoquest Corporation; San Jose, CA). Mixtures (1:1 v:v) of $AgNO_3$ and amino acid, prepared by mixing 0.5 mL of the respective stock solutions, were infused into the ESI-MS instrument using the incorporated syringe pump and a flow rate of 3−5 μL/min. The deuterium-exchanged amino acids were combined with an $AgNO_3$ solution prepared using D_2O. The atmospheric pressure ionization stack settings for the LCQ (lens voltages, quadrupole, and octapole voltage offsets, etc.) were optimized for maximum $(M + Ag)^+$ ion transmission to the ion-trap mass analyzer by using the auto-tune routine within the LCQ tune program. After the instrument was tuned, the spray needle voltage was maintained at +5 kV, the N_2 sheath gas flow at 25 units, and the capillary (desolvation) temperature at 200 °C. Though this capillary temperature caused some apparent thermal degradation of the phenylalanine, 200 °C provided the best signal-to-background ratios for these experiments. The ion-trap analyzer was operated at a pressure of $\sim 1.5 \times 10^{-5}$ Torr. Helium gas, admitted directly into the ion trap, was used as the bath/buffer gas to improve trapping efficiency and as the collision gas for CID experiments.

The studies of Ag^+ complex ion-dissociation pathways were performed as follows. To identify product ions that retained the Ag^+ cation, the $(M + Ag)^+$ complex ions derived from the various phenylalanine solutions were isolated by setting the parent mass (within the Define Scan window using the Advanced Scan Features of the LCQ operating software activated) between the ^{107}Ag and ^{109}Ag isotopic peaks. The isolation width was set at 6 *m*/*z* units to isolate the entire Ag^+/molecule isotope distribution. To induce collisional activation, the activation amplitude (which defines the amplitude of the radio frequency (RF) energy applied to the end-cap electrodes) for CID was set at 30−35% of the maximum "tickle" energy available (chosen empirically), the activation Q setting (as labeled by Thermoquest, used to adjust the frequency of the RF excitation voltage placed on the end caps) was set at 0.25 unit, and the isolation width was set at 6 *m*/*z* units. The activation time employed was 30 ms. In some cases, the dissociation pathways were also examined by isolating only the isotope peak corresponding to the fully deuterated amino acid and ^{109}Ag to remove any ambiguity in data interpretation.

The studies of the Ag^+ complex ion reactivity during storage within the ion-trap mass spectrometer were performed as described previously. Briefly, $(M + Ag)^+$ complex ions derived from solutions of phenylacetaldehyde, acetophenone, benzaldehyde, styrene, ethyl benzene, and *p*-xylene were isolated by setting the parent mass between the ^{107}Ag and ^{109}Ag isotopic peaks. Ions were isolated and stored with minimal activation by setting the activation amplitude at 0%. The isolation width and activation Q setting were set at 6 *m*/*z* units and 0.550 units, respectively. To monitor the formation of adduct ions, the Ag^+/amino acid complex ions, $(M + Ag)^+$, were isolated and stored

within the ion trap for periods ranging from 3 to 3000 ms; during this time, reactions occurred with H_2O and MeOH that was present within the ion-trap bath gas. As noted in a report by Vachet et al., both water and methanol will accumulate, due to their use as the ESI solvent, in the vacuum system at partial pressures sufficiently high to conduct ion/molecule reactions with these reagents in an ion trap.[9] After the isolation period, the precursor and product ions were scanned out of the trap and detected as part of the automated mass-analysis operation.

To measure the tendency for the product ions generated from the dissociation of the Ag^+/phenylalanine complex to form the adduct species, the ^{107}Ag and ^{109}Ag isotope peaks were isolated using an isolation width of 6 *m*/*z* units and subjected to collisional activation. The dissociation step was carried out as described above. After the dissociation step, selected product ions generated from the Ag^+/phenylalanine complexes were isolated (without further activation) using a width of 6 *m*/*z* units and an activation Q setting of 0.55 unit and subjected to H_2O and MeOH uptake studies as described above.

The data collected for the measurements of adduct-ion formation, including those following CID, represent the average of 20 isolation/reaction/scan sequences at isolation times ranging from 0.3 to 300 ms and the average of 5 isolation/reaction/scan sequences at an isolation time of 3000 ms. The mass spectra shown are the result of the averaging of 10−30 analytical scans. The spectra were generated by importing the peak intensity values for a particular experimental run, obtained from the Spectrum List option within the Xcalibur software (Thermoquest Corporation), into SigmaPlot version 4.1 (SPSS Science; Chicago, IL) and are plotted as intensity relative to that of the most abundant ion versus *m*/*z* in units of Thomson (Th). Though the Thomson unit refers to one atomic mass unit per unit positive charge,[73] we also use the Thomson unit in the discussion below to label all neutral losses. This was done for the sake of consistency in communicating the masses of fragment ion and neutral species.

Results and Discussion

ESI-MS and MS^n Spectra. Figure 1a shows the ESI mass spectrum derived from a H_2O/MeOH solution containing a 1:1 molar combination of $AgNO_3$ and phenylalanine. The prominent ions generated by ESI included the bare silver ion at 107 and 109 Th, the protonated molecule ion of phenylalanine at 166 Th, the $(M + Ag)^+$ ion at 272 and 274 Th, and an Ag^+-cationized dimer of the neutral phenylalanine at a *m*/*z* of 437 and 439 Th (not shown in Figure 1a). In addition, the immonium ion characteristic of phenylalanine at 120 Th was also observed. The MS/MS spectrum derived from the $(M + Ag)^+$ ion is shown in Figure 1b. Both Ag isotope peaks were isolated for the dissociation event to determine the number of fragment ions conserving the Ag cation. At the CID conditions employed here, four prominent fragment ions containing the Ag cation were generated, including pairs of peaks at 254 and 256, 227 and 229, 211 and 213, and 124 and 126 Th. The fact that several fragment ions retain the cation is indicative of the strong bonding between the amino acid and the Ag^+ ion. Similar observations were made in an investigation of the Ag^+ adducts of several peptides.[65,67−69] In the present case, the strong bonding is presumably due to interactions between the metal ion and the carbonyl oxygen atom and between the amine nitrogen atom and the aromatic ring. The CID of the Ag^+-cationized dimers of neutral phenylalanine produced the $(M + Ag)^+$ species as the predominant product ion. As a control experiment, the CID spectra of the $(M + K)^+$ and $(M + Cs)^+$ complexes with phenylalanine were also collected (spectra not shown). For either alkali metal ion, the only dissociation product observed was the bare cation. Figure 2 shows the MS^3 spectra produced from the isolation and CID of (a) the 254 and 256 Th and (b) the 227 and 229 Th fragment ions. The MS^3 spectra of the product ions at 211 and 213 Th and at 124 and 126 Th included only the bare Ag^+ ion. The proposed product-ion compositions and fragmentation pathways are discussed in the following section.

