Fragmentation Mechanisms of Glycine-Cu^+ in the Gas Phase. An Experimental and Theoretical Study†

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Electrosprayed copper complexes of glycine ($GlyCu^+$) were formed from a glycine/CuX_2 (X = Cl, CH_3CO_2) mixture, in methanol. The collision-induced fragmentation of the most abundant isotopic form $Gly^{63}Cu^+$ was studied as a function of collision energy. Four fragment ions are observed: $^{63}CuNHCH_2^+$, $^{63}Cu^+$, $CH_2NH_2^+$, plus a minor loss of H_2O. The potential energy surface for formation of these fragment ions has been investigated at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* level. Several types of mechanisms were considered, involving either metal insertion into covalent bonds (C−C, C−O, and C−N) or dissociative attachment whereby the metal ion catalyzes the fragmentation by its distant electronic influence. Mechanisms starting with copper insertion into the C−C bond account for the most favorable pathways for the formations of $CuNHCH_2^+$ and $CH_2NH_2^+$. Dissociative attachment cannot be excluded to participate to the formation of $CuNHCH_2^+$ and is the only way to explain H_2O loss. Finally, calculations on the possible mechanisms for NH_3 loss (not observed experimentally) indicate that the observed ions are the result of a competition between several fragmentation modes with relatively similar energetic requirements.

Introduction

The chemistry of metal ions bound to functionalized organic molecules in the gas phase has been a very active research area in the last two decades.[1−3] Within gas-phase biomolecular chemistry, the study of metal cation/molecule complexes started with the introduction of particle bombardment techniques [plasma desorption mass spectrometry (PDMS) or fast atom bombardment (FAB)] and has greatly benefited from the development of both matrix-assisted laser desorption−ionization (MALDI) and electrospray ionization (ESI). Gaseous biomolecules cationized with metal ions provide useful models to study the intrinsic properties of their liquid phase counterparts, including metalloproteins and metalloenzyme active sites. Since metal/molecule interactions are often strongly metal-specific, one may expect some relationship between metal binding sites, binding affinities and fragmentation reactions. In this context, the fragmentations of metal-cationized biomolecules in the gas phase may yield valuable structural information, such as peptide sequencing. Although the fragmentation of protonated peptides has long been used in sequencing strategies, it cannot yield full sequence information in general. Metal-specific fragmentations could provide a useful complement toward full structural characterization, and some significant success has been demonstrated along these lines with argentinated peptides.[4] The development of such methods has been slow, however, probably due to the lack of general understanding of the fragmentation pathways of cationized peptides. It is the purpose of this paper to provide a contribution toward this goal.

The gas phase reactivity of biomolecules attached to transition metals is dramatically dependent upon the oxidation state of the metal. Here we focus on the association of amino acids (AA) and peptides with transition metal cations in the +1 oxidation state. Studies on this topic mostly concern AA complexes (with some notable exceptions, including the peptide/Ag^+ work mentioned above[4]). Speir et al.[5] reported the first observation of a gas-phase reaction of a peptide with a transition metal ion: laser-desorbed neutral peptide molecules were allowed to react with Fe^+ in a Fourier transform ion cyclotron resonance mass spectrometer. The same group reported later a systematic study of the fragmentations of the Fe^+ and Cu^+ complexes of all twenty AA's occurring in the proteins of living systems.[6] Harrison and co-workers described the fragmentations of many AA complexes of Fe^+, Ni^+, and Cu^+ produced by FAB ionization.[7,8] Other systematic studies involving singly charged first row metal cations were published by Hoppilliard and co-workers using PDMS[9,10] and by Wesdemiotis and co-workers.[11] It was also shown that such ions can be produced by MALDI,[12] gas-phase ion−molecule attachment,[13] and electrospray.[4] These systematic studies established that the most common fragmentation reaction observed is the elimination of $[H_2,C,O_2]$, likely leading to the formation of a metal-cationized imine, and it has been suggested by several authors that this product ion is formed by M^+ insertion into the H(R)C−COOH bond of the amino acid, although this view has been challenged later on.[14,15] Several other reactions are often competitive with the elimination of $[H_2,C,O_2]$ and may sometimes be dominant. Isotope labeling experiments have partially unravelled the complexity of the fragmentation mechanisms. In particular, it helped establish that the hydrogen atoms involved in neutral losses are often, but not always, the labile hydrogens initially bound to heteroatoms.

Several other recent studies have also provided detailed insight into the possible fragmentation characteristics of AA−M^+ complexes. Although dealing with other neutrals, the gas-phase chemisty of Cu^+ complexes has been extensively studied by the groups of Yanez and Tortajada, including several organic molecules which are interesting mono- or di-functional models

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for biomolecules.[16,17] The most relevant of these studies describes the fragmentations of the Cu^+/glycolic acid complex.[17] Among the mechanisms considered, the elimination of $[H_2,C,O_2]$, formally identical to that observed with glycine (see below), was interpreted in terms of an elimination of formic acid rather than H_2O and CO. This pathway involves new intermediate structures bearing a dihydroxycarbene ligand, not hitherto considered. Other new pathways are associated with dissociative attachment, in which metal complexation perturbs the electronic structure of the neutral molecule sufficiently strongly so as to lead to low energy fragmentation without the need for metal insertion into covalent bonds. Such a mechanism, implying the formation of a three-membered lactam ring, was previously invoked to account for the fragmentations of the $GlyNi^+$ complex.[18] Analogous mechanisms were also proposed to account for the elimination of NH_3 and H_2O from the $PheAg^{+14}$ and $ProAg^{+15}$ complexes (denoted lactam and lactone routes, respectively). To the best of our knowledge, there has not yet been a full characterization of the potential energy profiles for such mechanisms for $AA-M^+$ complexes, nor have their energetic requirements been compared in details with those of other types of mechanisms.

In the present paper, we propose an interpretation of the low energy decomposition processes of $GlyCu^+$, formed in an electrospray source and fragmented in the collision cell of a triple quadrupole mass spectrometer. The fragmentation mechanisms are investigated by means of density functional calculations. We examine a number of different pathways, providing what is maybe the most comprehensive comparison of mechanisms to date for this type of gaseous species.

