Stable Gas-Phase Radical Cations of Dimeric Tryptophan and Tyrosine Derivatives

Yuyong Ke, Udo H. Verkerk, P. Y. Iris Shek, Alan C. Hopkinson, and K. W. Michael Siu*

Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

Received: January 2, 2006; In Final Form: March 13, 2006

Stable radical cations of dimeric amino acid derivatives of tryptophan and tyrosine were generated by collision-induced dissociation of $[Cu^{II}(\text{diethylenetriamine})(\text{amino acid derivative})_2]^{\bullet 2+}$. The yields of the dimer radical cations were dependent on both the auxiliary ligand and the tryptophan or tyrosine derivatives used. Amino acid derivatives with an unmodified carboxylic acid group did not generate dimer radical cations. For the amino acid derivatives Ac-Trp-OMe and Ac-Trp-NH_2 (Ac is *N*-acetyl; OMe and NH_2 are the methyl ester and amide modifications of the C-terminal carboxylic group), no auxiliary ligand was required for generating the dimer radical cations. Collision-induced dissociation of the $[Cu^{II}(\text{amino acid derivative})_4]^{\bullet 2+}$ precursor generated the dimer radical cation $[(\text{amino acid derivative})_2]^{\bullet +}$. Stabilizing interactions, most likely involving hydrogen bonding, between the two amino acid derivatives are proposed to account for observation of the dimer radical cations. Dissociation of these ions yields protonated or radical cationic amino acid derivatives; these observations are consistent with the expectation of proton competition between monomeric units, whose proton affinities were calculated using density functional theory.

Introduction

Copper is a cofactor in many enzymatic systems.[1−4] Central to the function of copper-based enzymes is the regulated variable-coordination environment created by the surrounding polypeptide matrix. In an attempt to understand, and subsequently exploit, the potential of copper-based catalysts, many coordination complexes of polypeptides and copper have been studied. The employment of electrospray ionization (ESI)[5] has considerably simplified transfer of such complexes into the gas phase, allowing analysis by mass spectrometry. The study of gas-phase copper complexes, in turn, has grown into an active and promising area of research.[6−11] Gas-phase copper complexes of amino acids, for example, were used to distinguish isomeric and isobaric amino acids[6,12] and to quantify amino acids.[7−9] Copper was also shown to direct polypeptide fragmentation to provide sequence and side-chain information.[10]

Previous work of our group[11,13,14] has centered on the fragmentation chemistries of $[Cu^{II}(L)(M)]^{\bullet 2+}$ complexes, where M is an oligopeptide containing the tryptophan (Trp) or tyrosine (Tyr) residue and L is an auxiliary tridentate ligand, such as diethylenetriamine (dien). Collision-induced dissociation (CID) of these copper complexes results in the formation of oligopeptide radical cations.[11,13,14] Radical cations of small peptides have conventionally been generated by electron impact[15] and UV photoionization,[16] with limited success. In the past few years, these methods were augmented by charge-stripping,[17] electron-capture dissociation,[18] and chemical modification and subsequent CID of the modified peptides.[19,20] The copper(II)-based method for generating radical cations compares favorably with existing methods of peptide radical cation generation, in terms of flexibility, scope, and ease of use, and has recently been extended to nucleobases[21] and peptides that do not contain residues of low ionization energies.[22]

In this present work, we explore the formation and fragmentation chemistries of radical cations of dimeric tryptophan and tyrosine derivatives. These novel dimeric radical cations, $M_2^{\bullet +}$, were formed via CID of $[Cu^{II}(\text{dien})(M)_2]^{\bullet 2+}$ or $[Cu^{II}(M)_4]^{\bullet 2+}$, where M = Ac-Trp-OMe, Ac-Trp-NH_2, Ac-Tyr-OMe, or Ac-Tyr-NH_2.

Experimental Section

Experiments were performed using a commercially available ion-trap mass spectrometer (Finnigan-MAT LCQ) equipped with an ESI source. Typical experimental conditions were: electrospray voltage, 4.5 kV; sheath-gas flow, 0.3 L/min of nitrogen; capillary temperature, 120 °C; ion-trap temperature, 25 °C. Samples, 100 μM in $Cu(ClO_4)_2$ and auxiliary ligand, and 200 μM in amino acid derivatives, were dissolved in 50:50 water/methanol solution and infused at a flow rate of 3 μL/min. Deionized water and methanol were of HPLC grade. For comparison, acetyl derivatives of tryptophan and tyrosine were also examined without the addition of the auxiliary ligand. All amino acid derivatives, Ac-Trp-OMe, Ac-Trp-NH_2, Ac-Trp-OH (C-terminal carboxylic acid), Ac-Tyr-OMe, Ac-Tyr-NH_2, Ac-Tyr-OH, Trp-OMe, Trp-NH_2, Trp-OH, Tyr-OMe, Tyr-NH_2, and Tyr-OH were purchased from Bachem BioSciences, Inc. (King of Prussia, PA) and were used as received. The hexahydrate salt of $Cu(ClO_4)_2$ and amine ligands diethylenetriamine, 2,2′:6′,2″-terpyridine (tpy), and 1,4,7-triazacyclononane (tacn) were available from Sigma-Aldrich (St. Louis, MO).

