The Reactions of Ground State Cu^+ and Fe^+ with the 20 Common Amino Acids

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A systematic investigation of the gas-phase reactions of Cu^+ and Fe^+ with the 20 common amino acids is reported. Metal ions are formed by laser ablation of a metal target and are trapped in the analyzer cell of a Fourier transform mass spectrometer. By using quadrupolar excitation to axialize the metal ions, tens of thousands of thermalizing collisions occur prior to their reactions with laser-desorbed amino acid neutral molecules. Amino acids with nonpolar side chains are found to be more reactive toward Cu^+ and Fe^+ than amino acids with polar side chain. Many of the nonpolar amino acids are found to undergo dissociative metal attachment with a neutral loss of 46 u. A ^{13}C -labeling experiment shows that the carboxyl group is lost during dissociative metal attachment to amino acids. Together these results suggest that these metal ions interact primarily with the carboxyl functional group in these molecules. (*J Am Soc Mass Spectrom* 1996, 7, 722–730)

Introduction

A number of ionization methods are available in mass spectrometry to measure the molecular weights of proteins and other large biomolecules such as matrix-assisted laser desorption ionization [1, 2], electrospray ionization (ESI) [3–7], and fast-atom bombardment (FAB) [8, 9]. To obtain structural details such as the amino acid sequence of a protein, fragmentation of the molecular ion is required. Collision-activated dissociation [10–12], photodissociation, [13–15], and surface-induced dissociation [16–18] have been used to induce the fragmentation of peptides and proteins. As an alternative to these approaches, we have developed methods to control the ionization and fragmentation of biomolecules by using gas-phase chemical reactions [19–27]. To accomplish this, reagent ions are formed and stored in the analyzer region of a Fourier transform mass spectrometer, and laser-desorbed neutral biomolecules are allowed to react with these ions. This two-step method for ion formations, called laser desorption–chemical ionization (LD-CI) [19–27], requires the production of gas phase, intact, *neutral* molecules, in contrast to most laser desorption mass spectrometry experiments in which *ions* are desorbed directly and detected. To efficiently desorb intact neutral molecules of peptides for chemical ionization studies, the technique of substrate-assisted laser desorption (SALD) was developed [21]. Using LD-CI, we have examined the gas-phase chemistry of peptide molecules and have used proton transfer [19], charge-exchange [22], and metal ion reactions [20, 22] to fragment peptides in a controlled fashion.

A number of workers have used mass spectrometry to examine metal ion–peptide complexes that are formed in the condensed phase. These earlier studies were carried out by mixing metal salts with biomolecules in solution and then transferring the sample into the gas phase via ionization methods such as FAB [28–32] or ESI [33–36]. Studies of peptides cationized with alkali, alkaline, and transition metal ions have provided an understanding of the relationship between fragmentation reactions [29–37], the metal ion binding site [32, 34], and metal-binding affinities [31, 37]. In contrast to such condensed phase studies, we examine here the reactions of metal ions with biomolecules in the gas phase. These studies probe the intrinsic reactivities of bare metal ions, that is, their chemical behavior in the absence of counterions or a solvent shell, such as are found in the condensed phase. Bare metal ions are known to be more reactive toward bond activation than their solvated counterparts [38]. Due to volatility limitations, previous investigations of gas-phase metal ion reactions have been limited to their reactions with small, volatile molecules [38–46]. The SALD method has made it possible to apply chemical ionization to laser-desorbed neutral molecules of low volatility [21].

A number of gas-phase metal ions have been used as chemical reagents, to obtain the molecular weight of compounds, and to induce the decomposition of molecules [38–46]. A large number of metal ion studies have focused on their chemistry with molecules such as hydrocarbons [47–53], carbonyl compounds [54–58], alkyl halides [59–61] and amines [62–64]. In these gas-phase studies, transition metal ions have exhibited a variety of reactivities. Electronic state specificity of the reactions of transition metal ions