There has been previous work on the structures and binding energies of $AA-Cu^+$ complexes. Cerda and Wesdemiotis[19] utilized the kinetic method to estimate relative Cu^+ ion affinities for all 20 common amino acids. Ab initio calculations have been carried out on the $Gly-Cu^+$, $Ser-Cu^+$, and $Cys-Cu^+$,[20] with relative binding energies in good agreement with experiments, leading to an anchoring of the relative into an absolute scale of Cu^+ affinity of most AA's. More refined calculations on $Gly-Cu^+$ [21] and other studies on $Pro-Cu^+$,[22] $Ala-Cu^+$,[23] and complexes of other amino acids[24] confirmed that copper binding to AA's is very strong and that the most stable forms of these cationized amino acids involve either bidentate or tridentate copper chelation. Copper binding sites in peptides containing basic residues were inferred from a combined experimental and theoretical study, with the conclusion that bidentate or multidentate binding of Cu^+ to basic side chains must be considered for a correct interpretation of metastable ion spectra.[25]

Experimental and Theoretical Procedures

Electrospray. Electrosprayed copper complexes of glycine were formed from glycine/$CuCl_2$ or glycine/$(CH_3CO_2)_2Cu$ mixtures (500 and 250 mM, respectively) in methanol. Such concentrations are typical for the formation of metal complexes of amino acids. In such conditions, the pH is in the 6.5−7 range. Labeling experiments were performed by H/D exchanging all exchangeable hydrogens of glycine in a D_2O/CH_3OD solution. Solutions were infused in the ion source with a syringe pump (Harvard, Southnatic, MA) at a flow rate of 10 μL/mn. L-Gly, anhydrous $CuCl_2$ and $(CH_3CO_2)_2Cu$ were purchased from Aldrich Chem. Co. (Saint Quentin Fallavier, France). All solvents were of HPLC grade.

All experiments were carried out on a triple quadrupole Quattro II mass spectrometer (Micromass, Manchester, UK). Source parameters were adjusted so as to optimize ion signals (such as $GlyCu^+$). Typical voltage values were: capillary 2.5−3.5 kV, counter electrode 0.1−0.3 kV, RF lens 0.7 V, skimmer 1.5 V. Source spectra were recorded with a sampling cone voltage of 40 V.

Among the two isotopes of Cu (^{63}Cu and ^{65}Cu), ^{63}Cu is the most abundant. Low energy collision-induced dissociation (CID) of $Gly^{63}Cu^+$ was performed with argon as the collision gas, and monitored as a function of collision energy in the laboratory frame (Elab). The breakdown graph associated with the abundances of the various fragment ions relative to the parent ion, *m*/*z* 138, is given in Figure 1, as a function of collision energy. The parent ion intensity is omitted in order to obtain a scale appropriate to low abundance product ions.

Computational. Calculations were initially performed at several levels of theory. Geometry optimizations and vibrational frequency calculations were carried out at both the B3LYP/basis1 and the MP2(FC)/basis1 level (where FC means that the frozen core approximation was used). Basis 1 is the 6-31G* basis for H, C, N, and O, and the Wachters [14s11p5d/8s6p3d] basis for Cu.[26] In several cases, intrinsic reaction path calculations were carried out to ascertain the identity of the minima connected by a given transition state. Final energetics were obtained with B3LYP/basis2 wave functions at the B3LYP/basis1 optimized geometries, and with MP2(FC)/basis2 at the MP2(FC)/basis1 geometries. Basis 2 consists of the 6-311+G-(2d,2p) for H, C, N, and O, and the extended Wachters basis [15s11p6d2f/10s7p4d2f] for Cu.

It was observed that in several cases, significant disagreement occurred between the MP2 and B3LYP relative energies. This trend is reminiscent of the ab initio failures for Cu^+ complexes described by Yanez et al.[27] To evaluate the potential problems associated with the use of perturbation theory and standard HF orbitals, we compared the relative energies of $Gly + Cu^+$, $GlyCu^+$ **1**, and $(H_2O)(CO)Cu^+(CH_2NH)$ at several perturbation orders [MPn(FC)/basis2//MP2(FC)/basis1, n=2−4]. Taking $Gly + Cu^+$ as the reference, the relative energies of $GlyCu^+$ were found to be −296, −252, and −331 kJ/mol at the MP2, MP3, and MP4 levels, respectively. It is clear therefore that convergence of the perturbation series is not reached. The situation was found to be even worse for $(H_2O)(CO)Cu^+(CH_2NH)$, whose energies relative to $Gly + Cu^+$ were found to be −360, −190, and −544 kJ/mol, respectively. Such spectacular errors are likely to be due to the use of inappropriate molecular orbitals, as shown by Lynch and Truhlar.[28] Therefore, in what follows, the relative energies are given at the B3LYP/basis2//B3LYP/basis1 level, after correction for the thermal energy at 298 K. The fact that the MPn failure is due to the use of inappropriate orbitals leads other post-Hartree−Fock methods, including CCSD(T), to fail also, even though not as badly as MP2.[27] As a result, the accuracy of the present B3LYP calculations cannot be checked against those of more traditional ab initio methods. It is reasonable to assume that B3LYP is able to yield *relative* energies with sufficient accuracy. All calculations were carried out using the Gaussian 98 program package.[29]

In the text and figures, cationized glycine and its rearranged forms are numbered. The numerals not followed by letters are reserved for the most stable forms (for instance, **1** is the most stable form of $GlyCu^+$). The numerals followed by letters are for less stable forms of a given species (for instance, **1A** and **1B** are forms of $GlyCu^+$ which are less stable than **1**).

Results and Discussion

Collisional activation experiments were carried out on $Gly^{63}Cu^+$ at *m*/*z* 138. Five new ions are observed on the collision

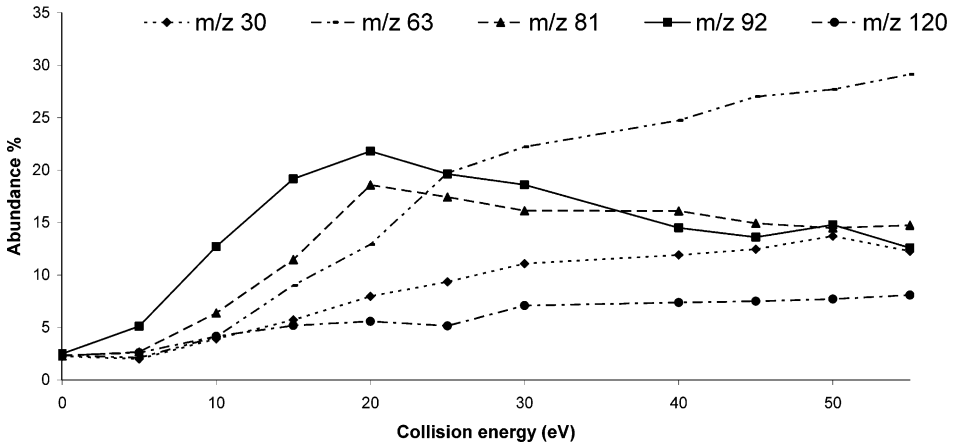


Figure 1. Relative abundances of the observed ions *m*/*z* 120 (loss of H_2O), *m*/*z* 92 (loss of 46 u) *m*/*z* 81 (Cu^+OH_2), *m*/*z* 63 (Cu^+) and *m*/*z* 30 ($NH_2CH_2^+$), after collisional activation of the parent *m*/*z* 138 ion $Gly^{63}Cu^+$, as a function of collision energy in the laboratory frame.

spectra: a minor *m*/*z* 120 corresponding to the elimination of H_2O, a major *m*/*z* 92 corresponding to the elimination of $[C,H_2,O_2]$, *m*/*z* 81 identified as H_2OCu^+, *m*/*z* 63, corresponding to Cu^+ as the result of $Gly^{63}Cu^+$ de-cationization, and *m*/*z* 30, the immonium ion $CH_2NH_2^+$, observed as the main fragment ion from $GlyH^+$.[30] The main fragmentations are also apparent in the high energy collision spectra of $Gly^{63}Cu^+$ formed by FAB in a sector instrument.[11] The breakdown graph associated with the decomposition of $Gly^{63}Cu^+$ is given in Figure 1.