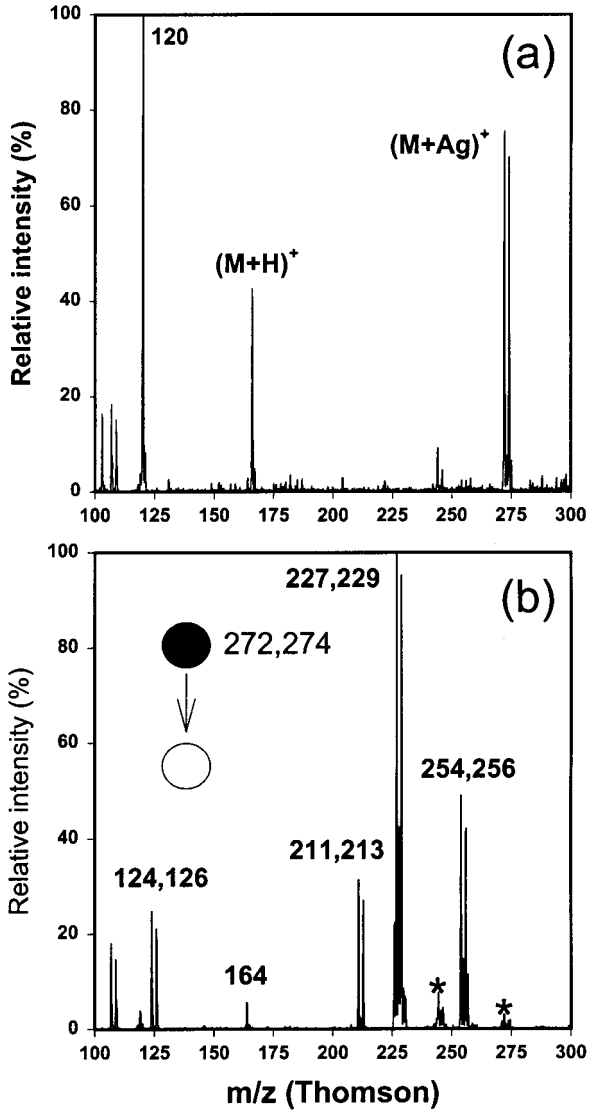


Figure 1. ESI mass spectra of a 50:50 mixture of phenylalanine (native) and $AgNO_3$: (a) full spectrum and (b) MS/MS spectrum of the $(M + Ag)^+$ precursor ion. Peaks labeled with asterisks correspond to water adducts to product ions formed by gas-phase reactions in the ion trap.

Collision-Induced Dissociation of the Ag^+ Complex with Phenylalanine. In our earlier investigation, molecular-modeling calculations [using MOPAC (PM3 level)] within the SPARTAN group of programs (version 5.1.3, Wave Function, Inc.; Irvine, CA) were used to determine the lowest-energy conformation of the Ag^+ complexes with alanine and phenylalanine. While the conformation of the Ag^+/alanine complex involved a coordination by the carbonyl oxygen and the amine nitrogen, the lowest-energy conformation predicted for the phenylalanine complex included the aromatic ring as a third coordinating group.[70] On the basis of gas-phase studies of the association reactions between Ag^+ ions and nitrogen- or oxygen-donor ligands, the interaction between the metal ion and the amine and carbonyl groups has been described as being electrostatic

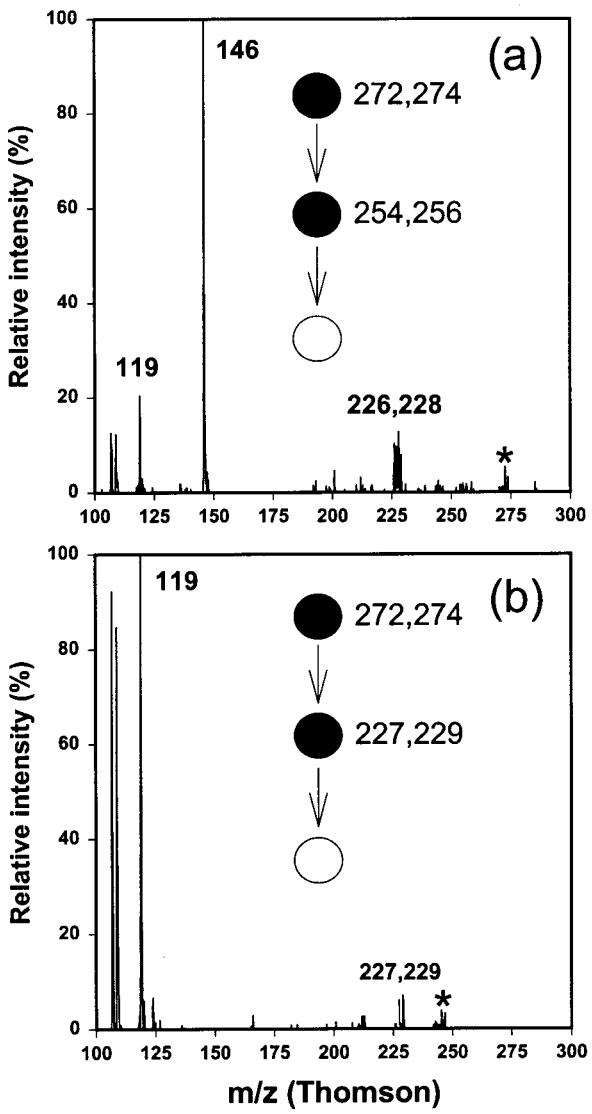


Figure 2. MS^3 spectra produced from the (a) 254 and 256 Th product ion and (b) 227 and 229 Th product ion generated from the CID of the $(M + Ag)^+$ ion derived from native phenylalanine. Peaks labeled with asterisks correspond to water adducts to product ions formed by gas-phase reactions in the ion trap.