All computations were performed using the Gaussian 98 program suite.[23] Density functional theory (DFT) was employed using the B3LYP correlation functional[24−26] with the 6-31G-(d,p) basis set[27,28] for calculating the proton affinities of Ac-Trp-OMe, [Ac-Trp-OMe − H]•, Ac-Tyr-OMe, and [Ac-Tyr-OMe − H]•.

Results and Discussion

Complexes of the type $[Cu^{II}(L)(M)_n]^{\bullet 2+}$ ($n = 1-3$) were readily observable under appropriate conditions by means of

* Author to whom correspondence should be addressed. Phone: (416) 650-8021. Fax: (416) 736-5936. E-mail: kwmsiu@yorku.ca.

10.1021/jp060029a CCC: $33.50
Published on Web 04/05/2006

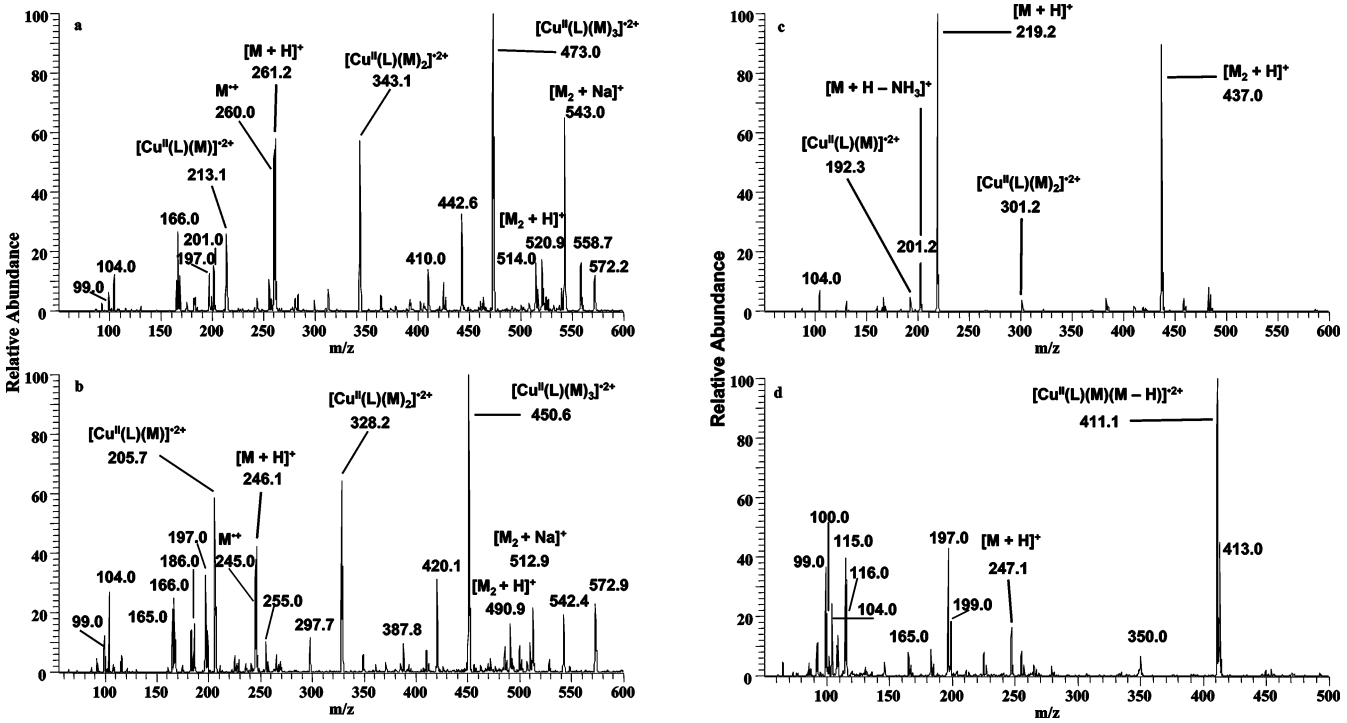


Figure 1. Mass spectra of $[Cu^{II}(dien)(M)_n]^{\bullet 2+}$, with (a) M = Ac-Trp-OMe, (b) Ac-Trp-NH_2, (c) Trp-OMe, and (d) Ac-Trp-OH, respectively. The ions at 201 Th in part a and 186 Th in part b are the products of $M^{\bullet +}$ after loss of acetamide. The ions at 99 Th and 115 Th are, respectively, $[Cu^{II}(dien)(MeOH)]^{\bullet 2+}$ and $[Cu^{II}(dien)(MeOH)_2]^{\bullet 2+}$ (MeOH = methanol). The ion at 104 Th is $[L + H]^+$. Those at 165 Th, 166 Th, and 197 Th are, respectively, $[Cu^{II}(dien - H)]^{\bullet +}$, $[Cu^{I}(dien)]^+$, and $[Cu^{II}(dien - H)(MeOH)]^{\bullet +}$. The remaining peaks have not been identified.

electrospraying a solution containing copper(II), the amine ligand L, and the amino acid derivative M. These were, however, only observed without ambiguity and with high abundances when M was an acetylated ester or amide (see Figures 1a−c; results for the ^{63}Cu isotope are shown in these and subsequent spectra). When M contained a C-terminal carboxylic acid, apparent