toward hydrocarbons and their derivatives has been reported [63-78]. The metal ions that are examined here, Fe^+ and Cu^+ , were first proposed as chemical ionization reagents by Freiser [39] and Gross [40]. Fe^+ ions have been observed to react with carbonyl compounds by cleavage of C—C bonds adjacent to the carbonyl group [78]. Cu^+ ion has been reported to react with esters to produce alcohol-ketone and acid-alkene pairs [39]. Such reactivities suggest that these metal ions may be useful to cleave peptides around their amide bonds. In prior work performed in this laboratory, Speir et al. [20] used LD-CI and Fourier transform mass spectrometry (FTMS) to investigate the reactions of Fe^+ with a few small peptides and observed the formation of metal-containing adducts and fragment ions [20]. However, in that study charge transfer was found to be the primary reaction channel between metal ions and the peptide molecule [20]. The authors concluded that excited state metal ions were responsible for the primary reactions, despite the fact that dozens of collisions between a buffer gas and the metal ions were used in an attempt to quench the excited states and that more collisions would be required to relax all metal ions to their ground state. However, collisions with neutrals cause ions to drift away from the principal axis of the FTMS analyzer cell [79]. Collision-induced radial loss of the ions restricted the number of collisions to less than a few hundred in this previous study in which ions were trapped in the FTMS analyzer cell in the standard manner.

One of the most promising recent advances in FTMS has been the development of quadrupolar excitation (QE) [79-86]. Ions that undergo quadrupolar excitation are returned to the center of the analyzer cell when they undergo collisions. Thus ions can experience thousands of collisions without being lost from the analyzer cell. By using QE, it is possible to completely quench the electronically excited states of metal ions formed by laser ablation and to trap only ground state ions for further investigation. In this article, we show the first example of the use of QE to examine ground state metal ion chemistry. Fe^+ and Cu^+ are prepared in their ground states and allowed to react with the 20 common amino acids.

Experimental

These experiments were performed with a Fourier transform mass spectrometer that was specifically designed and constructed for LD-CI experiments. An electromagnet with a 24-cm pole cap diameter produces a 1-T magnetic field. A 4.4-cm cubic analyzer cell is housed in an ultrahigh vacuum chamber and pumped to a base pressure of 2×10^{-10} torr by a 330-L/s turbomolecular pump. A pulsed valve mounted on the main vacuum chamber is used to introduce the collision gas that quenches the excited states of the metal ions. Figure 1 shows the instrument layout used for the metal ion LD-CI experiments.

Figure 1. Instrument layout for metal ion LD-CI experiments. For clarity, the electromagnet is not shown. The output of the excimer laser, shown by the dotted line, is split into two paths by a fused silica plate that transmits 92% and reflects 8% of the incident energy. The transmitted light is focused tightly onto a metal target to form Cu^+ or Fe^+ metal ions, which are then trapped in the analyzer cell. The reflected beam is focused on the amino acid-coated sample stub for the desorption of neutral intact molecules. Two electromechanical shutters block the laser beam that is directed to the sample stub and pass the laser beam that is focused on the metal target to form metal ions. The shutter positions are reversed to allow a second laser pulse to desorb neutral amino acid molecules from the sample stub.

Amino acids are introduced on a stainless steel sample stub that is mounted on the side of the analyzer cell. The amino acids were purchased from Sigma Chemical (St. Louis, MO) and used without further purification. ^{13}C -labeled amino alanine was purchased from Cambridge Isotope Laboratory (Cambridge, MA). The samples were prepared as thin films by electrospray deposition. The substrate-assisted laser desorption ionization (SALD) sample preparation method is used, in which a thin film (200 ng/mm², approximately 1000 monolayers) of an amino acid is coated over a thin film of sinapinic acid (200 ng/mm²) on the sample stub. The stub is introduced first into an antechamber, which is then evacuated to 10^{-7} – 10^{-8} torr. The sample is then transferred to the main vacuum chamber by a magnetically coupled manipulator where it is transferred to a stub holder on the analyzer cell. Copper and steel targets are mounted on a carousel that is located immediately below the analyzer cell. The carousel can be turned with a rotational motion vacuum feedthrough to permit selection of copper or steel targets for formation of Cu^+ or Fe^+ , respectively.

The output of an excimer laser (Questek model 2110) is split into two paths by a fused silica plate that transmits 92% of the incident energy and reflects 8%. The transmitted light is focused tightly onto a metal target to form Cu^+ or Fe^+ metal ions, which are then trapped in the analyzer cell. The reflected beam is focused on the amino acid coated sample stub for the desorption of intact neutral molecules. The KrF excimer laser ($\lambda = 248$ nm) provides approximately 200 mJ of energy per laser pulse. The laser irradiance at the metal target is approximately 10^7 – 10^8 W/cm² and at the sample stub is approximately 10^6 W/cm². Solenoid-driven electronic shutters pass or block the laser