in nature.[74,75] The cation−ring interaction may also be electrostatic in nature (i.e., via a cation−quadrupole interaction), but also has the potential for covalent character due to the overlap of p π-orbitals on the ring and a σ acceptor orbital on the metal ion. Currently, we have no quantitative data to describe the metal ion−ring interaction in detail, though we have discovered that changes in ring electron density influence the gas-phase reactivity of binary Ag(I)/*para*-substituted phenylalanine complex ions. As noted earlier, a "three-coordinate" type conformation, albeit one that would lack the covalent interaction between cation and ring, was reported by Dunbar, Wesdemiotis, and coworkers in a study of cation−π-orbital interactions in the formation of alkali metal ion adducts of phenylalanine.[71]

As mentioned above, four major fragmentation pathways were discerned for the CID of the $(M + Ag)^+$ complex ion. The fragment ions resulting from the MS/MS and selected MS^3 experiments are listed in Table 1. For the sake of clarity, the data shown in Table 1 summarize the isolation and dissociation of the $(M + {}^{109}Ag)^+$ isotope peak. Included in the table are the mass shifts for each peak observed when the deuterium-exchanged version of phenylalanine was subjected to CID (shown in boldface print). The mass shifts, or lack thereof, due to the incorporation of ^{13}C into the carboxy moiety or deuterium into the benzylic portion of phenylalanine (not shown in Table 1), are described where they are relevant to a particular fragmentation pathway. The intensities of the fragment ions, relative to that of the most abundant fragment ion observed in the CID spectrum, are included in parentheses.

The highest-mass product ion observed in the dissociation of the Ag^+ complex with native phenylalanine was the result of a neutral loss of 18 Th to produce the doublet of ions at 254 and 256 Th (the doublet is due to the ^{107}Ag and ^{109}Ag isotopes). A corresponding doublet of ions located at 255 and 257 Th, generated by the neutral loss of 20 Th, was observed following the CID of the deuterium-exchanged form of the complex. In either case, we propose that the fragmentation pathways lead to the production of the ion labeled as A′ in Scheme 1. The reaction pathways in Scheme 1, and those discussed later in Schemes 2 and 3, are shown using the complex composed of the deuterium-exchanged form of phenylalanine and the ^{109}Ag isotope. On the basis of the comparison of the native and deuterium-exchanged forms of the amino acid, the loss of 18 or 20 Th corresponds to the elimination of H_2O or D_2O as the neutral fragment. The production of A′ can be explained most simply as a novel cyclization to yield an aziridinone (also known as an α-lactam) with the Ag cation attached. We note that our earlier semiempirical calculations indicated that the lowest-energy conformation for the Ag^+/phenylalanine complex ion was one in which the aromatic ring, amine nitrogen atom, and carbonyl oxygen atom coordinate the metal ion. This conformation has also been found to be the lowest in energy using Hartree−Fock (HF/3-21G*) and density-functional theory (B3LYP/3-21G*) calculations.[72] The coordination of the amine functionality is plausible on the basis of the affinity of Ag^+ ions, weak Lewis acids, for nitrogen-donor ligands. The loss of H_2O or D_2O from the complex ion, however, requires that the carboxylic acid proton be located in close proximity to the amine functional group, i.e., a coordination by the hydroxyl oxygen instead of the carbonyl oxygen. Our continuing ab initio investigation of the complex geometry should provide accurate estimates of the relative energies associated with the two conformations. We assume, however, that the collisional activation imposed to induce fragmentation may give the complex enough energy to rearrange and thereby facilitate the loss of H_2O or D_2O. The formation of the closed-ring structure may also be aided by the close coordination of Ag^+ ions by the $-ND_2$ group, thus enhancing its acidity and tendency to shed D^+ (in conjunction with DO^- from the carboxyl moiety).

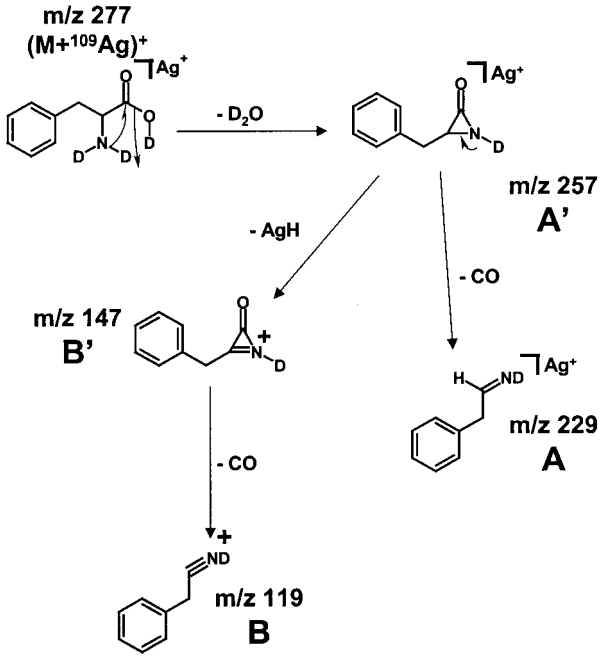
Isolation and subsequent CID (MS^3) of A′ produced four product ions. One product ion was the bare Ag^+ ion. The highest-mass fragment produced from ion A′ corresponded to the loss of 28 Th to afford what may be the ion labeled as A in Scheme 1. Because the loss of CO is a known process in the decomposition of aziridinones, for instance, upon photochemical irradiation (by a Norrish "type 1" process) or upon electron impact,[76] we postulate that ion A′ undergoes a loss of CO to yield A, which corresponds to a complex of an imine ($PhCH_2$-CH=ND) with Ag^+ ions. When the same product ion was generated from the *carboxy*-^{13}C phenylalanine complex with Ag^+ ions, the neutral CO that was released in the fragmentation reaction contained the ^{13}C label, in agreement with our proposed pathway. The same type of product ion was reported following the reaction of Cu^+ ions with neutral phenylalanine[44] and that of the CID of a Ni^+ complex with the amino acid.[40] In these

TABLE 1: MS and MS³ Pathways for the Dissociation of the (M + Ag)⁺ Species[a]

	product ions				
		MS/MS			
274 precursor (M + Ag)⁺	109 (18%)	126 (23%) **129**	213 (26%) **213**	229 (100%) **229**	256 (50%) **257**
		MS³			
256 precursor	109 (40%)	119 (20%) **121**	146 (100%) **148**	228 (12%) **229**	
229 precursor	109 (100%)	119 (60%) **119**			
213	109 (100%)				