facile proton abstraction in the lens region of the mass spectrometer was observed. As a consequence, a high abundance of $[Cu^{II}(L)(M)(M - H)]^{\bullet +}$ was observed (Figure 1d, L = dien, M = Ac-Trp-OH). The receptor of the proton was probably perchlorate, the counterion of Cu^{2+}, or an M that was eliminated.[29] The absence of this channel in C-terminal-modified amino acids suggests strongly that the proton involved is the carboxylic hydrogen; the loss of which is probably facilitated by the preferred binding of Cu^{2+} with the carboxylate anion.[30−32]

In accordance with previous observations,[11,13,14] four major channels were observed in the CID of the generated $[Cu^{II}(L)(M)_n]^{\bullet 2+}$: peptide radical cation formation (reaction 1), dissociative proton addition to the peptide (reaction 2), dissociative proton abstraction from the peptide (reaction 3), and peptide fragmentation (reaction 4).

$$[Cu^{II}(L)(M)_n]^{\bullet 2+} \rightarrow [Cu^{I}(L)(M)_{n-1}]^{+} + M^{\bullet +} \quad (1)$$

$$[Cu^{II}(L)(M)_n]^{\bullet 2+} \rightarrow [Cu^{II}(L)(M)_{n-1} - H]^{\bullet +} + [M + H]^{+} \quad (2)$$

$$[Cu^{II}(L)(M)_n]^{\bullet 2+} \rightarrow [Cu^{II}(M)_n - H]^{\bullet +} + [L + H]^{+} \quad (3)$$

$$[Cu^{II}(L)(M)_n]^{\bullet 2+} \rightarrow [Cu^{II}(L)(M - b_m)(M)_{n-1}]^{\bullet +} + b_m^{+} \quad (4)$$

Shown in Figure 2 are the fragmentation reactions of $[Cu^{II}(L)(M)_{1-3}]^{\bullet 2+}$ for L = dien and M = Ac-Trp-OMe. The major pathway in the CID of $[Cu^{II}(L)(M)]^{\bullet 2+}$ is radical cation formation (Figure 2a), while the CID of $[Cu^{II}(L)(M)_3]^{\bullet 2+}$ instead results in peptide loss as the major fragmentation channel (Figure 2c).