beams, which allows the formation of metal ions and the desorption of neutral molecules with alternate pulses of a single laser. In a typical experiment, shutters first are set to block the laser beam that is directed at the sample stub and to pass the laser beam that is focused upon the metal target. After the metal ions are formed by laser ablation of the metal target, a pulsed valve introduces the buffer gas (N_2 or CH_4) into the cell in four short (30-ms) bursts. The pressure of the buffer gas reaches a peak value between 10^{-5} and 10^{-3} torr 150 ms after the pulsed valve is opened. The buffer gas was introduced in four bursts to provide enough collisions to efficiently relax the electronically excited metal ions to their ground states. Figure 2 shows a plot of the pressure versus time for the pulsed valve introduction of the buffer gas that was obtained by converting the current output of an ion gauge tube to a voltage and recording the signal with a transient digitizer. The peaks and valleys in the pressure plot are highly reproducible and result from the pulsed introduction of the collision gas into the vacuum system in four discrete bursts separated by 120 ms. Numerical integration of the plot in Figure 2 indicates that the metal ions have 11,000 collisions. To thermalize metal ions in these experiments 10,000–100,000 collisions were used.

Quadrupolar excitation is achieved by using methods that were described previously [81]. A switching relay for the excitation and detection plate cell connections is activated for quadrupolar excitation shortly after the pulsed valve for the collision gas has been triggered. The quadrupolar excitation signal (50 mV p-p) is applied to the cell plates for 2.4 s. The frequency of the quadrupolar excitation signal that is applied corresponds to the mass of the selected metal ion, $^{63}Cu^+$, $^{65}Cu^+$, or $^{56}Fe^+$. This is a mass-selective process in which all the ions other than the selected metal ion are eliminated by radial diffusion. After the metal ions are thermalized, the position of both shutters is reversed and neutral molecules are desorbed by

Figure 2. A plot of pressure versus time for the pulsed introduction of the buffer gas to thermalize metal ions. N_2 or CH_4 is admitted through a pulsed valve and used as a collision gas in all quadrupolar excitation experiments to cool metal ions. The base pressure is 10^{-8} torr and the peak pressure is 10^{-4} torr. Integration of the area from 300 to 800 ms gives 11,000 collisions per ion.

a second laser pulse. Metal ions are then allowed to react with the neutral amino acid molecules in the analyzer cell. The ions are then detected by standard FTMS methods.

Ammonium LD-CI is achieved with experimental procedures that were described previously [19, 21, 23]. Ammonia is admitted into the vacuum system through a pulsed valve and is ionized by 70-eV electron bombardment. Ion-molecule reactions of the initially formed ammonia ion with ammonia gas leads to the exclusive formation of the ammonium ion. SALD of the amino acids into the trapped ammonium ions leads to the formation of the protonated molecule of the sample.

Results and Discussion

The desorption of intact neutral molecules was established for each of the amino acids by using LD-CI with the reagent ion NH_4^+ . Because ammonia has a lower proton affinity than any of the amino acids [26], proton transfer from the ammonium ion to the laser-desorbed amino acids will occur with unit collision efficiency [87]. Figure 3a shows the mass spectrum that results from laser desorption of leucine without chemical ionization and Figure 3b shows the mass spectrum obtained by laser desorption of leucine with chemical ionization by ammonium ions. Without chemical ionization, only peaks that arise from electronic noise are observed in the laser desorption mass spectrum at m/z 80, 96, 120, 160, and 240. This observation demonstrates that no ions are formed directly by the laser desorption process. With NH_4^+ as a reagent ion, laser-desorbed leucine neutrals react to form the protonated molecule, as seen in Figure 3b. No fragment ions are

Figure 3. (a) Mass spectrum that results from the laser desorption of the amino acid leucine without chemical ionization. No ions are formed by laser desorption. The small peaks observed at m/z 80, 96, 120, 160, and 240 are due to electronic noise and are found in all the mass spectra. (b) Laser desorption-chemical ionization mass spectrum of leucine with NH_4^+ reagent ion. The protonated molecule is formed and no fragment ions are observed.

observed, which demonstrates that laser desorption yields only intact neutral molecules, that is, no neutral fragments are produced. This test was repeated for each amino acid to permit the adjustment of the proper laser power and sample preparation conditions for the desorption of intact neutral molecules.