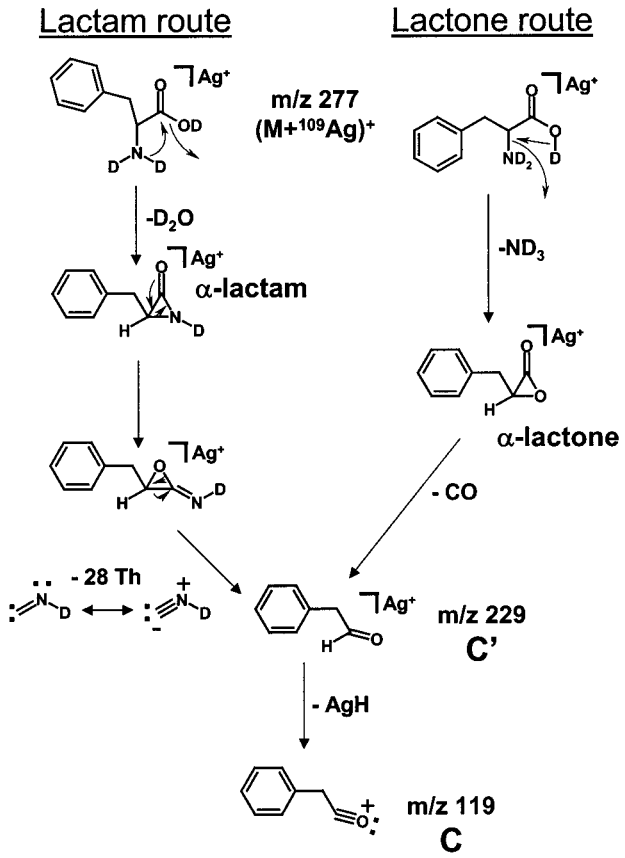
[a] Boldface type indicates the shift in mass observed for the deuterium-exchanged version of phenylalanine. Numbers in parentheses indicate the relative intensity of each fragment ion relative to that of the most abundant product.

SCHEME 1: Proposed Reaction Pathways for the Dissociation of the (M + Ag)⁺ Ion Derived from Phenylalanine[a]



[a] Scheme includes the MS/MS pathway to produce the 255 and 257 Th product ions (deuterium-exchanged version of phenylalanine) and associated MS³ pathways.

SCHEME 2: Proposed Reaction Pathways for the Dissociation of the (M + Ag)⁺ Ion Derived from Phenylalanine[a]



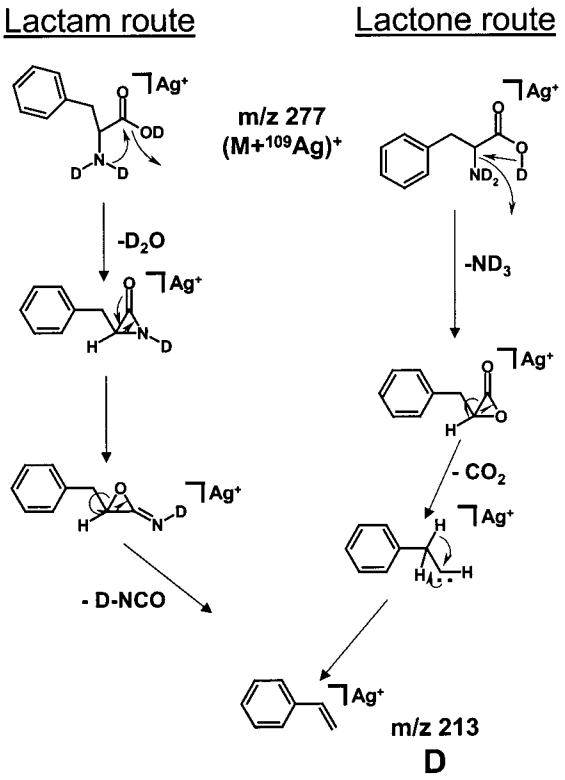
[a] Scheme includes the MS/MS pathway to produce the 227 and 229 Th product ions (deuterium-exchanged version of phenylalanine) and associated MS³ pathways.

two cases, however, the product ion was not demonstrated in the MS³ spectrum derived from the dissociation of a stable intermediate such as the aziridinone complex proposed above.

The third and most prominent product ion observed in the MS³ spectrum of ion A′ corresponded to the loss of 108 and 110 Th to yield ion B′. The MS⁴ of this ion in turn produced ion B through the loss of 28 Th. We suggest that B′ is the result of the introduction of an endocyclic double bond into the aziridinone through the loss of AgH (108 and 110 Th), which would make ion B′ an azirinone. Azirinones are isoelectronic with the well-characterized cyclopropenones (which can be considered to be 2π aromatic systems when the carbonyl group is highly polarized). By analogy with aziridinones, an azirinone could lose CO and yield ion B, a nitrilium ion.

A fourth and minor fragmentation pathway observed in the CID of ion A′ resulted in the production of ion G (121 Th) with the deuterium-exchanged form of phenylalanine and the puzzling but probably related production of an ion at 119 Th for the native form of phenylalanine; both ions were devoid of silver. The proposed compositions of the ions at 121 (labeled as ion G) and 119 Th (labeled as ion H) are provided in Figure 3. Because the formation of ion A′ resulted in the loss of two deuterium atoms from the (M + Ag)⁺ ion, ion G (121 Th) should contain only one deuterium atom. Ion G should therefore differ from the ion at 119 Th, observed following the CID of the native phenylalanine/Ag⁺ complex, by one mass unit if G and H have the same structure. Because G and H differ instead by two mass units, we suggest that the two ions have somewhat different structures, namely, that ion G may be a radical cation (deriving its stability by being allylic in nature) and that ion H may be produced from the protium version of G by the loss of a hydrogen atom. Ion H may then represent a protonated ketene, as shown in Figure 3. An isotope effect may be responsible for the difference in the behavior of G and that of its protium analogue. Ion H, when generated from the Ag⁺ complex with the β,β-d_2-phenylalanine, was observed at a *m*/*z* value of 121

SCHEME 3: Proposed Reaction Pathways for the Dissociation of the $(M + Ag)^+$ Ion Derived from Phenylalanine[a]



[a] Scheme includes the MS/MS pathway to produce the 211 and 213 Th product ions (deuterium-exchanged version of phenylalanine).