The most stable form of copper-cationized glycine is **1**. Therefore, the energy of **1** will be taken as the reference throughout the paper. This form involves metal chelation between the nitrogen atom and the oxygen atom of the carbonyl group.[20] From this structure, the energy of dissociation into Gly and Cu^+ is calculated to be 296 kJ/mol at the B3LYP/basis2//B3LYP/basis1 level of theory. This process (formation of Cu^+ at *m*/*z* 63) is observed in the collision spectra of $GlyCu^+$ (see Figure 1). It has a higher apparent threshold than for the elimination of 46 u. At higher collision energies, it is the only fragment with rising intensity, while all others decline. This is likely due to the fact that direct processes become favored over rearrangements involving one or several energy barriers at higher internal energies. Therefore the de-cationization energy is taken as an approximate limit for the interpretation of fragmentations: transition or final states with energies above this limit will be considered as unfavorable.

Formation Mechanisms of the Cationized Imine: $Cu^+NH{=}CH_2$. It is clear from Figure 1 that the fragmentation of lowest activation energy is the loss of 46 u. The elemental composition associated with this loss is $[C,H_2,O_2]$, which may correspond to several chemical formulas: (1) water and carbon monoxide (H_2O + CO); (2) formic acid (HCOOH); (3) dihydroxycarbene $[C(OH)_2]$; or (4) carbon dioxide and dihydrogen (CO_2 + H_2). The total H/D exchange of the acidic hydrogens shows that the hydrogen atoms eliminated in the 46 u are labile hydrogens. This loss of 46 u is also observed as the main fragmentation from $GlyH^+$ and it was demonstrated experimentally and theoretically that it corresponds exclusively to the sequential losses of H_2O and CO.[30] However, there is no reason the mechanisms should be the same from $GlyCu^+$ and $GlyH^+$. Therefore, a variety of mechanisms were investigated and are described below.

Formation of $Cu^+NH{=}CH_2$ Associated with Losses of H_2O and CO. There are several ways by which a transition metal ion may influence the course of a gas-phase chemical reaction. First, it is well established that certain gas-phase transition-metal ions activate C−C and C−H bonds of hydrocarbons.[1−3] After insertion, the archetypical mechanism proposed in the literature is a transfer of a β-H atom followed by a reductive elimination producing a bis-olefin complex.[1−3] By analogy with this mechanism, copper insertion into bonds was proposed to interpret the losses of H_2O and CO from $GlyCu^+$.[9,10] It may either start with insertion into the C−C bond, followed by insertion into the C−OH bond, or these two elementary steps may occur in the reverse order (mechanisms A and B below, respectively). In both cases, the third step is a hydrogen atom migration from NH_2 to OH, to form a three-ligand intermediate $(H_2CNH)Cu(CO)(OH_2)^+$, from which eliminations of CO and H_2O can occur.

Alternatively, strong binding of the metal cation, which acts as a Lewis acid, to one or more electron-rich sites of a molecule may perturb the covalent bonds strongly enough that reactivity is enhanced without metal insertion. This alternative is all the more plausible as the $3d^{10}$ ^1S ground state of Cu^+ and the high lying $4s^13d^9$ ^3D first excited state (262 kJ/mol above the ground state) do not make insertion as favorable as it is for most open shell transition metal cations. Two different patterns of such dissociative attachment may be envisioned. In both cases, the $GlyCu^+$ complex first isomerizes into a form in which the copper ion interacts only with the amino group. A hydrogen transfer from N to OH may then occur, leading to the formation of a water molecule. In the first case, this transfer is assisted by a cyclic rearrangement of electrons, leading to the breaking of C−C and C−OH bonds, thereby forming the three-ligand complex in a single step (mechanism C below). In the second mechanism, hydrogen transfer from N to OH is assisted by the formation of a N−C bond, leading to a three membered ring lactam with elimination of a water molecule. Further ring rearrangement leads to the extrusion of a CO molecule and to the formation of the copper-imine final product (mechanism D below).

Mechanism A. The successive steps of mechanism A are summarized in Figure 2. The insertion of Cu^+ into the C−C bond leads to the two-ligand form $(H_2CNH_2)Cu(COOH)^+$ **2A**, which rearranges in turn into a three-ligand isomer (H_2CNH_2)-$Cu(CO)(OH)^+$ **3A**, after copper insertion into the C−OH bond. From **3A**, the transfer of a labile hydrogen from NH_2 to OH leads to a very stable species, **4**, located −34 kJ/mol below **1**, in which Cu^+ interacts with three ligands, which are neutral molecules: H_2O, CO, and $NH{=}CH_2$. At this point, complex **4** may decompose by elimination of one or several of these ligands, depending on its internal energy. The limiting step of this mechanism is the transition state associated with the initial

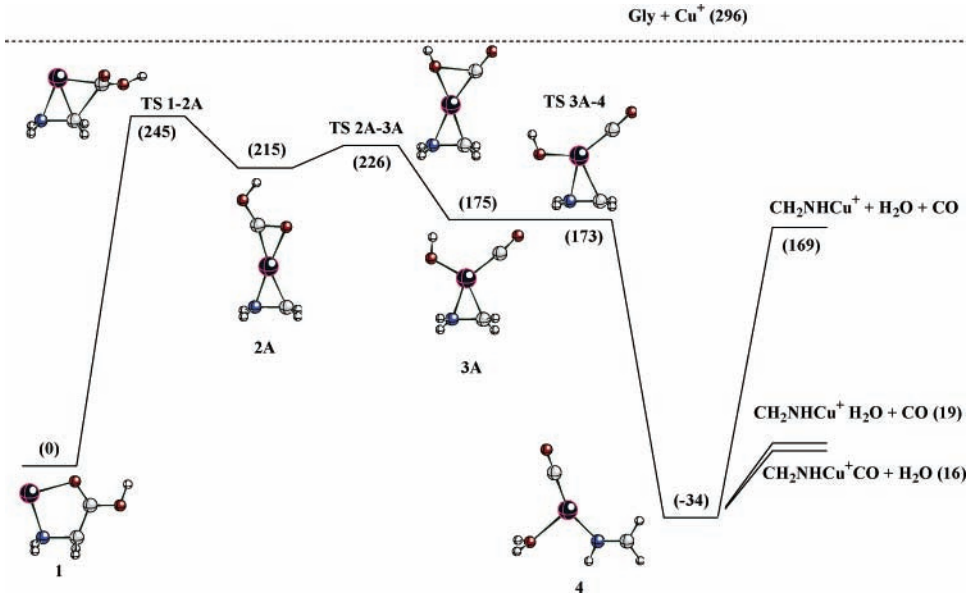


Figure 2. Potential energy profile for mechanism A: Formation of $CH_2{=}NHCu^+$ by loss of (H_2O + CO) via an insertion of Cu^+ into the C−C followed by an insertion into the C−OH bond. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