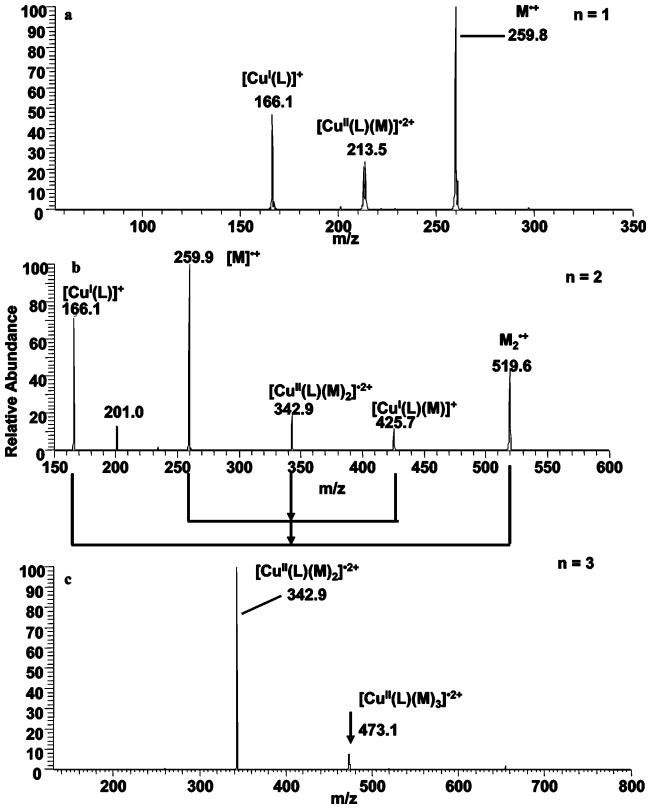


Figure 2. CID mass spectra of $[Cu^{II}(dien)(Ac\text{-}Trp\text{-}OMe)_n]^{\bullet 2+}$ (a) n = 1, (b) n = 2, and (c) n = 3 at relative collision energies of 6%, 7%, and 9%, respectively. The ion at 201 Th in part b is the product of $M^{\bullet +}$ after loss of acetamide.

$$[Cu^{II}(L)(M)_3]^{\bullet 2+} \rightarrow [Cu^{II}(L)(M)_2]^{\bullet 2+} + M \quad (5)$$

Additionally and significantly, a new channel is evident in the CID of $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ that results in the formation of a dimeric radical cation $M_2^{\bullet +}$ (Figure 2b and Scheme 1).

$$[Cu^{II}(L)(M)_2]^{\bullet 2+} \rightarrow [Cu^{I}(L)]^{+} + M_2^{\bullet +} \quad (6)$$

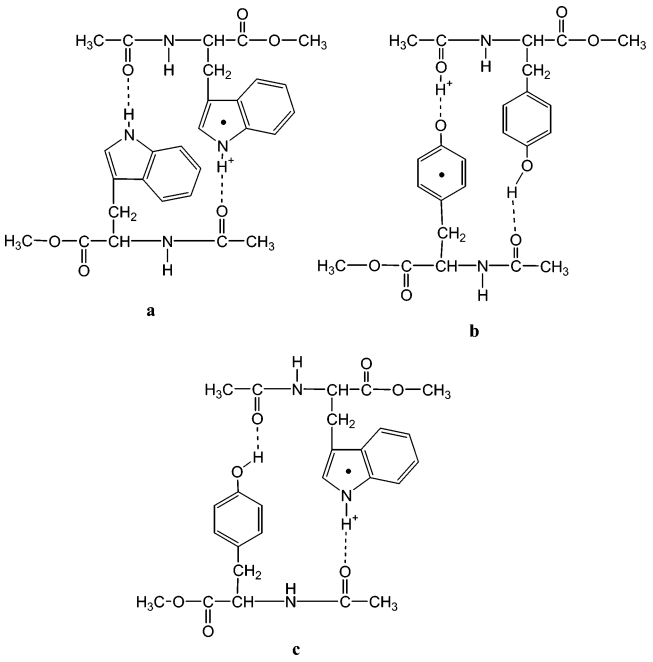
Experiments using Ac-Trp-OMe or Ac-Trp-NH_2 and dien as the auxiliary ligand consistently yielded the dimer radical cations. In addition, monomeric radical cations were also observed. Dimer radical cations of low abundances were observed with tpy as the ligand, while complexes with tacn as the auxiliary ligand did not produce dimeric radical cations. Similar results were obtained using the corresponding tyrosine derivatives with the tridentate ligands. In this report, all spectra from complexes that contained an auxiliary ligand used dien as that ligand.

Dimer radical cations are more prevalent with the ester derivatives (Figure 2b) than with the amide derivatives (Figure 3a) in the CID of $[Cu^{II}(L)(M)_2]^{\bullet 2+}$. For esters the formation of radical cations $M^{\bullet +}$ and/or $M_2^{\bullet +}$ predominates, while for amides dissociative proton transfer prevails. The dimer radical formation, under otherwise identical experimental conditions, is suppressed for the nonacetylated tyrosine and tryptophan derivatives (Figures 3b and 3c). Instead, the major fragmentation channel for the nonacetylated derivatives is proton transfer, resulting in formation of protonated M and/or protonated L. A readily available proton source in both the amino acid and dien is the NH group. The proton-donating capacity of diethylenetriamine has been reported previously.[11,13,14,33,34] The formation of $[L + H]^+$ and $[M + H]^+$, when M is an amide derivative, is a consequence of the fact that the amide is not only a proton acceptor but also a proton donor, in contrast with the ester group which only functions as a proton acceptor. The observed difference between the amide and the ester derivatives strongly suggests that the proton source for $[L + H]^+$ is the amide group in the amidated M.