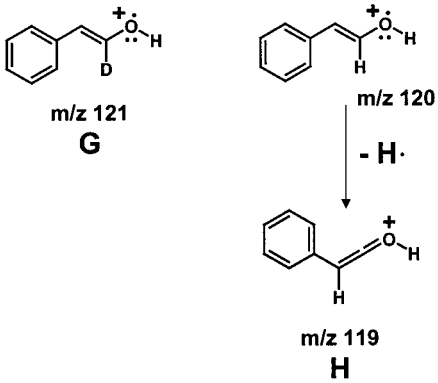


Figure 3. Proposed structures for product ions at 119 and 121 Th generated from the MS^3 of ion A′ [254 and 256 (native)/255 and 257 (deuterium-exchanged)].

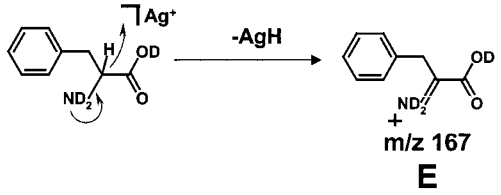
Th, indicating the retention of the two deuterium atoms at the benzylic position and in agreement with our postulated structure shown in Figure 3.

The major fragmentation pathway observed in the CID of the $(M + Ag)^+$ ion derived from native phenylalanine was the loss of 45 Th to furnish what we propose is ion C′ (227 and 229 Th) shown in Scheme 2. This species was produced at the same *m*/*z* value following the dissociation of the deuterium-exchanged form of the complex (via the loss of 48 Th). This indicates that (a) the product ion is devoid of deuterium and (b) the amine functional group is lost in the dissociation reaction. Less-intense peaks were observed at 226 and 228 Th for the native version of phenylalanine, consistent with the formation of ion A (Scheme 1) through an intermediate step involving ion A′. The loss of 48 Th from the Ag^+ complex with deuterium-exchanged phenylalanine to afford the more prominent ions at 227 and 229 Th can be accounted for by the loss of ND_3 and CO (20 and 28 Th, respectively) or the loss of D_2O and D−NC (also 20 and 28 Th, respectively) as shown in Scheme 2. The corresponding loss of 45 Th from the complex formed from the native version involves the loss of the protonated versions of the same neutral species. The loss of ND_3 and CO (NH_3 and CO) suggests the formation an α-lactone intermediate, whereas the loss of D_2O and D−NC (H_2O and H−NC) suggests the intervention of an α-lactam. The latter lends further support to its probable involvement as ion A′, as discussed in the preceding fragmentation route (Scheme 1). Either the loss of CO from an α-lactone or the loss of an isocyanide from an α-lactam (a known process in the thermal degradation of α-lactams[77,78]) would result in the formation of an Ag^+ complex with phenylacetaldehyde (227 and 229 Th), labeled as ion C′ in Scheme 2. The observation of a possible α-lactam complex of Ag^+ (A′ in Scheme 1) but not of an α-lactone would be consistent with the greater stability of the lactam in general. The formation of phenylacetaldehyde is the most cogent argument in favor of an α-lactam as an intermediate as it is difficult to envisage other plausible pathways for the production of an aldehyde from the amino acid (except via an α-lactone). When ion C′ was generated from the Ag^+/*carboxy*-^{13}C phenylalanine complex ion, the ^{13}C label was removed with the neutral species (i.e., as $H^{13}CN$ or $D^{13}CN$ via the α-lactam), in agreement with the proposed reaction pathway. The isolation and CID of ion C′ (MS^3) resulted in either the loss of 108 and 110 Th (AgH) to afford ion C or the loss of the organic component of the complex altogether to furnish the bare Ag ion. In the case of ion C, we assume that the loss of 1 Th results in the formation of an acylium ion. Production of the bare Ag^+ ion implies the release of the neutral phenylacetaldehyde molecule.

A third fragmentation route observed in the dissociation of the $(M + Ag)^+$ ion involved a loss of 61 Th from the complex derived from native phenylalanine and a corresponding loss of 64 Th from the complex generated from the deuterium-exchanged form to produce what we propose is ion D (Scheme 3). The *m*/*z* values for this product ion, whether generated from the native or deuterium-exchanged forms of phenylalanine, were 211 and 213 Th, indicating a composition lacking the amine and carboxyl functional groups of the original amino acid. As shown in Scheme 3, we suggest that a loss of 64 Th from the Ag^+ complex with the deuterium-exchanged form of phenylalanine can be achieved by a loss of D_2O (20 Th) to afford an α-lactam, followed by a loss of D−NCO (44 Th) to furnish ion D. Alternatively, the same result can be obtained by an initial loss of ND_3 (20 Th), followed by the loss of CO_2 (44 Th), in this case implicating an α-lactone. The same process was observed for the Ag^+ complex with native phenylalanine. Assuming the reaction pathways shown in Scheme 3 are correct, we can regard ion D as a complex of the silver ion either with benzylcarbene or, by rearrangement of the carbene, with styrene. Ion D, when generated from the Ag^+/β,β-d_2-phenylalanine complex, was observed at 213 and 215 Th and at 211 and 213 Th when generated from the Ag^+ complex with *carboxy*-^{13}C phenylalanine. The former indicates that the benzylic portion of the original amino acid is retained while the latter suggests that the carboxylic acid functionality is lost during the dissociation reaction. Both observations support the proposed reaction pathways outlined in Scheme 3.

A fourth pathway in the dissociation of the $(M + Ag)^+$ ion is a loss of 108 and 110 Th, corresponding to the ejection of AgH as the neutral fragment. The resulting product is likely an

SCHEME 4: Proposed Reaction Pathway for the Dissociation of the $(M + Ag)^+$ Ion Derived from Phenylalanine[a]



[a] Scheme includes the MS/MS pathway to produce the 167 Th product ion (deuterium-exchanged version of phenylalanine).

immonium ion, corresponding to the proposed species labeled as E in Scheme 4. The *m*/*z* value for ion E when generated from the native form of phenylalanine was 164 Th, and the mass of the ion shifted to a value of 167 Th derived from the deuterium-exchanged form of phenylalanine. The latter observation suggests that the amine functional group is retained in the product ion. The *m*/*z* value for the same ion when generated from the β,β-d_2 form of phenylalanine was 166 Th, demonstrating the retention of both benzylic deuterons. Therefore, the proton that is removed during the loss of AgH to form the immonium ion is likely to be the one located in the α-carbon position. The formation of an immonium ion at 120 Th ($C_6H_5-CH_2-CH{=}NH_2^+$) via the loss of H_2O and CO is the most common fragmentation route for the protonated version of phenylalanine. Unlike the dissociation of the $(M + H)^+$ ion, however, the immonium product ion that is generated from the $(M + Ag)^+$ precursor apparently retains the carboxyl moiety.

