Copper(II) Amino Acid Complexes in the Gas Phase

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We report on the novel gas-phase chemistry of amino acid complexes with copper(II) and 2,2′-bipyridyl (bpy). Metal complexes of amino acids and peptides in the gas phase have been of much recent interest as models for metal binding sites.[1] Metal cations have also been sought to direct amino acid and peptide fragmentations to provide information on both the side chains and amino acid sequence.[2] We have discovered recently[3] that Cu^{II} complexes can be obtained efficiently in the gas phase by electrospray ionization[4] of aqueous methanol solutions of Cu^{II} or Cu^{I} salts and carboxylic acids in the presence of bpy.

We find that electrospraying CH_3OH/H_2O (50/50) solutions of amino acids, $CuSO_4 \cdot 5H_2O$, and bpy produces abundant gas-phase cations of the $[Cu^{II}(RCOO)bpy]^+$ type (Table 1, Figure 1a).[5] These complexes are known to exist in solution with large stability constants, $\beta > 10^{16}$.[6] The bpy ligand is essential for the efficient formation of gaseous Cu^{II} ions. Electrospray ionization of Cu^{II} salts with amino acids in the absence of bpy gives only weak $[Cu^{II}(RCOO)(RCOOH)]^+$ ions which are 10^2–10^3 times less abundant than $[Cu^{II}(RCOO)bpy]^+$ obtained at the same amino acid concentration. The ionization efficiencies for the formation of gas-phase $[Cu^{II}[RCOO)bpy]^+$ from nonbasic amino acids are 1–2 orders of magnitude greater than those for protonation (Table 1), as determined by electrospraying solutions containing Cu^{II}, bpy, and a 4-fold molar excess of the amino acid whose $(M + H)^+$ ion was used as internal reference. Detection of $[Cu^{II}(RCOO)bpy]^+$ from phenylalanine at 10^{-7} M concentration (1 pmol total sample consumption) is thus readily achieved at neutral nominal pH.[7] Detection at a 10^{-5} M level of amino acids through $(M + H)^+$ ions at nominal pH 2.9 has been reported.[8] $[Cu^{II}(RCOO)(bpy)_2]^+$ ions are also formed with relative abundances <10% of those of $[Cu^{II}(RCOO)bpy]^+$. Cysteine is oxidized with Cu^{II} to form cystine, which affords $[Cu^{II}(RCOO)bpy]^+$ ions in electrospray (Table 1). Reoxidation of Cu^{I} to Cu^{II} is probably driven by the stability of the $[Cu^{II}(RCOO)bpy]^+$ complex,[9] similar to the formation of $[Cu^{II}(CH_3COO)bpy]^+$ from Cu^{I}, acetic acid, and bpy in electrospray.[3] Amino acid deuteronation with CD_3OD and D_2O increases the relative yields of $[Cu^{II}(RCOO)bpy]^+$ (Table 1). This effect is due to the combined enhancement of the formation of gas-phase $[Cu^{II}(RCOO)bpy]^+$ from the deuterated solvents and suppression of amino acid deuteronation, as determined for proline.[10]

Basic amino acids lysine and arginine form doubly charged ions (Table 1), $[Cu^{II}(H + RCOO)bpy]^{2+}$, due to protonation at the basic residue. The formation of doubly charged ions from amino acids is noteworthy, as protonation in electrospray of peptides typically requires several amino acid residues to accommodate two protons.[11] Double charging provides facile distinction of lysine from the isobaric glutamine, which forms singly charged ions only.