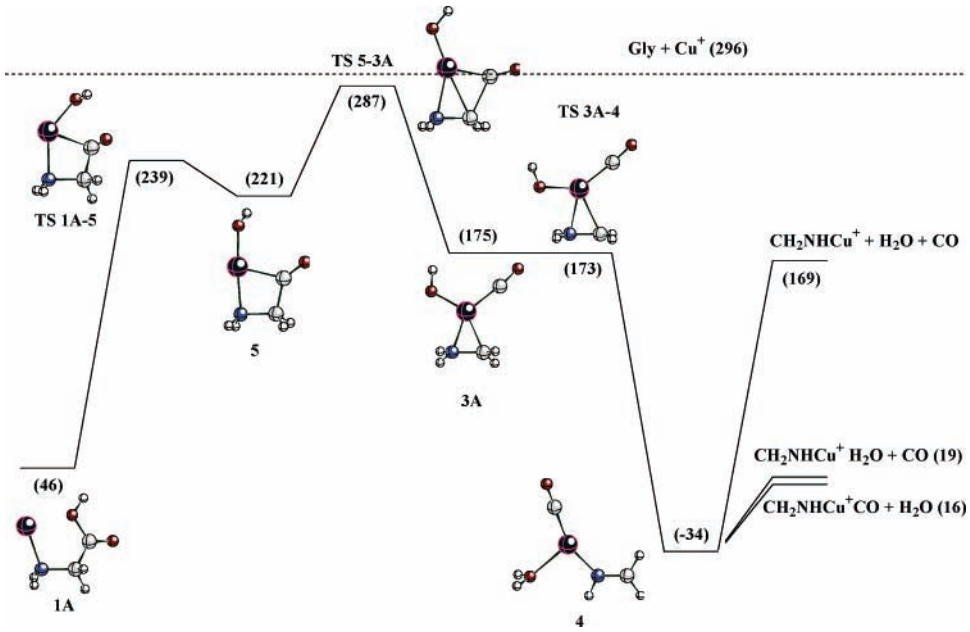


Figure 3. Potential energy profile for mechanism B: Formation of $CH_2{=}NHCu^+$ by loss of (H_2O + CO) via an insertion of Cu^+ into the C−OH bond followed by an insertion into the C−C bond. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

insertion into the C−C bond (**TS 1−2A**) located at 245 kJ/mol, i.e., more than 50 kJ/mol below the dissociation energy to Cu^+ + Gly. The cationized glycine reaching this barrier leads to the rearranged form **4** with enough internal energy to give directly $Cu^+NH{=}CH_2$ by losses of H_2O and CO (the loss of CO may either precede or follow the loss of H_2O). This final state is located 169 kJ/mol above **1**. Losses of $NH{=}CH_2$ + H_2O and of $NH{=}CH_2$ + CO, leading to Cu^+OH_2 and Cu^+CO respectively, are also computed to be energetically viable. However Cu^+CO is not observed experimentally, suggesting that out of the three possibilities, the only product ion formed is the most stable.

A loss of H_2O of very low abundance is also detected on CID spectra. The energy of the final state associated with this fragmentation is close to that of the initial state at 16 kJ/mol. No loss of CO is observed in CID spectra although the final state corresponding to this decomposition is very stable (19 kJ/mol). It is therefore likely that H_2O loss proceeds via a different mechanism (see below).

Mechanism B. The successive steps of mechanism B are summarized in Figure 3. Starting from isomer **1A** of $GlyCu^+$, the insertion of Cu^+ into the C−OH bond yields the split form **5**, which rearranges in turn into the three-ligand isomer seen above, **3A**, after insertion into the C−C bond. The insertion into the C−OH bond is less energy demanding (239 kJ/mol) than that into the C−C bond (287 kJ/mol) which is the critical energy for this mechanism. Mechanism B is energetically less favorable than mechanism A since its rate-limiting barrier is 42 kJ/mol higher in energy. In both cases, insertion into the C−C bond requires more activation energy than does insertion into the C−OH bond.

Mechanism C. This mechanism also starts from **1A** (Figure 4) in which Cu^+ interacts with the nitrogen atom and with the hydroxyl oxygen. Direct transfer of a labile hydrogen from the

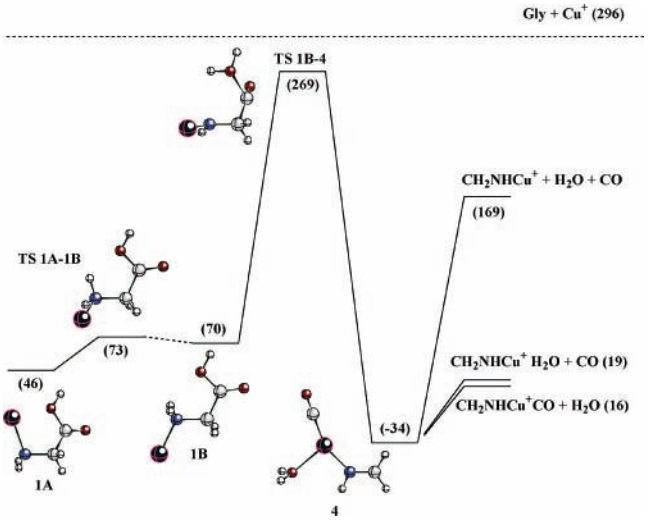


Figure 4. Potential energy profile for mechanism C: Formation of CH_2═$NHCu^+$ by loss of (H_2O + CO) without insertion of Cu^+ into the C−OH or C−C bonds. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

amine function to the hydroxyl requires de-complexation of OH to give **1B**, in which the moving hydrogen (bound to N) is now pointing toward OH. The transition state associated with the de-complexation of OH is located 73 kJ/mol higher than **1**. From **1B**, H transfer may occur from N to OH, to form an H_2O molecule. This rearrangement is assisted by C−OH and C−C stretches, forming the three molecules CH_2NH, CO, and H_2O in a single step, therefore yielding directly the three-ligand intermediate **4**. The transition state associated with this migration is located 269 kJ/mol above **1**. Among the three mechanisms described above, A is the one requiring the smallest critical energy, and B has the largest.