The last major pathway for fragmentation of the $(M + Ag)^+$ ion is due to the neutral loss of 148 Th from the complex derived from native phenylalanine and a corresponding loss of 149 Th from the deuterium-exchanged version. This ion (*m*/*z* 124 and 126 Th, shifted to *m*/*z* 127 and 129 Th in the deuterated form) corresponds to a complex of Ag^+ with ammonia.

In all, the fragmentation pathways discerned for the Ag(I) complex with phenylalanine are distinct from those reported for the protonated and transition-metal-cationized phenylalanine ions. For example, Wei and Amster have examined the reactions of neutral phenylalanine with Cu^+ and Fe^+ ions in their ground states in an ion cyclotron mass spectrometer.[44] The reaction between Fe^+ ions and phenylalanine leads to the generation of an Fe^+ adduct of the amino acid modified by the loss of CO_2. The reaction between Cu^+ ions and phenylalanine, however, produced Cu^+ adducts of the amino acid modified by the loss of CO and the loss of CO and H_2O. Similar results were obtained in our laboratory following the dissociation of the Cu^+ complex with phenylalanine generated by ESI. In the dissociation of N-protonated glycine, the reaction involving the loss of CO produces a product that has been characterized as a water/immonium complex species, which involves an ion−dipole interaction.[79] In the case of the Cu^+-cationized species derived from phenylalanine, the H_2O molecule may instead coordinate the metal ion if we assume that the product species is characterized, in part, by a cation/imine complex. During an investigation of the metastable ion dissociation of a Cu^+ complex with phenylalanine generated by fast atom bombardment (FAB), three fragmentation pathways were observed, involving (a) the loss of H_2O, (b) the loss of CO_2, and (c) the loss of H_2O and CO.[41] Though not as relevant to the present case, the dissociation of Cu^{2+} complexes containing an amino acid and a third coordinating ligand has also been reported.[37,38,80] In general, the CID of these complexes caused the loss of CO_2 to leave Cu^{2+}-cationized product species. The dissociation of the Ni^+ complex with phenylalanine (also formed by FAB) led to a major product ion that was consistent with the formation of a Ni^+-cationized imine (similar to the results for the Cu^+ adduct), again via the loss of CO and H_2O.[40] In an investigation of the CID of Al^{3+} complexes composed of amino acids and glycerol (generated by FAB), the major fragmentation route that was observed for the phenylalanine complex involved the loss of H_2O and CO along with neutral species from the glycerol ligand.[39]

It has also been observed that the fragmentation of Ni^+, Cu^+, and Co^+ complexes with aliphatic amino acids also proceeded via the net loss of 46 Th.[81] The current consensus is that the elimination of H_2O and CO from amino acids occurs via the rapid, consecutive loss of the two species. The net elimination of 46 Th is not thought to occur via the loss of intact dihydroxycarbene, $C(OH)_2$, because the calculated reaction barrier for this mechanism is higher than that for the simple loss of H_2O and CO.[79] Instead, it has been proposed that a proton at the amine moves to the carbonyl oxygen to effect the fragmentation reaction. The elimination of discrete H_2O and CO has been shown experimentally through the use of neutralization/reionization mass spectrometry (NRMS) to be the predominant mechanism for the dissociation of, for instance, glycine protonated at the amine group.[79]

As discussed above, we have determined through MS^n experiments with isotope-exchanged and -labeled versions of phenylalanine that the dissociation of the $(M + Ag)^+$ ion derived from phenylalanine is unique, and that three of the major dissociation products generated by MS/MS arise via the loss of (a) H_2O, (b) H_2O and HCN *or* NH_3 and CO, and (c) H_2O and HNCO. The more commonly observed elimination of H_2O and CO is only a minor pathway in the MS/MS spectrum of the Ag^+ complex and is significantly less favored than the loss of H_2O and HCN *or* NH_3 and CO. This former pathway is observed, however, following the dissociation of the ion we propose to be the Ag^+ complex with an aziridinone. The dissociation reactions for the Ag^+ complex are also somewhat surprising in light of the fact that the loss of NH_2 or NH_3 is not a favored process in the CID of protonated phenylalanine. This can apparently be attributed to the instability of the resulting phenonium ion, which can be stabilized by electron-donating ring substituents in the *para*-position, as in the case of tyrosine.[82] The loss of NH_3 from the Ag^+ complex with phenylalanine may be facilitated by the formation of the proposed aziridinone intermediate structure.

We note that there is a precedent for the formation of aziridinones in CID experiments involving protonated peptides and derivatized amino acids.[55,83−85] For example, as a result of CID and NRMS experiments, Wesdemiotis and co-workers postulated that the neutral species formed in dissociation reactions of peptides protonated at the N-terminus correspond to aziridinone structures.[84] Support for the formation of an aziridinone structure was provided by later work reported by Klassen and Kebarle in which ab initio calculations and threshold CID measurements were used to investigate the dissociation reactions of model amino acids and peptides.[85] In addition, the formation of an aziridinone has been invoked to explain the mechanism behind the charge remote fragmentation of cyclic peptides[55] and the mechanism for the formation of b_n-type fragments from protonated peptides.[86] Though not explicitly stated, a mechanism appearing to involve the formation of an aziridinone was also postulated by Russell and coworkers for the generation of $(b_n + Cu-H)^+$ product ions

following the metastable dissociation of Cu^+-cationized peptides containing arginine at the N-terminus.[87]

Reactivity toward Water and Methanol in the Gas Phase. As noted before, we have shown that certain binary complexes composed of Ag^+ ions and neutral amino acids will react when stored in the ion-trap mass spectrometer to form water and methanol adduct ions.[70] The formation of the adduct species, which we assume is best described as an association reaction between the binary complex and H_2O or MeOH that is present as a contaminant in the ion-trap bath gas, is dependent on the composition of the coordinating molecule. Aliphatic amino acids such as alanine and valine will form the adduct species, whereas complexes composed of phenylalanine, tyrosine, and tryptophan will not. We have also shown that the CID of the Ag^+/phenylalanine complex ion, to produce the fragment ions listed above, will promote adduct formation due either to a change in conformation or to the loss of a coordinating group.[70] To provide further experimental evidence to support the reaction pathways and product ions for the CID of the phenylalanine/Ag^+ complex proposed here, we compared the reactivity of the Ag^+-cationized CID products to that of complexes composed of Ag^+ and known organic compounds. Our premise was that the product ion at 227 and 229 Th, attributed to the formation of an Ag^+ complex with an aldehyde, would exhibit a reactivity similar to that of an Ag^+ complex with phenylacetaldehyde. The latter complex can be generated by the ESI of a solution containing the neat aldehyde and $AgNO_3$. Likewise, we predicted that the product ion at 211 and 213 Th, if it is indeed an Ag^+/carbene or styrene complex, would have a reactivity toward H_2O and MeOH similar to that of a complex composed of Ag^+ ions and styrene; the latter was generated by the ESI of a solution containing $AgNO_3$ and neat styrene. Though we have found that the 254 and 256 Th product ion (native form of phenylalanine) is slightly reactive toward H_2O and MeOH,[70] a sample of the intact α-lactam or lactone is not commercially available, nor would one be expected to be sufficiently stable for isolation. Therefore, an analogous experiment to further confirm the formation of the Ag^+/aziridinone complex ion was not possible.

