Oxidation of Peptide−Copper Complexes by Alkali Metal Cations in the Gas Phase

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Interactions of copper^{II} and other transition metal ions with amino acid residues in peptides and proteins are important for the functioning of several redox enzyme systems.[3] Such interactions have been modeled in the gas phase in studies that have been focused on elucidating the structure and reactivity of peptide−metal complexes and the role of the metal ion in promoting cleavages of the peptide backbone and amino acid side chains.[4a,b,e] In addition, redox reactions of copper−peptide complexes have been studied intensively because of the role of copper in the formation and dissociation of peptide radicals relevant to radiation damage of biomolecules.[5]

We have found recently that the gas-phase chemistry of Cu^{II}−amino acid[6] and peptide ternary complexes[7] showed a surprising similarity to the radiolytic processes in solution.[8] In particular, the gas-phase complexes were found to undergo intramolecular electron transfer from the amino acid to the metal, which in some instances resulted in sequential metal reduction, $Cu^{II} \rightarrow Cu^{I} \rightarrow Cu^{0}$, accompanied by oxidation and fragmentation within the amino acid residue or the peptide chain.[6,7] In this communication we report on an unprecedented oxidation of gas-phase dipeptide−Cu^{I}(bpy) complexes with Na^+, K^+, and Rb^+ cations in the course of dissociation.

Dipeptides, e.g., Phe−Leu, Leu−Phe, Ala−Leu, and Leu−Ala, form ternary complexes with Cu^{II} and 2,2′-bipyridyl (bpy), which are obtained in the gas phase as [Cu^{II}(peptide − H)-bpy)]$^{+\bullet}$ ions[7] by electrospray ionization (ESI)[9] in (50/50) aqueous methanol. In solution the complexes give absorption spectra (λ_{max} = 660 nm, ϵ_{max} = 50 for Phe−Leu), which are typical of tetracoordinated Cu^{II} with square planar or rhombic geometries, or an elongated octahedron geometry with loosely bound apical ligands.[3,10] The complexes deprotonate in solution when treated with 1 equiv of 10^{-4} M alkali hydroxide (XOH, X = Li, Na, K, Rb, Cs) to give new species, which show identical absorption spectra, λ_{max} = 630 nm and ϵ_{max} = 75 for Phe−Leu, with all alkali metal cations.

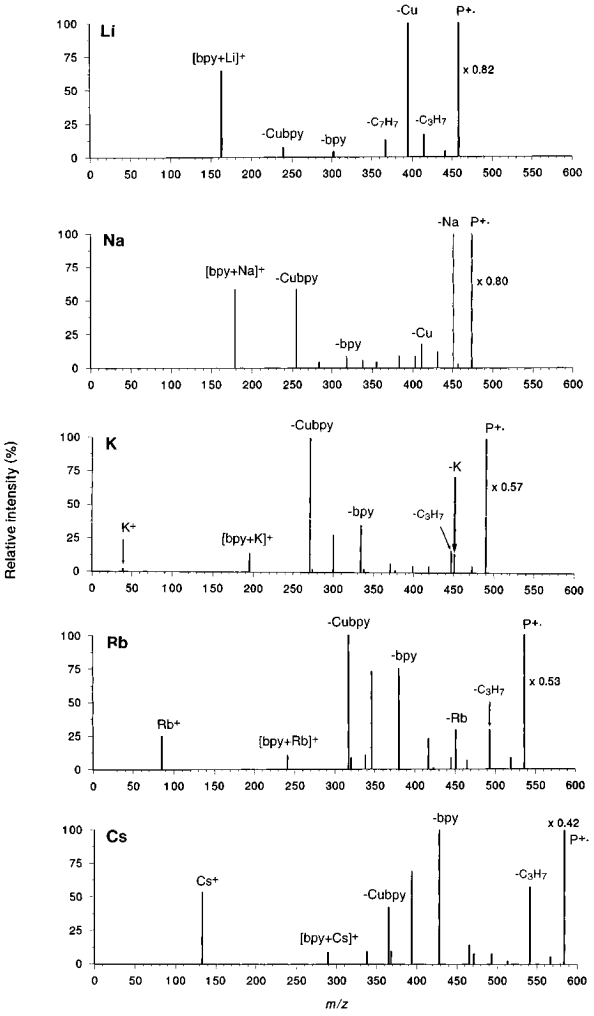

Figure 1. CAD spectra (E_{LAB} = 10 eV) of mass-selected [^{63}Cu(Phe−Leu − 2H + X − CO_2)bpy]$^{+\bullet}$ ions for X = ^{7}Li, ^{23}Na, ^{39}K, ^{85}Rb, and ^{133}Cs.