Mechanism D. Another mechanism avoiding metal insertion into a covalent bond has been proposed previously for the reactions of Ag^+ + Phe,[14] Ag^+ + Pro,[15] and Ni^+ + Gly.[18] It involves the concerted migration of a hydrogen atom from N to the hydroxyl oxygen as in mechanism C; however, it is now assisted by C−N bond formation, yielding an aziridinone ligand bound to copper via the carbonyl oxygen. To our knowledge, the potential energy profile for this mechanism (shown in Figure 5), including the transition states, has not been computed before for a reaction of a transition metal cation with an amino acid. It was however considered for the reaction of Cu^+ with glycolic acid. We find that this process starts from the **1B** isomer of $GlyCu^+$. It leads to the elimination of H_2O via **TS 1B-9**, in a direction which is away from the copper ion. It might either lead to a complex **9** in which Cu^+ is bound to aziridinone and water and then expel water (since there is nearly enough internal energy in the complex to do so) or else eliminate water directly to form **9-H_2O**. The exact mechanism is likely to depend on the amount of internal energy available in excess of H_2O binding to Cu^+. After water loss, aziridinone may fragment into CH_2-NH and CO, preparing for CO elimination. It may be seen in Figure 5 that the critical energy required to eliminate water is 282 kJ/mol, close to that necessary to separate Gly from Cu^+, whereas the transition state for subsequent formation of CO is iso-energetic with Gly + Cu^+. These results are consistent with the observation of a minor loss of H_2O: water loss is less favorable than elimination of (H_2O + CO) via mechanism A above, and further elimination of CO is in direct competition with the formation of Cu^+ which is an entropically favored, one-step process.

Formation of Cu^+NH═CH_2 Associated with Losses of Other Neutral Species. *Formation of Cu^+NH═CH_2 Associated with Loss of Formic Acid.* The elimination of formic acid via the insertion of Cu^+ into the C−C bond was also examined. The various steps of this mechanism are given in Figure 6. It starts, as mechanism A above, with C−C insertion yielding **2A**. In a second step, H-transfer from NH_2 to COOH leads to the cleavage of both Cu−C bonds and to the formation of a very stable complex **6** (−33 kJ/mol relative to **1**), involving methanimine and formic acid ligands. The critical step of this fragmentation is the transition state between **2A** and **6**. The energy of this transition state (342 kJ/mol) is much higher than that of glycine decationization, and consequently, this process cannot be competitive. Decomposition of **6** would give either Cu^+NH═CH_2 or Cu^+(HCOOH), the later final state being energetically less favorable (133 versus 196 kJ/mol, respectively).

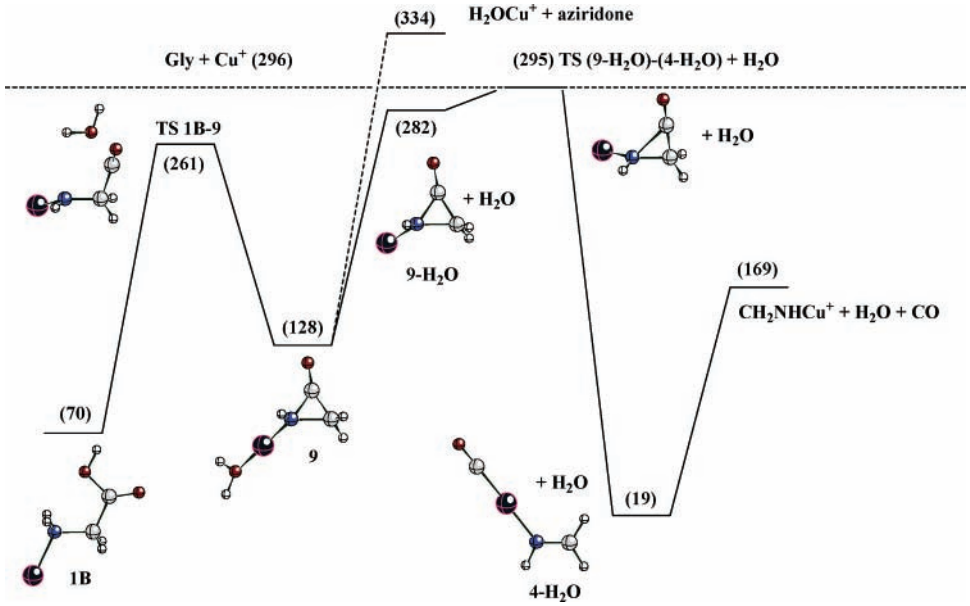


Figure 5. Potential energy profile for mechanism D: Formation of CH_2═$NHCu^+$ by loss of (H_2O + CO) via formation of a copper-lactone complex. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

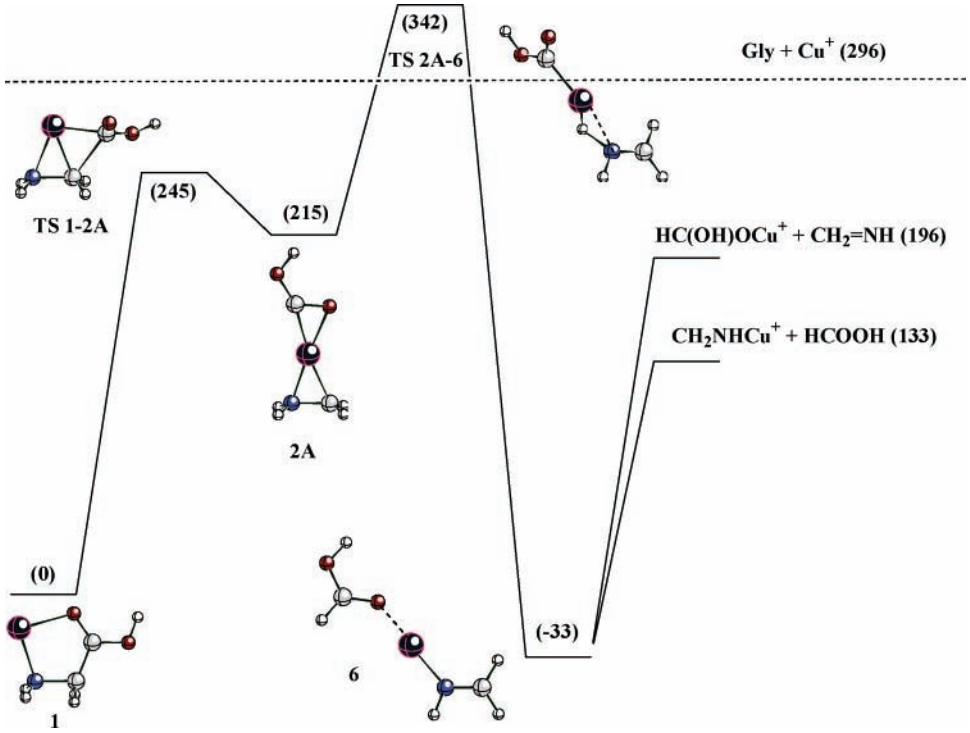


Figure 6. Potential energy profile for the formation of $CH_2{=}NHCu^+$ by loss of HCOOH via an insertion of Cu^+ into the C−C bond. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

Another mechanism was considered, based on recent work on the Cu^+ + glycolic acid reaction.[17] It starts with a hydrogen migration from N to the carbonyl oxygen, followed by copper insertion into the C−C bond, yielding a $(CH_2NH)CuC(OH)_2^+$ intermediate. Direct dissociation of this complex into $Cu^+NH{=}CH_2$ and a dihydroxycarbene would be unfavorable (see below); however, the intermediate complex should have enough internal energy for the dihydroxycarbene to isomerize to formic acid during dissociation. Although this pathway was found to be favorable in the reaction of Cu^+ with glycolic acid,[17] we could locate neither the transition state for N to O hydrogen transfer, nor the resulting minimum, on the $GlyCu^+$ potential energy surface (hydrogen back-transfer from O to N always occurred spontaneously). Although this does not necessarily imply that such a mechanism does not exist in the present case, it may suggest that the relative N−H and O−H bond strengths make the glycolic acid and glycine cases different.