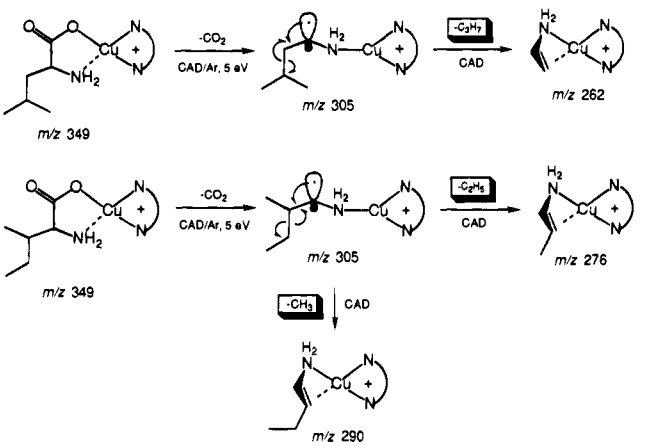
The gas-phase dissociations upon collisional activation[12] of $[Cu^{II}(RCOO)bpy]^+$ are critically dependent on the position of

Table 1. $[Cu^{(II)}(RCOO)bpy]^+$ Ions from Amino Acids

amino acid	$[Cu^{(II)}(RCOO)bpy]^+$ *m/z* (^{63}Cu, ^{65}Cu)	rel sensitivity[a]
glycine	293, 295	8
alanine	307, 309	>10[b]
valine	335, 337	>10[b]
leucine	349, 351	20–40[c]
leucine-*N*-d_3	351, 353	60–80[c]
isoleucine	349, 351	28
proline	333, 335	6
proline-*N*-d_2	334, 336	87
phenylalanine	383, 385	40–200[c]
serine	323, 325	>10[b]
threonine	337, 339	60–120[c]
methionine	353, 355	15
cysteine	458, 460[d]	6–15[c]
aspartic acid	351, 353	30–60
glutamic acid	365, 367	>30
glutamine	364, 366	50–120[c]
glutamine-*N*,*N*′-d_5	368, 370	130
histidine	373, 375	10–27[c]
lysine	182.5, 183.5[e]	2
arginine	196.5, 197.5[e]	1.5–5[c]
β-alanine	307, 309	13
γ-aminobutyric acid	321, 323	6–20

[a] Calculated as the relative abundance ratio $\{[^{63}Cu(RCOO)bpy]^+ + [^{65}Cu(RCOO)bpy]^+\}/[RCOOH + H]^+$. $[RCOOH + H]^+$ relative abundances are sensitive to the presence of Na^+ ions that give $[RCOOH + Na]^+$ adducts. [b] Estimated because of interference at low *m/z* for $[RCOOH + H]^+$. [c] From measurements on the magnetic sector and triple-quadrupole spectrometers at different interface potentials. [d] Oxidized to cystine. [e] Doubly charged ions.

Scheme 1



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(10) From independent measurements of ion yields for $[proline + H]^+$ (*m/z* 116), $[proline\text{-}d_2 + D]^+$ (*m/z* 119), and stoichiometric complexes $[Cu(Pro - H)(bpy)]^+$ (*m/z* 333 + 335) and $[Cu(Pro\text{-}d_2 - D)(bpy)]^+$ (*m/z* 334 + 336) relative to Rhodamine-G as internal standard.