Metal ions underwent thousands of thermalizing collisions before they were allowed to react with the amino acids. The effect of collisional relaxation on their reactivity can be seen in Figure 4. Cu^+ formed by laser ablation, but without collisional thermalization reacts with isoleucine as shown in Figure 4a. The protonated molecule at m/z 132 is the only product ion observed, but unreacted Cu^+ also appears in the mass spectrum. The formation of a protonated ion has been reported before for LD-CI with excited state metal ions [21]. This formation is postulated to occur by a two-step mechanism in which the metal ion first undergoes charge transfer with a laser-desorbed neutral to form an odd electron ion, which then reacts with a second neutral molecule to form a protonated molecule, as illustrated by Equations 1 and 2 for isoleucine:

The recombination energy of Cu^+ in its ground state ($^1S_0, 3d^{10}$) is 7.7 eV [88, 20]. The ionization energy of isoleucine is estimated to lie between 8.9 and 9.1 eV [89, 20]. Therefore charge transfer between ground state Cu^+ and amino acids is an endothermic process. The product that has been observed suggests a reaction by electronically excited Cu^+ ions, because an endothermic ion-molecule reaction should occur at too

slow a rate to form an appreciable abundance of a product ion under the experimental conditions used here. This reaction is not driven by the kinetic energy of the metal ions. If the metal ions are thermalized by 100 collisions with a buffer gas, the protonated ion is still the only product observed. One hundred collisions are more than adequate to relax the kinetic energy of the ions, but not enough to relax the internal energy. We find that thousands of thermalizing collisions are necessary to quench the charge-transfer reaction, and this quenching requires the application of quadrupolar excitation to prevent the loss of the metal ions from the analyzer cell through collision-induced radial diffusion. The observation of the protonated ion rather than an odd electron ion implies that several collisions occur between the initially formed ions and the desorbed neutral amino acid molecules [20]. It previously was estimated that the initially formed ions undergo 1–1000 collisions with laser desorbed neutrals [20]. The pressure in the vacuum chamber, as monitored by the ion gauge, does not rise during the laser desorption of the neutrals, and so the number of collisions between ions and neutrals cannot be calculated as was done for the pulsed introduction of the buffer gas. The pressure rise due to the laser desorption of neutral molecules is contained within the analyzer cell, because the low volatility amino acid molecules will probably stick to the first surface with which they collide. We estimate that ions undergo fewer than 10 collisions, because there is little evidence of radial diffusion in the surviving metal ions.

The mass spectrum that results from the reaction of ground state $^{63}\text{Cu}^+$ with isoleucine is shown in Figure 4b. More than 10,000 collisions with N_2 were used to quench the excited states of Cu^+ ions after laser ablation. Quadrupolar excitation was applied to the copper ions while the bath gas was pulsed into the vacuum system. Except for the unreacted $^{63}\text{Cu}^+$, the only signal that is observed in the mass spectrum occurs at m/z 148; it arises from the elimination of a 46-u neutral species from the $^{63}\text{Cu}^+$ -Ile adduct. Either of the two copper isotopes $^{63}\text{Cu}^+$ or $^{65}\text{Cu}^+$ can be isolated selectively by quadrupolar excitation prior to the metal ion reaction with the neutral amino acid molecules. The reaction between ground state $^{65}\text{Cu}^+$ with isoleucine is shown in Figure 4c. Similar to the reaction of $^{63}\text{Cu}^+$ with isoleucine, unreacted $^{65}\text{Cu}^+$ is the dominant peak in the spectrum, and a signal at m/z 150 arises from the $^{65}\text{Cu}^+$ -Ile adduct with the elimination of a 46-u neutral species. Experiments with the two isotopes of Cu^+ can be used to distinguish the peaks that contain metals from those that contain no metal atoms by observing which product peaks undergo a 2-u mass shift. The protonated isoleucine signal observed in Figure 4a, which is produced by charge transfer followed by hydrogen transfer, is much stronger relative to the residual Cu^+ ions than is the signal that comes from dissociative attachment of copper to isoleucine in Figure 4b. The difference in the relative abundances

Figure 4. Mass spectra of the reactions between Cu^+ and isoleucine. (a) Cu^+ ions, formed by laser ablation without collisional thermalization, are allowed to react with isoleucine. Excited state metal ions undergo charge transfer to form an odd electron molecular ion of isoleucine, which abstracts a hydrogen from other isoleucine neutral to form a protonated molecule. (b) Thermalized $^{63}\text{Cu}^+$ reacts with isoleucine to form a metal-containing fragment ion. (c) Thermalized $^{65}\text{Cu}^+$ reacts with isoleucine to form a metal-containing fragment ion 2 u higher than in (b).

for the product ions from the two experiments suggests that metal ions in their excited states have a larger reaction cross section than do metal ions in their ground state. The enhanced reactivity of excited state metal ions is observed for the reactions of Cu^+ and Fe^+ with all 20 amino acids.