Interpretation of the observed differences among auxiliary ligands and between acetylated and nonacetylated derivatives of Trp and Tyr requires a discussion on the mechanism of radical cation formation, the structure of the copper complex, and the binding modes of the ligands.Several studies on the coordination and chemistry of Cu^{II} complexes in the gas phase indicate a preference for a square planar arrangement of the ligands surrounding the Cu^{II} nucleus.[12,35−38] If such an arrangement is maintained in the systems studied here, then displacement of one or two dien nitrogens by tyrosine or tryptophan ligands would have to take place.[39] However, alternative binding modes whereby the tridentate dien ligand forces one or two of the coordinating amino acids into an apical or equatorial position, or forces at least one of the amino acids into a secondary complexation shell, cannot be excluded.[39−41] Indeed, X-ray crystallographic examination of $Cu^{II}(tpy)Cl_2.nH_2O$ ($n = 0, 1$) reveals a distorted square pyramidal structure.[42] Coordination of the acetylated amide (Ac-Trp-NH_2, Ac-Tyr-NH_2) and ester derivatives (Ac-Trp-OMe, Ac-Tyr-OMe) with Cu^{II} most likely involves the acetyl or amide carbonyl group.[43]

As a result of the difference between the second ionization energy (IE) of Cu, 20.29 eV, and the first ionization energies of the ligands, all <10 eV, electron transfer from the ligand to Cu^{2+} is exothermic.[44,45] To observe $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ complexes on the mass spectrometry time scale, a sufficiently large potential-energy barrier must exist between the complex and the charge-separated products.[45] The height of this barrier is determined by the crossing of the attractive potential-energy curve of $[Cu^{II}(L)(M)]^{\bullet 2+} + M$ with the repulsive curve (due to Coulombic repulsion) of $[Cu^{I}(L)(M)]^{+} + M^{\bullet +}$. A large barrier is favored by a large binding energy (a deep well), which may be promoted by stabilization of the complex through multiple, electron-donating, functional groups.

SCHEME 1: Proposed Structures of Dimeric Radical Cations of Ac-Trp-OMe and Ac-Tyr-OMe



Alternatively, stabilization can also be achieved by multiple ligation, which facilitates delocalization of the Cu^{2+} charges onto the polydentate ligands. However, introduction of additional ligands also increases the likelihood of competing dissociation pathways (reactions 3−5). In the CID of $[Cu^{II}(M)_n]^{\bullet 2+}$ ($n \geq 4$), elimination of M is competitive with dissociative electron transfer (Figure 4). The potential well of $[Cu^{II}(M)_{n-1}]^{\bullet 2+}$ is sufficiently deep (or the barrier against electron dissociation to form $M^{\bullet +}$ and $[Cu^{I}(M)_{n-2}]^{+}$ is sufficiently large) to render this complex kinetically stable and observable on the mass spectrometry time scale.[44−48] Kinetic stability is reduced as n decreases, as a result of decreased charge delocalization and decreased stabilization. Thus, stabilization of the double charge on copper is a sensitive balancing act—removal of a single, critical ligand by CID will render the resulting complex unstable and unobservable.

The following CID results of $[Cu^{II}(M)_n]^{\bullet 2+}$ ($n \geq 4$), where M = Ac-Trp-NH_2 (Figures 4a and 4b) and Ac-Trp-OMe (Figures 4c and 4d), hint at the importance of the coordination environment in determining the fragmentation chemistry observed. For $[Cu^{II}(M)_4]^{\bullet 2+}$ where M = Ac-Trp-NH_2, the following product ions are evident, $[Cu^{II}(M)_3]^{\bullet 2+}$, $[Cu^{I}(M)_2]^{+}$, $M_2^{\bullet +}$, and $M^{\bullet +}$. $[Cu^{II}(M)_n]^{\bullet 2+}$ complexes with $n = 5-7$ display a higher degree of fragmentation complexity. In general, $[Cu^{II}(M)_{n-1}]^{\bullet 2+}$ and $[Cu^{II}(M)_{n-2}]^{\bullet 2+}$ are observed ($n = 5$, M = Ac-Trp-NH_2, Figure 4b; $n = 6$, M = Ac-Trp-OMe, Figure 4c). However, the $[Cu^{II}(M)_7]^{\bullet 2+}$ complex of M = Ac-Trp-NH_2 gives only abundant $[Cu^{II}(M)_5]^{\bullet 2+}$ and no $[Cu^{II}(M)_6]^{\bullet 2+}$, suggesting strongly a neutral loss of dimeric M_2. In addition, $[Cu^{I}(M)_2]^{+}$ and $M_2^{\bullet +}$ ions of varying abundances are typically observable in the CID spectra, depending on collision energies. Observations of $M_2^{\bullet +}$ and