In the gas phase, the complexes appear as singly charged [Cu^{II}(peptide − 2H + X)bpy]$^{+\bullet}$ ions, which are obtained by electrospray ionization. Deuterium labeling in Phe−Leu and Leu−Phe showed that the alkali metal cation displaced one of the active peptide protons for X = Li through Cs. Hence the peptide ligands can be depicted as having imidate, −C(−O$^-$)=N−, and not enolate, =C(−O$^-$)−NH−, structures, as the latter would have retained the exchangeable amide deuteron. Collisional activation of mass-selected gas-phase complexes resulted in clean decarboxylation for Phe−Leu and Leu−Phe (X = Li through Cs) and for Ala−Leu and Leu−Ala (X = Na). However, the dissociations of the decarboxylated ions,[11] denoted as P(peptide, metal)$^{+\bullet}$, were found to depend critically on the peptide structure and the alkali ion (Figure 1). For Na, K, and Rb complexes of Phe−Leu and Na complexes of Ala−Leu and Leu−Ala, the P$^{+\bullet}$ ions showed losses of *neutral alkali metals*, which competed with other dissociations (Figure 1). No complementary Na^+ ions were formed in the range of E_{LAB} = 5−20 eV, while the (P − Na)$^+$ ions represented the *most abundant fragments*. Losses of K and Rb showed

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(11) P(peptide,X)$^{+\bullet}$ ions were prepared by CAD/decarboxylation of [Cu^{II}-(peptide − 2H + X)bpy]$^{+\bullet}$ complexes in the ESI ionizer, selected by mass, and allowed to collide with Ar at 30% ion beam transmittance and 5−20 eV laboratory kinetic energies (E_{LAB}).

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distribution of positive charge between the (P − X) fragment and the alkali metal, whereas P(Phe−Leu, Cs)$^{+\bullet}$ produced Cs^+ only (Figure 1).[12] The $(P - X)^+$ and X^+ ion abundances did not change appreciably relative to each other when the collision energy was varied within 5−20 eV. CAD of mass-selected $(P - X)^+$ ions resulted in loss of the neutral peptide residue to yield $[Cu(bpy)]^+$ as the sole product.

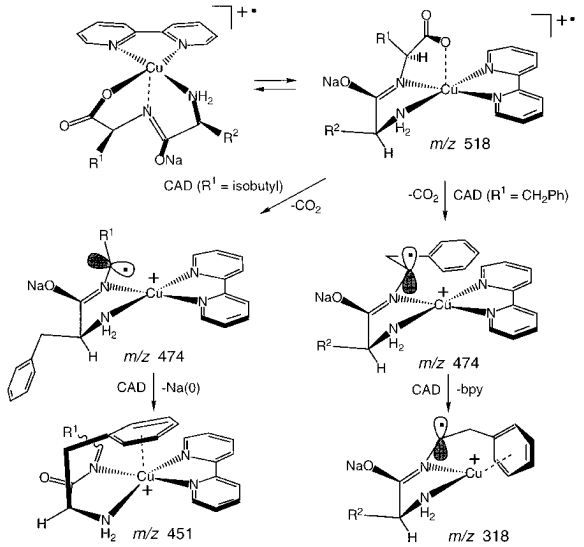
In contrast to the $P^{+\bullet}$ ions derived from Phe−Leu, Ala−Leu, and Leu−Ala, those from Leu−Phe uniformly eliminated bpy as the predominant dissociation channel at $E_{LAB} = 5-10$ eV, which produced 76, 94, 98, and 99% of total fragment ions for Na, K, Rb, and Cs, respectively. P(Leu−Phe, Na)$^{+\bullet}$ showed a minor loss of Na (1%), whereas no losses of K, Rb, and Cs were observed from the corresponding P(Leu−Phe, X)$^{+\bullet}$ ions.

Very different dissociations were found for P(peptide, Li)$^{+\bullet}$ ions of Phe−Leu, Ala−Leu, and Leu−Ala, which eliminated Cu^0 and formed abundant $[bpy + Li]^+$ ions (Figure 1). P(Leu−Phe, Li)$^{+\bullet}$ dissociates chiefly by loss of bpy (46%) but also shows loss of Cu(0) (7%) and forms $[bpy + Li]^+$ (22%).