Table 1 lists the products of the reactions of the 20 amino acids with Cu^+ . Mass spectra that are representative of the reactions of Cu^+ with the 20 amino acids are displayed in Figure 5. The reaction of Cu^+ with amino acids that bear a hydrocarbon side chain generally are found to form a copper-attached ion with the loss of a 46-u neutral, which is assigned as $[\text{M} + \text{Cu}^+ - \text{H}_2\text{CO}_2]$. This ion can be seen in the reaction of Cu^+ with valine in Figure 5a, as well as for the reaction of isoleucine in Figure 4b and c. Formation of a copper adduct with loss of a 28-u neutral, which is assigned as $[\text{M} + \text{Cu}^+ - \text{CO}]$, is observed for the reaction of some, but not all of the amino acids with a hydrocarbon side chain. Other products of the reaction of valine with Cu^+ found in Figure 5a include the adduct species $[\text{M} + \text{Cu}^+]$ and $[\text{M} + \text{Cu}^+ + \text{H}_2\text{O}]$. The adduct $[\text{M} + \text{Cu}^+]$ likely results from the sequential reaction of trapped ions with two or more valine neutrals. It is uncommon in ion-molecule reactions to form an adduct without the loss of a neutral species. However, a fragment species formed by the reaction of Cu^+ with valine could transfer Cu^+ to a second valine molecule

Figure 5. Mass spectra that results from the reactions of $^{63}\text{Cu}^+$ with (a) valine, (b) methionine, and (c) glutamic acid. The intensity scale has been expanded by a factor of 10 in (c). Noise peaks are marked with an asterisk.

to form the adduct ion. Unfortunately, double resonance experiments cannot be used to establish the identity of the intermediate, because the neutrals are desorbed by the laser in a short burst (10–100 ns). The time between consecutive collisions of the metal ion with valine neutral molecules is too short to permit resonance ejection of the intermediate. The $[\text{M} + \text{Cu}^+$

Table 1. Reactions of the 20 amino acids with Cu^+

Amino acid	MW	$^{63/65}\text{Cu}^+ + \text{A.A.}$	$^{63/65}\text{Cu}^+ + \text{A.A.} - 28 \text{ (CO)}$	$^{63/65}\text{Cu}^+ + \text{A.A.} - 46 \text{ (H}_2\text{CO}_2\text{)}$	Others
Glycine	75			192/194	
Alanine	89	152/154	124/126	106/108	
Valine	117	180/182	152/154	134/136	$\text{M} + \text{Cu}^+ \text{H}_2\text{O}$
Leucine	131	194/196		148/150	
Isoleucine	131			148/150	
Proline	115	178/180		132/134	
Phenylalanine	165		200/202	182/184	
Methionine	149			166/168	
Cysteine	121				$\text{M} + \text{Cu}^+ - (\text{H}_2\text{O})$ $\text{M} + \text{Cu}^+ - 35$
Aspartic acid	133			No reaction	
Glumatic acid	147	210/212			$\text{M} + \text{Cu} - \text{CO}_2$
Asparagine	132			No reaction	
Glutamine	146			No reaction	
Serine	105			No reaction	
Threonine	119		154/156 (weak)		$\text{M} + \text{Cu}^+ - 18(\text{H}_2\text{O})$
Tyrosine	181	244/246 (weak)			
Tryptophan	204	267/269 (weak)			
Lysine	146			No reaction	
Histidine	155		190/192		$\text{M} + \text{Cu}^+ - \text{H}_2\text{O}$
Arginine	174		209/211		