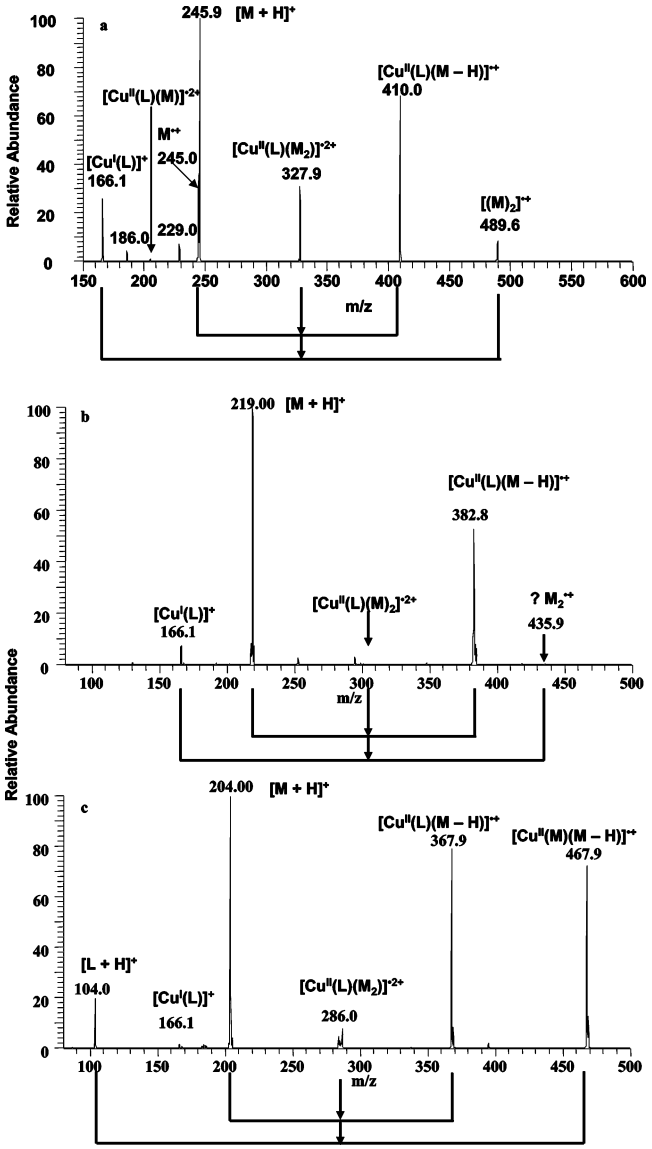


Figure 3. CID mass spectra of $[Cu^{II}(dien)(M)_2]^{\bullet 2+}$ (a) Ac-Trp-NH_2, (b) Trp-OMe, and (c) Trp-NH_2 at relative collision energies of 7%, 8%, and 8%, respectively. The ions at 186 Th and 229 Th in part a are, respectively, the product of $M^{\bullet +}$ after loss of acetamide and that of $[M + H]^+$ after the loss of ammonia.

indirect evidence of M_2 losses are strong indications that there are considerable intermolecular interactions (between the ligands) in $[Cu^{II}(M)_n]^{\bullet 2+}$. Given the nature of the ligands—derivatized amino acids—and the steric constraints within a Cu^{2+}-centered complex, the most probable ligand−ligand interactions involved are hydrogen bonding. Indeed, hydrogen bonding among smaller ligands, including water and ammonia, has been reported for Cu^{2+} complexes.[35] A Cu^{2+} nucleus can accommodate a large number of protic ligands, as a result of ligand−ligand interactions. For example, the $[Cu^{II}(H_2O)_n]^{2+}$ complexes display maximum stability for eight water molecules—four directly coordinated to the metal and four in the secondary solvation shell—as a result of increased strength in the hydrogen bonds.[35,36] Collisional activation results in loss of neutral ligands until the number of ligands has decreased sufficiently for electron or proton transfer to become competitive.[44−48] Our current data indicate that four Ac-Trp-OMe or Ac-Trp-NH_2 ligands can stabilize the Cu^{2+} sufficiently and that an auxiliary, multidentate ligand L is not required to reduce the chelating effect of the amino acid derivative.[33]