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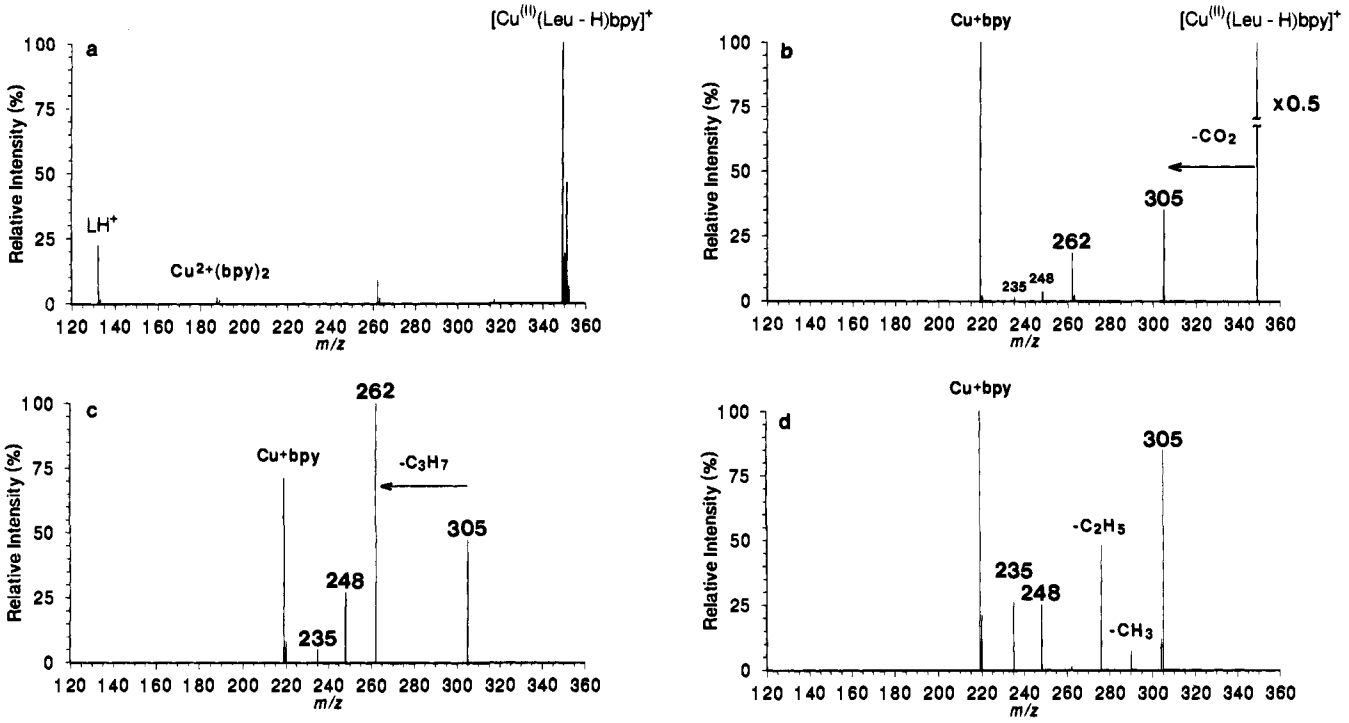
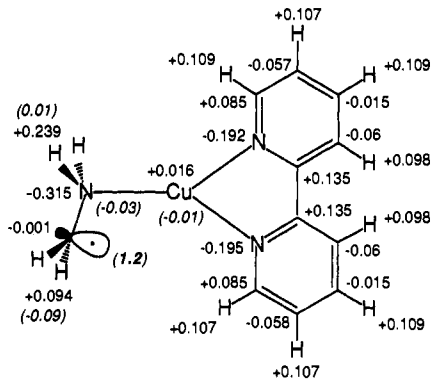


Figure 1. (a) Electrospray spectrum of leucine, $CuSO_4$ and bpy (4/1/1) in 50/50 CH_3OH/H_2O. (b) Collisionally activated dissociation (CAD, Ar, 30% precursor ion transmittance, 5 eV E_{LAB}) spectrum of $[^{63}Cu^{II}(Leu - H)(bpy)]^+$. CAD spectra of $RCu^{I}bpy^+$ from (c) leucine and (d) isoleucine.