+ H_2O] ion found in Figure 5a provides further evidence that trapped ions undergo multiple collisions with the amino acid neutrals, because this species clearly could not be produced by the reaction of Cu^+ with a single valine molecule. It is possible that the water adducts are produced by reaction of metal-amino acid complexes with water molecules present as a trace contaminant in the electrosprayed thin films and liberated by laser desorption. Figure 5b shows the mass spectrum that results from the reaction of methionine with Cu^+ . The thiomethyl side chain of methionine is nonpolar and this amino acid is found to have a reactivity that is similar to that of the amino acids with a hydrocarbon side chain. The two products are the adduct species $[\text{M} + \text{Cu}^+]$, and the fragment ion $[\text{M} + \text{Cu}^+ - \text{H}_2\text{CO}_2]$. The products of the reaction of Cu^+ with glutamic acid, an amino acid with a polar side chain, are shown in Figure 5c. An adduct ion $[\text{M} + \text{Cu}^+]$ and a fragment ion that results from loss of a 44-u neutral and is assigned as $[\text{M} + \text{Cu}^+ - \text{CO}_2]$, are the two principal products. To observe the product ions, the intensity scale was expanded by a factor of 10; that is, the ratio of the product ions to the unreacted copper signal is approximately 10 times smaller for the reaction of glutamic acid compared to that of the amino acids with hydrocarbon side chains. The reaction of NH_4^+ with neutrals of glutamic acid produced an abundant protonated ion, which shows that the laser desorption process produces ample quantities of neutrals. The low abundance of product ions for the reaction of Cu^+ indicates that this is an inefficient reaction. In general, amino acids with polar side chains

were found to be far less reactive toward Cu^+ than those with a nonpolar side chain. For cysteine, in addition to the $[\text{M} + \text{Cu}^+ - \text{H}_2\text{O}]$ product, we have observed copper attachment with a neutral loss of 35 u, which corresponds to the combined loss of H_2O and NH_3 .

Table 2 lists the products of the reactions of the 20 amino acids with Fe^+ . Mass spectra that are representative of the reactions of Fe^+ with the 20 amino acids are shown in Figure 6. As with Cu^+ , the amino acids with polar side chains were found to be less reactive or unreactive compared to the amino acids with nonpolar side chains. The formation of an adduct $[\text{M} + \text{Fe}^+]$, was observed as the principal product for many of the reactive amino acids, in contrast to the reactions of Cu^+ . Similar to the reactions of Cu^+ , metal attachment accompanied by neutral losses of 46 (H_2CO_2), 44 (CO_2), 28 (CO), or 18 (H_2O) u were observed. Valine and methionine, which are typical of the amino acids that have a nonpolar side chain, have been found to form an iron-attached ion with neutral losses of 28 u and 46 u, shown in Figure 5a and b. Methionine and lysine react with iron by dissociative attachment with the loss of a 46-u neutral (H_2CO_2), whereas proline and phenylalanine form an adduct with a 44-u neutral loss (CO_2). An 18-u neutral loss (H_2O) has been observed only for alanine. Other reactive amino acids have shown the formation of stable adduct ions, as represented by glutamic acid in Figure 6c, with a low reaction efficiency. The low reactivity or unreactive behavior of the polar amino acids with ground state Fe^+ is similar to their reactivity with Cu^+ . In condensed phase studies

Table 2. Reaction of the 20 amino acids with Fe^+

Amino acid	MW	$^{56}\text{Fe}^+ + \text{A.A.}$	$^{56}\text{Fe}^+ + \text{A.A.} - 28 (\text{CO})$	$^{56}\text{Fe}^+ + \text{A.A.} - 46 (\text{H}_2\text{CO}_2)$	Others
Glycine	75			85	
Alanine	89	145			$\text{M} + \text{Fe}^+ - \text{H}_2\text{O}$
Valine	117		145	127	
Leucine	131	187			
Isoleucine	131	187			
Proline	115	171			$\text{Pro} + \text{Fe}^+ - \text{CO}_2$
Phenylalanine	165				$\text{Phe} + \text{Fe}^+ - \text{CO}_2$
Methionine	149	205		159	
Cysteine	121	177			
Aspartic acid	133	189			
Glutamic acid	147	203			
Asparagine	132		No reaction		
Glutamine	146		No reaction		
Serine	105		No reaction		
Threonine	119		No reaction		
Tyrosine	181		No reaction		
Tryptophan	204		No reaction		
Histidine	155		No reaction		
Lysine	146			156	
Arginine	174		No reaction

Figure 6. Mass spectra that results from the reactions of $^{56}\text{Fe}^+$ with (a) valine, (b) methionine, and (c) glutamic acid. Noise peaks are labeled with an asterisk.

of copper affinity toward the amino acids, it has been found [31] that amino acids that have side chains that can be described as soft Lewis donors, such as methionine and phenylalanine, produce a more stable copper-amino acid bond than amino acids that have strongly polar side chains. This study is consistent with our observations that the low polarity amino acids are found to be more reactive toward the two metal ions. As discussed previously for Cu^+ , the Fe^+ -amino acid adduct ion observed for many of the reactive amino acids most likely results from a sequential reaction in which a fragment species transfers the metal ion to a second neutral molecule to form the adduct ion, $[\text{M} + \text{Fe}^+]$.