Formation of $Cu^+NH{=}CH_2$ Associated with Loss of $C(OH)_2$. Since the energy required to reach the final state corresponding to this fragmentation (311 kJ/mol) is higher than that of Gly + Cu^+, the mechanism corresponding to this fragmentation has not been considered.

Formation of $Cu^+NH{=}CH_2$ Associated with Consecutive Losses of CO_2 and H_2. Two mechanisms may be considered. The successive elimination of CO_2 and H_2 from **1** would require, after cis−trans isomerization of the carboxyl group, a mechanism in two steps: first, transfer of the labile hydrogen from OH to CH_2 leads to the formation of a two-ligand complex, **7**, with methylamine and carbon dioxide ligands; then after CO_2 loss, a 1,2-dehydrogenation of methylamine leads to $Cu^+NH{=}CH_2$. Alternatively, the activation could start from the salt bridge isomer of $GlyCu^+$ **1C** (see Figure 7). Transfer of a labile hydrogen from the ammonium to CH_2 is assisted by the rupture of the C−C bond, leading to **7**.

These processes can be discarded on the grounds of experimental as well as theoretical arguments. After H/D exchange of all labile hydrogens and collisional activation of the *m*/*z* 141 ion (138+3), *m*/*z* 92 is entirely and exclusively shifted to *m*/*z* 93. The mechanisms above would lead to a mixture of *m*/*z* 93 (1/3) ($-CO_2$, $-D_2$) and *m*/*z* 94 (2/3) ($-CO_2$, −HD), which is not observed. These mechanisms were nevertheless determined

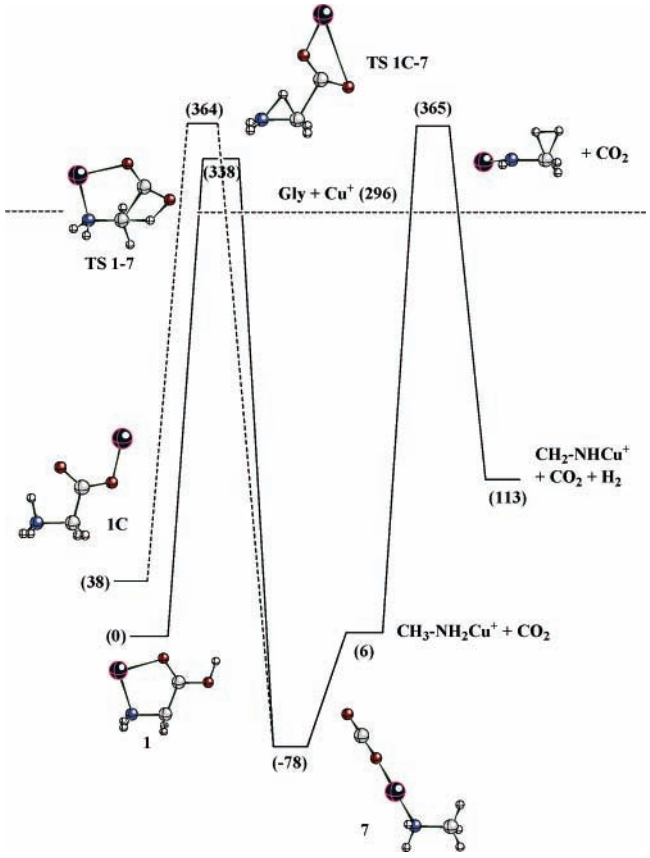


Figure 7. Potential energy profile for the formation of $CH_2{=}NHCu^+$ by loss of CO_2 and H_2. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

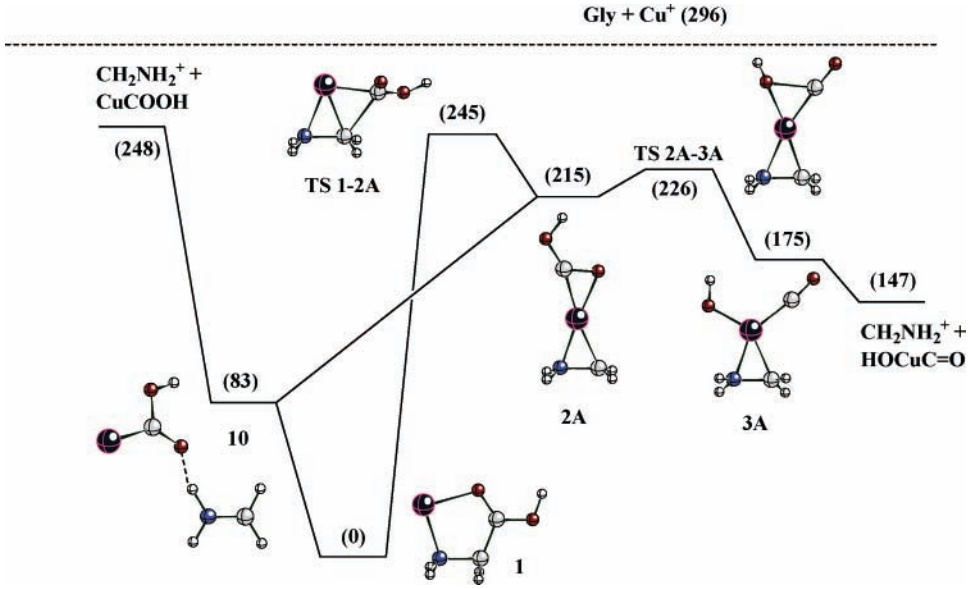


Figure 8. Potential energy profiles for the formation of $CH_2{=}NH_2^+$. All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

(see Figure 7). It was found that all transition states associated with hydrogen transfer and with elimination of H_2 from complexed methylamine are significantly less stable than Gly + Cu^+. Therefore, these mechanisms can be ruled out, in good agreement with labeling experiments.

In summary, both experimental and theoretical results indicate that the formation of $Cu^+NH{=}CH_2$ is exclusively associated with the loss of (H_2O+CO). Among the several mechanisms considered herein to account for this reaction, the most favorable involves copper insertion into the C−C and C−OH bonds. Dissociative attachment, on the other hand, is found to account for the minor loss of H_2O.