Figure 4 shows the data comparing the apparent reactivity of the 227 and 229 Th and the 211 and 213 Th product ions derived from the CID of the Ag^+ complex with native phenylalanine to that of the Ag^+ complexes with phenylacetaldehyde and styrene. The plots in Figure 4 show (a) the decrease in abundance of the initially isolated ions, whether they are $(M + Ag)^+$ ions or the product ions from the CID of the phenylalanine complex, (b) the increase in the abundance of the H_2O adduct ion, and (c) the increase in the abundance of the MeOH adduct ion. The abundance of each, relative to that of the total stored ion abundance, is plotted as a function of isolation and storage time in the ion-trap mass spectrometer. The data are plotted using a logarithmic scale on the time axis to better illustrate the apparent trends in reactivity. Our studies to date suggest that the reactions are pseudo-first order. In Figure 4a−c, the curves for the 227 and 229 Th product ion and those for the Ag^+ complex with phenylacetaldehyde are remarkably similar. We take this observation as further evidence that the dissociation product observed at 227 and 229 Th, following the CID of the Ag^+ complex with phenylalanine, is in fact the Ag^+ complex with phenylacetaldehyde. The curves for the 211 and 213 Th product ion and those for the Ag^+ complex with styrene are also similar. The slight difference in reactivity between the dissociation product and the complex with styrene may be due to the possibility that the fragment is actually the Ag^+ complex with the benzyl carbene or a mixture of the two species.

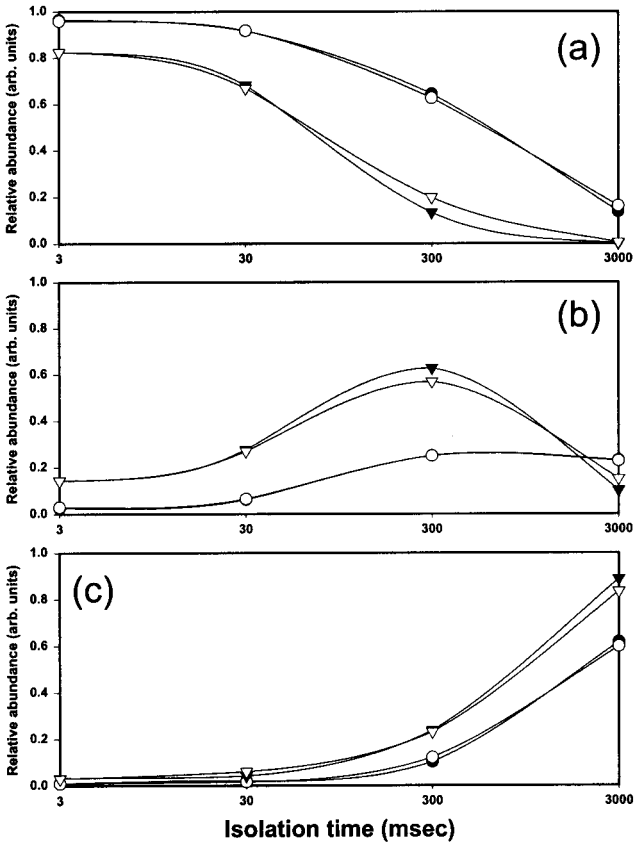


Figure 4. Apparent association reactivities of the 227 and 229 Th and the 211 and 213 Th product ions generated from the CID of the $(M + Ag)^+$ ion (native form of phenylalanine), and the Ag^+ complexes with phenylacetaldehyde and styrene following isolation and storage in the ion-trap mass spectrometer without imposed activation. (a) Change in the abundance of the $(M + Ag)^+$ ion (M = phenylacetaldehyde, styrene) and the 227 and 229 Th product ion. (b) Increase in the abundance of the H_2O adduct peak. (c) Increase in the MeOH adduct peak. Symbols: ●, 227 and 229 Th product ion from the CID of the $(M + Ag)^+$ ion (native form of phenylalanine); ▼, 211 and 213 Th product ion from the CID of the $(M + Ag)^+$ ion (native form); ○, Ag^+ complex with phenylacetaldehyde; and ▽, Ag^+ complex with styrene. Solid lines are given to guide the eye.

To test the dependence of the apparent reactivity of the complexes on stoichiometry and structure, we compared the reactivity of the 227 and 229 Th product ion to that of the Ag^+ complexes with phenylacetaldehyde, acetophenone, and benzaldehyde (Figure 5). Acetophenone was included in this investigation because the nominal *m*/*z* value of this molecule is the same as that of phenylacetaldehyde. The structures of the two molecules, however, are obviously different. Benzaldehyde was included to determine the specificity of the reactivity to the stoichiometry of the coordinating aldehyde. For instance, in phenylacetaldehyde, the carbonyl and aromatic ring moieties are separated by $-CH_2-$, whereas there is no such spacing between the two groups in acetophenone and benzaldehyde. As shown in Figure 5a−c, the 227 and 229 Th product ion exhibits a reactivity similar to that of the Ag^+ complex with phenylacetaldehyde and quite different from the reactivities of Ag^+ complexes with either acetophenone or benzaldehyde. When the 211 and 213 Th product ion was compared to the Ag^+ complexes with various small organic species, the apparent reactivity was closer to that of the Ag^+ complex with styrene than, for instance, the reactivities of the Ag^+ complexes with ethylbenzene and *p*-xylene, which are molecules with masses similar to that of styrene but with different structures (data not