Charge distribution between complementary fragments formed by a unimolecular dissociation of a single precursor is a sensitive probe of fragments' relative ionization energies (IE),[13] and thus the formation of $(P - X)^+$ and X^+ ions can be used to compare the electronic properties of their corresponding neutral counterparts. The IE of the P(Phe−Leu), P(Leu−Ala), and P(Ala−Leu) neutral fragments are bracketed from above by the IE of Na (5.14 eV),[14] as Na^+ irreversibly oxidizes the peptide complexes. Rb^+ and $(P - Rb)^+$ are formed at about equal rates from P(Phe−Leu, Rb)$^{+\bullet}$ (Figure 1), indicating that the (P − Rb) residue has an IE close to that of Rb (4.18 eV). The exclusive formation of Cs^+ and absence of $(P - Cs)^+$ (Figure 1) show that the peptide−Cu(bpy) complex has an IE higher than that of Cs (3.89 eV). These data indicate that the peptide−Cu^I(bpy) fragments are electron-rich species of extremely low ionization energies. By comparison, the lowest IE among organic compounds (5.3−5.4 eV) have hitherto been attributed to α-aminoalkyl radicals[15a,b] and tetrakis(dimethylamino)-ethylene.[15c]

For Na^+ through Cs^+, the $P^{+\bullet}$ ions are postulated to have the alkali metal coordinated to oxygen, whereas the peptide imine and amine groups and bpy provide ligands to tetracoordinated Cu^I (Scheme 1). The peptide−X residue in $P^{+\bullet}$ can be viewed as a salt of the alkali metal cation and an *N*-alkylidene amide anion−radical coordinated to Cu^+bpy. The simplest prototype of an *N*-alkylidene amide, $CH_2{=}N{-}CH{=}O$, is found to have a small positive electron affinity of 0.5 eV.[16] The anion−radical is best represented as $^{\bullet}CH_2{-}N{=}CH{-}O^-$ with 90% of unpaired spin density localized in the p_z-orbitals on the terminal methylene, which is slightly pyramidized, and 75% of the negative charge localized on the oxygen atom. Analogous structures are considered for the peptide residues (Scheme 1). In the absence of $Cu(bpy)^+$, a gaseous $^{\bullet}CH_2{-}N{=}CH{-}O^-Na^+$ salt would

Scheme 1



spontaneously dissociate by exothermic electron transfer to form $CH_2{=}N{-}CH{=}O$ and Na. Hence the observed stability of $P^{+\bullet}$ ions must be due to delocalization of the electron density from the formally anionic peptide residue to Cu^I and formation of a partially covalent alkali metal−oxygen bond. Oxidation by the alkali metal cation thus can be viewed alternatively as a homolysis of the polarized X−O bond.

The gas-phase dissociations show a few trends depending on the alkali metal ion. Lithium is exceptional in promoting loss of Cu^0 and formation of $[bpy + Li]^+$, while loss of Li^0 is not observed at all. Loss of Cu^0 is very rare in gas-phase Cu^I complexes and has been observed only for ions in which Cu^I was dicoordinated to electron-rich ligands, such as dehydrohistamine or α-histaminyl radical.[6b] This fact and the high affinity of lithium to nitrogen ligands[17] indicate that bpy is coordinated to Li^+ in the gas-phase $P^{+\bullet}$ ions.

The differences in dissociations in $P^{+\bullet}$ ions from Phe−Leu and Leu−Phe most likely reflect an effect of the aromatic ring in the C-terminal Phe residue. Aromatic rings are known to coordinate to transition metal ions in binary and ternary peptide complexes.[18] In $P^{+\bullet}$ ions, the C-terminal benzyl group is positioned such that the phenyl ring can reach to one of the equatorial coordination sites at Cu^I and displace the bpy ligand, which is lost on CAD. In contrast, an N-terminal benzyl allows the phenyl ring to attain an axial coordination site at Cu^I, whereas an equatorial approach is sterically impossible (Scheme 1). In the absence of a C-terminal Phe, electron transfer to Na^+ is the dominant reaction. However, loss of bpy occurs increasingly in the P(Phe−Leu, X)$^{+\bullet}$ ions for K^+, Rb^+, and Cs^+ (Figure 1). This likely reflects an increasing polarization of the $X^{(+)}-O^{(-)}$ bond, which elevates the electron density in the $R^1{-}C^{\bullet}H{-}N{=}C(O)$ moiety, making it an even stronger donor ligand for Cu^I. Loss of bpy from ternary Cu^I complexes is known to be promoted by strongly binding ligands, such as imidazole or α-aminoalkyl radicals.[6b,c]

In conclusion, the eliminations of neutral Na, K, and Rb atoms from the gas-phase dipeptide complexes are equivalent to oxidation by Na^+, K^+, and Rb^+ of the Cu^I-coordinated peptide residues. To our knowledge, this represents the first case of oxidation of organic substrates by alkali metal cations.

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(12) The absence of Na^+ ions is not due to discrimination by the quadrupole mass analyzer. The Fisons Quattro instrument used in these measurements shows flat ion transmission characteristics in the *m/z* 10−200 range, and abundant Na^+ ions from other sodiated complexes are routinely observed.

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