An isotopic labeling experiment was conducted to verify the composition of the major neutral losses in the Cu^+ reactions. A neutral loss of 28-u from the Cu^+ -amino acid adduct could result from a loss of C_2H_4 or from CO, whereas the 46-u neutral loss might correspond to $\text{C}_2\text{H}_4 + \text{H}_2\text{O}$, $\text{CO} + \text{H}_2\text{O}$, or H_2CO_2 . The loss of C_2H_4 would require cleavage of the side chain by the metal ion. The observation of a 46-u loss from glycine suggests that the neutral loss involves the carboxyl group rather than the side chain. Carboxyl loss was verified by examining the reaction of Cu^+ with ^{13}C -alanine, labeled at the carboxyl carbon. Figure 7 compares mass spectra of the products of the reactions of $^{63}\text{Cu}^+$ with unlabeled and labeled alanine. If cleavage occurs between the α -carbon and carboxyl group, then the neutral mass losses for ^{13}C -labeled alanine should be 47 u and 29 u rather than the 46 u and 28 u mass losses observed for unlabeled alanine. If the cleavage results in the loss of the side chain and the α -carbon of the amino acid, then there should be no difference between the mass losses of labeled alanine versus unlabeled alanine. ^{13}C -labeled alanine was found to react with $^{63}\text{Cu}^+$ to yield a 47-u loss, which produces a peak at m/z 106, and a 29-u loss, which produces a peak at m/z 124. This proves that the

Figure 7. Mass spectra of the reaction of (a) Cu^+ with unlabeled normal alanine and (b) Cu^+ with ^{13}C -labeled alanine. Noise peaks are marked with an asterisk.

neutral loss involves the carboxyl carbon and that the cleavage occurs at the $\text{C}_\alpha-(\text{COOH})$ bond. This result is consistent with findings by Freiser and co-workers [78] that the cleavage of carbonyl compounds by Fe^+ occurs at the $\text{C}-\text{C}$ bonds adjacent to the carbonyl group. A proposed intermediate in the reaction of Cu^+ or Fe^+ with the amino acids is shown in Structure 1. The proposed intermediate can lose CO_2 , H_2CO_2 , CO, and H_2O by hydrogen or hydroxyl transfer. These results suggest that the ground state Cu^+ and Fe^+ may be useful reagent ions for the dissociation of peptides, by activation of bonds near the amide bond rather than by cleaving the side chains of the amino acids. The loss of CO is much greater for the labeled alanine (30%) versus the unlabeled alanine (1%) in Figure 7. It is tempting to assign this difference to an isotope effect, which would be consistent with a structure such as 1 below. However, the error bars for the relative abundance of product ions in these studies is approximately 25%, and so it would be incautious to interpret ion abundance differences in terms of fragmentation mechanisms.

Amino acids with a nonpolar side chain have a higher reactivity toward Cu^+ and Fe^+ compared with those that have polar side chains. Based on relative product ion intensities, the efficiency of metal attachment or dissociative attachment is 10-50% that of proton transfer for the amino acids with nonpolar side chains. For many of the amino acids with polar side chains, the efficiency of the metal insertion is very small and it is difficult to observe any products. It is a curious feature of these observations that the reactivity of the amino acids toward Cu^+ and Fe^+ is influenced

Structure 1

by the polarity of the side chains, but that the site of attack by the metal ions is around the carboxyl group.

Conclusion

Ground state transition metal ions Fe^+ and Cu^+ react with the amino acids primarily by dissociative attachment. Quadrupolar excitation successfully has been used to relax the excited state metal ions to their ground state, which completely eliminates the charge-exchange reactions that were observed previously. Amino acids that have nonpolar side chains have higher reaction efficiencies toward the ground state metal ions than do amino acids with polar side chains. Bond cleavage of the metal-amino acid complexes occurs at the $\text{C}\alpha\text{--CO}_2\text{H}$ bond and suggests that sequence information can be obtained from the reactions of peptides with Cu^+ or Fe^+ .

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