Formation Mechanisms of the Immonium Ion I: $CH_2NH_2^+$. *Formation of $CH_2NH_2^+$ Associated with Loss of CuCOOH.* This mechanism starts from **1**, with copper insertion into the C−C bond as for mechanism A above, leading to intermediate **2A** with an activation energy of 245 kJ/mol. Direct elimination of $CH_2NH_2^+$ would lead to **10** and then to **I** + CuCOOH (Figure 8). The energy of this final state is similar to that of **TS 1**−**2A**.

Formation of $CH_2NH_2^+$ Associated with Loss of HO−Cu−C=O. The three-ligand complex **3A**, generated from **2A** as in mechanism A above, is a good precursor for the formation of **I** + HO−Cu−C=O (Figure 8), by simple dissociation involving no activation barrier. This final state is rather stable at 147 kJ/mol. The overall critical energy along this path is that corresponding to the initial insertion of Cu^+ into the C−C bond (**TS 1**−**2A**, 245 kJ/mol higher in energy than **1**).

Formation of $CH_2NH_2^+$ Associated with Loss of HCOOCu. Two mechanisms have been considered for the formation of **I** + HCOOCu.

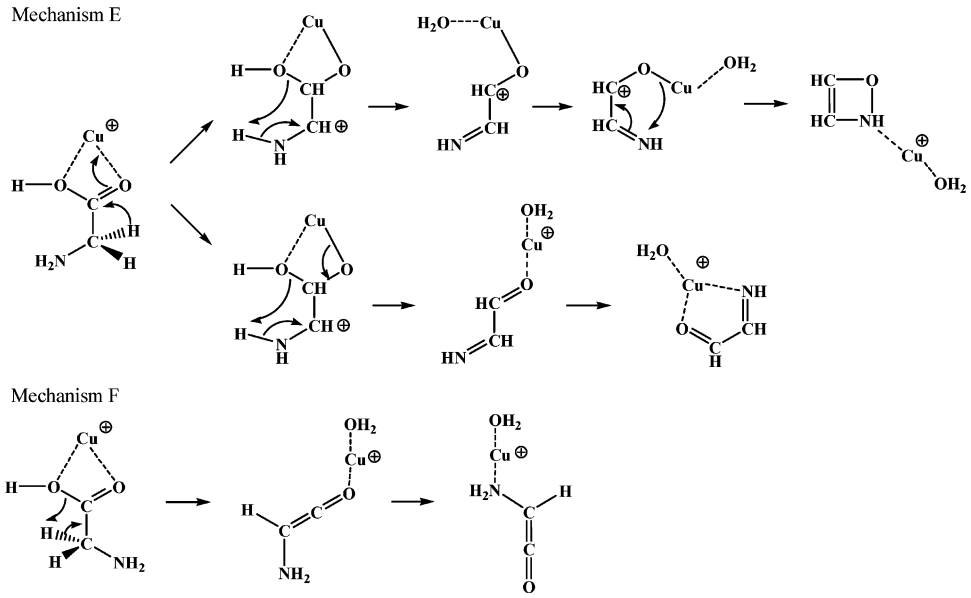
Both involve the heterolytic rupture of the CH_2−C bond associated either with a 1,2 H transfer of the hydroxylic hydrogen to the carboxyl carbon or from the salt bridge form, with a 1,3 H transfer of the protonating hydrogen to the carboxyl carbon. The transition states associated with these hydrogen transfers are located 397 and 364 kJ/mol respectively above **1**, much higher than Gly + Cu^+. Such high energies rule out these mechanisms as reasonable competitors.

In summary, formation of the immonium ion $CH_2NH_2^+$ has the same energetic requirement as that for the formation of $Cu^+NH{=}CH_2$. Among the various structures proposed to describe the neutral species associated to the formation of **I**, HO−Cu−CO is the complex giving the most stable final state. Its critical step is initial insertion of Cu^+ into the C−C bond, which is common to this path and mechanism A for loss of [H_2O+CO]. It is not clear why the relative abundances of $CH_2NH_2^+$ and $Cu^+NH{=}CH_2$ are somewhat different at low energies, however such relatively small differences may not be quantitatively significant.

Formation of $Cu^+(H_2O)$. The breakdown graph in Figure 1 shows that at relatively low collision energies a significant amount of $Cu^+(H_2O)$ is formed. At these energies, elimination of H_2O is not observed, whereas it is seen at higher energies in minor amounts. Several mechanisms may be invoked to account for the formation of $Cu^+(H_2O)$. All involve an intermediate of the form $[C_2,O,N,H_3]Cu^+(H_2O)$. They differ by the isomer of $[C_2,O,N,H_3]$ being considered. The first is aziridinone, previously discussed to account for the minor loss of H_2O. Elimination of H_2O via intermediate **9** was shown to require 282 kJ/mol (see Figure 5). If elimination of aziridinone is considered instead, formation of $Cu^+(H_2O)$ is computed to require 334 kJ/mol; therefore, this process requires more energy than reaching Gly + Cu^+ and must be discarded.

The other mechanisms which we have considered are gathered in Scheme 1. The first (mechanism E in Scheme 1) may lead to the formation of several different final structures, the first of which is a four-membered ring. This molecule can bind copper via the oxygen or nitrogen atom, the latter being the most favorable. The energy required to eliminate this isomer of $[C_2,O,N,H_3]$ from $[C_2,O,N,H_3]Cu^+(H_2O)$ is 222 kJ/mol larger than that needed to detach Cu^+ from $GlyCu^+$ **1**, and therefore, this mechanism is highly unfavorable. Mechanism E in Scheme 1 may also yield a conjugated isomer of $[C_2,O,N,H_3]$, of the form O=CH−CH=NH, which again can bind copper either via the oxygen or nitrogen atom. If the most stable isomer (chelated between N and O) is formed, elimination of O=CH−CH=NH to form $Cu^+(H_2O)$ is less favorable than H_2O detachment by 106 kJ/mol and thus cannot be observed. It is also possible that the O-bound isomer is formed, from which H_2O detachment may proceed before isomerization to the more stable isomer. In this case, we find that elimination of O=CH−CH=NH is more favorable than that of water; however, it is so by only 8 kJ/mol. Therefore, competitive losses of either ligand would be predicted, contrary to experimental findings (see Figure 1).

SCHEME 1



An analogous conclusion arises from mechanism F in Scheme 1. In this case, an amino-ketene isomer of $[C_2,O,N,H_3]$ is bound to copper. As with its O═CH−CH═NH isomer, the most stable attachment to Cu^+ is via nitrogen, leading to the elimination of water being much more favorable than that of amino-ketene (by 53 kJ/mol). If the less stable O-bound isomer is formed, loss of amino-ketene is now more favorable than loss of H_2O by only 8 kJ/mol, and here again both processes would be predicted to be competitive, in disagreement with experiments.