Elimination of the dimeric radical cation $M_2^{\bullet +}$ from $[Cu^{II}(M)_4]^{\bullet 2+}$ is facilitated by strong binding of the first two ligands, while the third and the fourth ligands bind much more weakly, as a result of 4s−3d−σ hybridization in Cu^+ complexes.[49] Conceptually, electron transfer within collisionally activated $[Cu^{II}(M)_4]^{\bullet 2+}$ leads to an excited $[Cu^+(M)_3(M^{\bullet +})]$ transient, which dissociates by eliminating $M_2^{\bullet +}$. Presumably, elimination of $M_2^{\bullet +}$ is facilitated by ligand−ligand hydrogen bonding (vide infra). Such interactions within $[Cu^{II}(M)_4]^{\bullet 2+}$ are strengthened as a result of delocalization of the dipositive charge of Cu onto the ligands.[35] Indeed, hydrogen bonding has been observed even within the neutral dimers of Ac-Trp-OMe and Ac-Phe-OMe.[50−52] The Ac-Phe-OMe dimer was found to form a β-sheet in the gas phase through two NH(amide) and C═O(amide) hydrogen bond interactions,[51,52] while the Ac-Trp-OMe dimer instead forms an asymmetric arrangement through NH(indole)•••O═C(ester) and NH(indole)•••O═C(amide) hydrogen bonding.[50]

Establishing ligand−ligand interactions requires the proximity of the two amino acid derivatives in the Cu^{II} complex. Within $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ complexes, such proximity is probably achieved via a cis configuration of the two amino acid derivatives. We speculate that the observed low abundance or absence of dimeric radical cations in CID of $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ complexes for L = tpy or tacn is a result of limited or weak inter-amino-acid-derivative interactions in such complexes. Tpy and tacn complexes are considerably more rigid (sterically hindered) than dien and may force the ligated amino acid derivatives into orientations that are less than optimal for hydrogen bonding.[53] A corollary of this hypothesis is that the derivatives need to have both sufficient length and appropriate composition to develop such interactions.[50−52] Additionally, increasing the size of ligated amino acid derivatives would increase the effectiveness of charge redistribution and lead to increased complex stability. Accordingly, the Trp-OMe, Trp-NH_2, Tyr-OMe, and Tyr-NH_2 derivatives do not give observable, stable $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ complexes (Figure 1c). Extending the "reach" of these derivatives by means of an acetyl group results in the formation of stable $[Cu^{II}(L)(M)_2]^{\bullet 2+}$ complexes (Figures 1a and 1b).

CID of Dimer Radical Cations $M_2^{\bullet +}$

The $M_2^{\bullet +}$ thus produced are stable species on the mass spectrometer time scale and can be mass-selected for further fragmentation reactions. The fragmentation chemistry observed is found to depend on the nature of the amino acid derivatives. For instance, CID of the $M_2^{\bullet +}$ of a tryptophan derivative produces $M^{\bullet +}$ by presumably elimination of a neutral M (Figure 5a)

$$M_2^{\bullet +} \rightarrow M^{\bullet +} + M \qquad (7)$$

By contrast, CID of the $M_2^{\bullet +}$ of a tyrosine derivative produces $[M + H]^+$ and presumably $[M - H]^{\bullet}$ via dissociative proton transfer (Figure 5b).

$$M_2^{\bullet +} \rightarrow [M + H]^+ + [M - H]^{\bullet} \qquad (8)$$

Dimeric radical cations of mixed M, i.e., $[M_aM_b]^{\bullet +}$, can be produced via CID of $[Cu^{II}(L)(M_a)(M_b)]^{\bullet 2+}$. For $[M_aM_b]^{\bullet +}$ in which M_a is a Trp derivative and M_b is a Tyr derivative (Figure 5c), CID results in