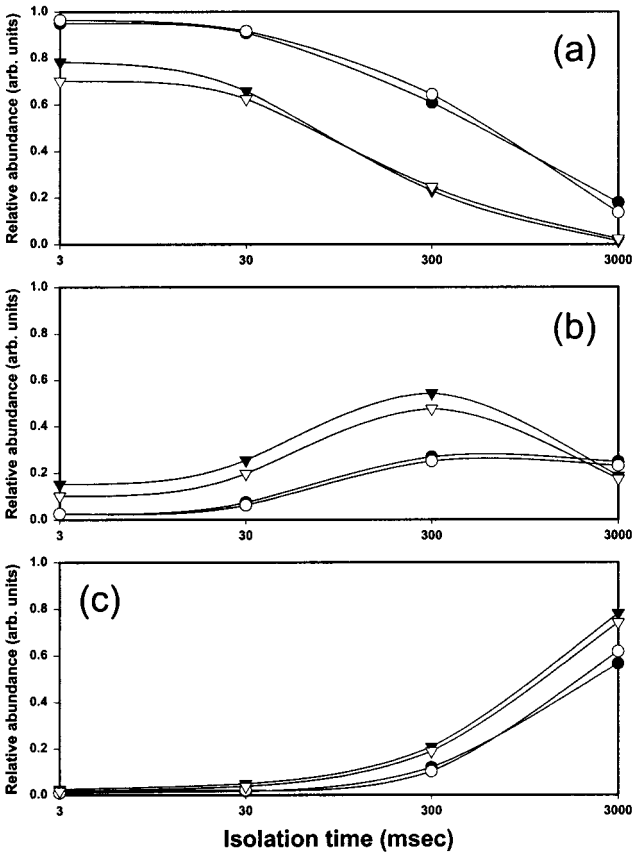


Figure 5. Apparent association reactivities of the 227 and 229 Th product ion generated from the CID of the $(M + Ag)^+$ ion (native form of phenylalanine) and the Ag^+ complexes with phenylacetaldehyde, acetophenone, and benzaldehyde following isolation and storage in the ion-trap mass spectrometer without imposed activation. (a) Change in the abundance of the $(M + Ag)^+$ ion (M = phenylacetaldehyde, acetophenone, benzaldehyde) and the 227 and 229 Th product ion. (b) Increase in the abundance of the H_2O adduct peak. (c) Increase in the MeOH adduct peak. Symbols: ●, Ag^+ complex with phenylacetaldehyde; ▼, Ag^+ complex with acetophenone; ○, 227 and 229 Th product ion from the CID of the $(M + Ag)^+$ ion (native form of phenylalanine); and ▽, Ag^+ complex with benzaldehyde. Solid lines are shown to guide the eye.

shown). Taken together, the observed ion/molecule chemistry provides further supporting evidence for the proposed product ion compositions and thus the reaction pathways. In addition, the differences in reactivity demonstrate the sensitivity of the H_2O and MeOH adduct formation to the composition and structure of the reacting species. A previous study by Wu and Brodbelt demonstrated that the gas-phase formation of an H_2O adduct to Ag^+ ligand complexes (in this case involving heteroaromatic ligands) was also sensitive to the composition and structure of the coordinating ligand.[8] We have observed similar trends for a variety of nitrogen- and oxygen-donor ligands[88] and for the Ag^+ complexes with *para*-substituted versions of phenylalanine.[72]

Conclusions

By comparing the product ions generated in the CID of Ag^+ complexes incorporating phenylalanine, phenylalanine with exchangeable protium replaced with deuterium, phenylalanine with the carboxlic acid group labeled with ^{13}C, and phenylalanine with the benzylic group labeled with deuterium, we have elucidated several novel fragmentation pathways for the binary Ag^+ complex with phenylalanine. The major product ion observed in the MS/MS spectrum of the $(M + Ag)^+$ ion is an Ag^+ complex with phenylacetaldehyde. This product ion is markedly different from those observed following the CID of phenylalanine cationized by the attachment of, for instance, Cu^+, Ni^+, or Fe^+. Complex ions composed of the latter metal ions dissociate primarily via the loss of H_2O and CO to produce an immonium product ion. The apparent reactivity of the product ions toward H_2O, following isolation and storage in the ion trap, matches that which is observed for a complex generated by the ESI of a solution containing $AgNO_3$ and the neat aldehyde.

We suggest that the formation of the aldehyde complex ion and the other product ions observed implicates the formation of an Ag^+ complex with either an aziridinone (α-lactam) or an α-lactone during or following the collisional activation of the $(M + Ag)^+$ ion. MS^3 experiments involving the 254 and 256 Th product ion from the CID of $(M + Ag)^+$, consistent with the Ag^+ complex with either an α-lactam or lactone, indicate that the former is the more likely species. An alternative explanation for the CID patterns observed for the $(M + Ag)^+$ ion may involve the insertion of the metal ion into a carbon−carbon or carbon−nitrogen bond to mediate the fragmentation pathways that were observed.[89,90] The insertion of metal ions into C−C bonds in the gas phase has been studied extensively.[91] In all of our investigations to date, a universal observation is that ternary complexes with Ag^+ such as those composed of nitrogen-donor ligands (e.g., $Ag(bpy)_2^+$) or two amino acids are unreactive toward H_2O and MeOH in the gas phase. Similar observations were reported by Wu and Brodbelt.[8] In addition, we found that Ag^+ incorporated into porphyrin structures also failed to produce H_2O adducts upon isolation and storage. We attribute the lack of reactivity observed for ternary complexes to (a) the fact that the most-stable Ag^+ complexes are linear due to the overlap of ligand orbitals with collinearly oriented Ag *σ*sd hybrid orbitals and (b) the low enthalpy and free-energy changes associated with the addition of third and fourth coordinating ligands.[92] In our previous investigation of the reactivity of the $(M + Ag)^+$ complex ion toward H_2O and MeOH we noted that the CID product ion consistent with the formation of the Ag^+-cationized aziridinone was reactive, though less so than other product ions.[70] We assume that the insertion of the Ag ion into a bond within the phenylalanine molecule would "quench" the reactivity of the species in the ion trap, i.e., by saturating the Ag acceptor orbital through the formation of C−Ag−C, C−Ag−O, or C−Ag−N types of covalent bonds. It appears, therefore, that the most cogent mechanism involves a ring-closure reaction, mediated by the Ag^+ ion, to form the aziridinone, which subsequently decomposes to form the product ions observed. To the best of our knowledge, this is the first such indication of a metal-ion-mediated conversion of an amino acid to an aldehyde that has been observed in the gas phase.

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