Yet another possibility is that the *m/z* 81 ion is not $Cu^+(H_2O)$ but rather $HCuOH^+$, i.e., that copper is inserted into the O−H bond. All attempts to characterize such a species failed; it was found to be kinetically stable within a $HCuOH^+(CO)(CH_2NH)$ complex provided that the hydride and hydroxyl ligands are in trans positions; however, the energy of this complex is more than 400 kJ/mol less stable than Gly + Cu^+. Such an energy demand is of course incompatible with the formation of $HCuOH^+$ in the low energy conditions used. One more hypothesis is that $Cu^+(H_2O)$ arises from losses of CO and CH_2-NH from **4** (see Figure 1). This dissociation channel requires 254 kJ/mol; that is, it is lower than Cu^+ detachment from **1** by 42 kJ/mol. Although this route to $Cu^+(H_2O)$ is energetically viable, it is difficult to understand why losses of H_2O+CO *and* of CO+CH_2NH should be observed, but not losses of H_2O+CH_2-NH (no *m/z* 91 ion is seen in the spectra). The conclusion from these investigations is that, as long as the B3LYP calculations are considered to be accurate enough, $Cu^+(H_2O)$ is likely not to be a fragment ion arising directly from the decomposition of $GlyCu^+$, but rather comes from the attachment of background H_2O to Cu^+, the latter being formed by metal detachment from $GlyCu^+$ under collisional activation. Repeated experiments at different periods of time showed a rather variable amount of $Cu^+(H_2O)$ formed, in agreement with this interpretation.

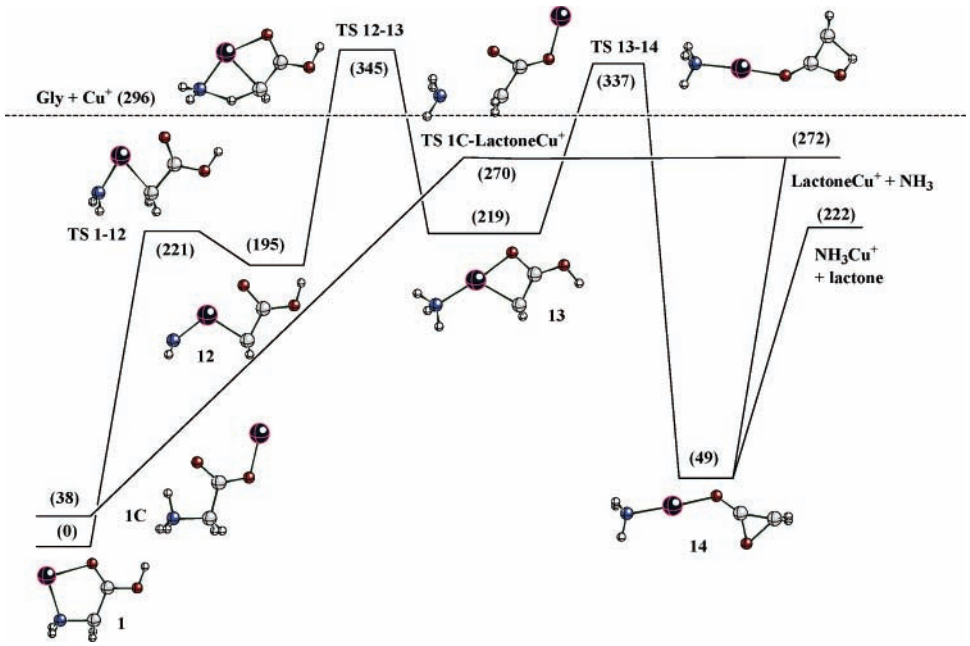


Figure 9. Potential energy profiles for the elimination of NH_3 (not observed experimentally). All energies are relative to that of **1**, computed at the B3LYP/basis2//B3LYP/basis1 level.

Mechanisms for the Elimination of NH_3 (Not Observed Experimentally). Although NH_3 loss is not observed among the collision-induced fragmentations of $GlyCu^+$, it is of interest to consider the mechanism and energy requirement of a fragmentation which is observed for amino acid complexes of Ag^+.[14] The latter is a weaker binder than is Cu^+ and is even less prone to insertion into covalent bonds; however, the general electronic properties of these two coinage metals are similar. We were therefore curious to learn how unfavorable the elimination of ammonia may be in the $GlyCu^+$ case.

As was done for the elimination of H_2O, two different types of mechanisms were considered. The corresponding potential energy profiles are reported in Figure 9. The first mechanism starts with the insertion of copper into the C−N in **1**, leading to an inserted minimum **12** via a reasonable energy barrier (221 kJ/mol). Forming an ammonia molecule (as in **13**) then requires the migration of a hydrogen atom from C to N, with a high energy barrier of 345 kJ/mol (**TS 12**−**13**). A second high barrier (**TS 13**−**14**) is associated with the ring closure leading to a very stable lactone complex **14**, prior to elimination of NH_3. Clearly the rate-limiting activation barriers for this mechanism are much too high for it to be competitive. It may be noted that elimination of lactone from **14** is predicted to be easier than that of ammonia by ca. 50 kJ/mol.

There is however a second mechanism, inspired from that previously proposed in the fragmentations of silver complexes of Phe,[14] which starts from the salt bridge isomer of $GlyCu^+$ **1C**. Here the formation of an ammonia molecule is assisted by ring closure to the three-membered ring lactone. The transition state for this one-step process lies 270 kJ/mol above **1**. It may lead either to the two-ligand copper complex of lactone and ammonia **14**, or directly to the elimination of ammonia (272 kJ/mol above **1**) since the formation of NH_3 is distant from the metal in this mechanism. The present results suggest that loss of ammonia is nearly competitive with the losses of H_2O and of (H_2O + CO), explaining why changing either the metal cation or the amino acid may be enough to reverse the preference for these product ions.

Conclusions

In this work, we have shown that the main decomposition process of $GlyCu^+$ is the loss of H_2O and CO to give the cationized imine CH_2NHCu^+. This elimination arises from a rearranged form of $GlyCu^+$ which is a complex of Cu^+ with three neutral molecules H_2O, CO, and $NH{=}CH_2$. Three mechanisms were explored for the formation of this intermediate. The mechanism of lowest critical energy involves successive insertions of Cu^+ into the C−C and C−OH bonds. This result indicates that despite the large promotion energy of Cu^+, copper insertion into covalent bonds is a viable alternative to dissociative attachment. The energetic requirements being relatively similar, it is difficult to rule out the latter type of mechanism. In fact, the minor loss of H_2O can only be interpreted in terms of a non insertive attachment, with the formation of a lactone ligand.

None of these mechanisms is particularly easy (the lowest rate-limiting activation barrier is computed to be 245 kJ/mol), so that direct detachment of Cu^+ is a competitive process, in good agreement with experiment. Formation of the fourth fragment ion, $CH_2NH_2^+$, is found to occur via the same C−C insertion step as for the formation of CH_2NHCu^+. Finally, the elimination of NH_3 was considered, although not observed experimentally. The best mechanism is a non insertive one, in which a lactone ligand is formed.

The large panel of reaction pathways described in this paper should help rationalize the seemingly scattered data on the fragmentations of related complexes with different amino acids and metal cations.

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