the amino group, side chain branching, and presence of polar functional groups. In the first step, collision-induced dissociation of $[Cu^{II}(RCOO)bpy]^+$ from α-amino acids results in reductive decarboxylation to give $[Cu^{I}(R^{\bullet})bpy]^+$ ions (Figure 1b, Scheme 1). The ion from [1-^{13}C]leucine loses specifically $^{13}CO_2$. The stability of the $[Cu^{I}(R^{\bullet})bpy]^+$ formed depends on the amino acid side chain. $[Cu^{I}(R^{\bullet})bpy]^+$ ions from glycine and α-alanine eliminate α-amino alkyl radicals, $^{\bullet}CH_2NH_2$ and $CH_3CH^{\bullet}NH_2$, respectively, to form stable $[Cu^{I}bpy]^+$.[3] $[Cu^{II}(RCOO)bpy]^+$ ions from β-alanine and γ-aminobutyric acid do not give stable decarboxylation intermediates, but eliminate the entire amino acid moiety to form $[Cu^{I}bpy]^+$. $[Cu^{I}(R^{\bullet})bpy]^+$ ions from higher aliphatic α-amino acids (valine, leucine, and isoleucine) lose readily alkyl radicals in a process resembling the α-cleavage dissociation of cation radicals (Scheme 1). These dissociations distinguish unambiguously leucine from isoleucine (Figure 1c,d), as the $[Cu^{I}(R^{\bullet})bpy]^+$ ion from leucine loses specifically $C_3H_7^{\bullet}$, while the ion from isoleucine competitively eliminates $H^{\bullet}$, $CH_3^{\bullet}$, and $C_2H_5^{\bullet}$ (Scheme 1). In addition, $[Cu^{I}(NH_2)bpy]^+$ and $[Cu^{I}(CH_2{=}NH)bpy]^+$ are formed, which on further collisional activation dissociate to $[Cu^{I}bpy]^+$. CAD of deuterium-labeled [Cu(Leu-d_3 − D)(bpy)]$^+$ of ~99% d_2 content showed very specific (99%) retention of two deuterium atoms in the product ions containing the original amino group. By contrast, $[Cu^{I}(CH_2{=}NH)bpy]^+$ was formed after intramolecular H,D exchange between the ND_2 group and the hydrocarbon side chain, resulting in d_1- and d_0-containing ions.

The radical character of the $[Cu^{I}(R^{\bullet})bpy]^+$ dissociations follows from the ion electronic structure,[13] which shows the unpaired electron to be largely localized at the α-carbon atom as illustrated with $[Cu^{I}(^{\bullet}CH_2NH_2)bpy]^+$ (Chart 1). The Cu atom is calculated to be electroneutral, and it is surrounded by three negatively charged nitrogen ligands. The positive charge is delocalized among the hydrogen atoms and the 2,2′-carbons of bpy. The directing effect on the amino acid fragmentations of

Chart 1



Cu^{I} is due to the tendency of the metal atom to retain tri- or tetracoordination with one soft nitrogen ligand[14] provided by the amino acid fragment. α-Aminoalkyl radicals and enamines have very low ionization energies[15] and thus represent stabilizing soft ligands for the gaseous $[Cu^{I}bpy]^+$ ion. The calculated binding energy for $CH_2{=}CHNH_2$ (188 kJ mol^{-1})[13] suggests significant stabilization on complexation.

The ability of the Cu^{I} atom to bind O- and N-ligands affects the dissociations of $[Cu^{I}(R^{\bullet})bpy]^+$ ions derived from amino acids with polar groups in the side chains. For example, glutamine gives dominant fragments due to the migration of the $Cu(bpy)^+$ moiety onto the amide group followed by expulsion of $C^{\beta}H_2{=}C^{\alpha}HNH_2$ as established by deuterium labeling. Hence, Cu(bpy)-induced fragmentations of gas-phase ions may have potential for sequential cleavages of amide bonds in peptides starting with coordination at the C-terminus carboxylate and proceeding as Cu(bpy) migrates along the peptide backbone. Research in this direction is in progress in these laboratories.

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(12) Collisionally activated dissociations (CAD) of mass-selected ions were carried out in the collision hexapole (Ar, 30% transmittance, E_{LAB} = 5 eV) and/or without mass selection in the electrospray interface (0.6−12 Torr of N_2, 25−500 V acceleration).

(13) From UHF/STO-3G* calculations (3d and 4d orbitals on Cu, optimized geometry). Total atomic charges (Chart 1) and spin densities (in parentheses) are from Mulliken population analysis.

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(15) Ionization energies: $^{\bullet}CH_2NH_2$, 6.1 eV (Griller, D.; Lossing, F. P. *J. Am. Chem. Soc.* **1981**, *103*, 1586); $CH_2{=}CHNH_2$, 8.20 eV (Albrecht, B.; Allan, M.; Haselbach, E.; Neuhaus, L.; Carrupt, P.-A. *Helv. Chim. Acta* **1984**, *67*, 216).