$$[M_aM_b]^{\bullet +} \rightarrow M_a^{\bullet +} + M_b \qquad (9)$$

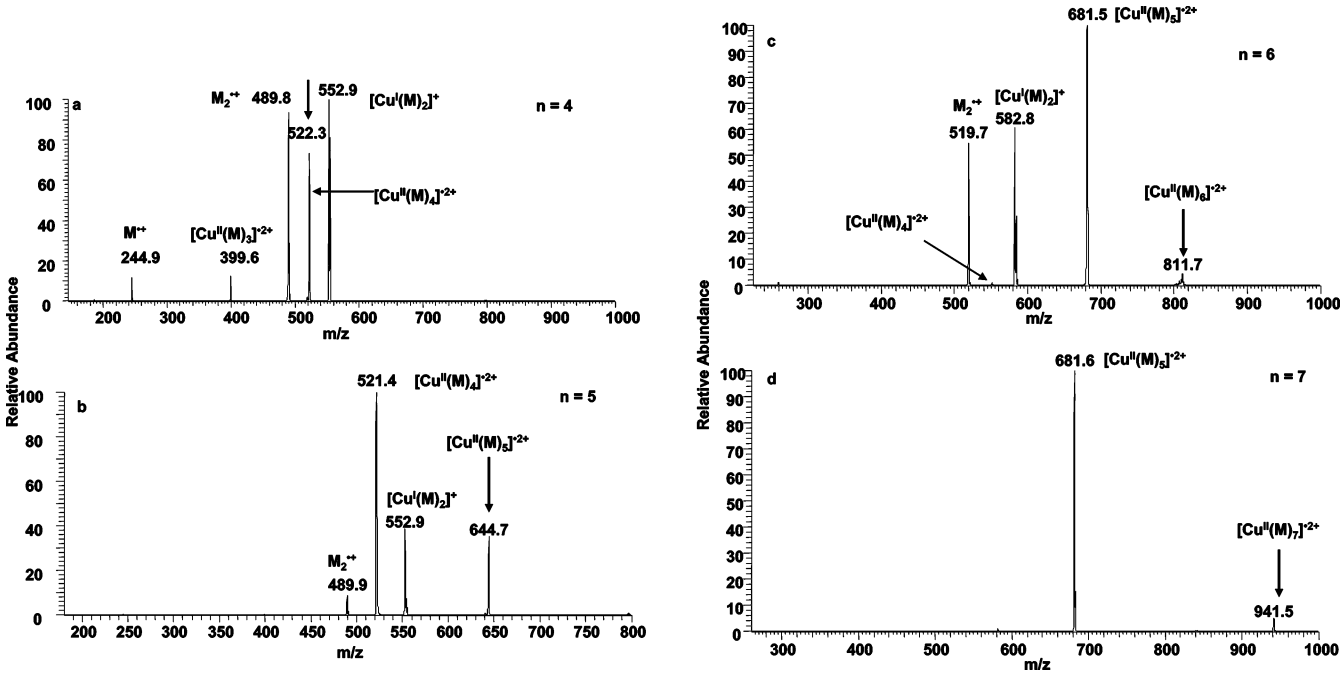


Figure 4. CID mass spectra of $[Cu^{II}(Ac\text{-}Trp\text{-}NH_2)_n]^{\bullet 2+}$ (a) $n = 4$ and (b) $n = 5$, and CID mass spectra of $[Cu^{II}(Ac\text{-}Trp\text{-}OMe)_n]^{\bullet 2+}$ (c) $n = 6$ and (d) $n = 7$. Relative collision energies: 8%, 8%, 12%, and 14%, respectively.

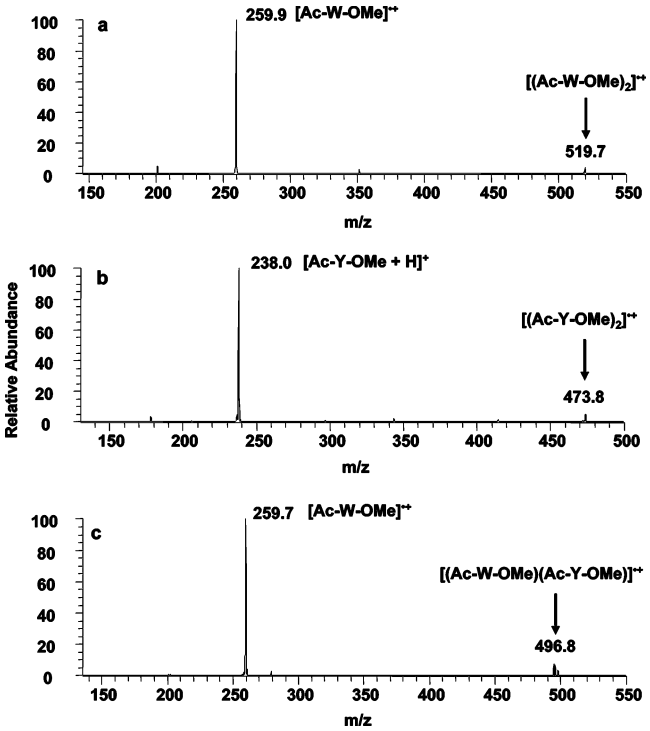


Figure 5. CID mass spectra of dimeric radical cations: (a) $(Ac\text{-}Trp\text{-}OMe)_2^{\bullet +}$, (b) $(Ac\text{-}Tyr\text{-}OMe)_2^{\bullet +}$, and (c) $[(Ac\text{-}Trp\text{-}OMe)(Ac\text{-}Tyr\text{-}OMe)]^{\bullet +}$ at relative collision energies of 9%, 11%, and 9%, respectively. W = Trp; Y = Tyr.

These results can be rationalized in terms of proton competition between the incipient neutral and the incipient radical components after collisional activation of the dimeric radical cation (Scheme 1 and vide infra). As shown in Table 1, the proton affinity(PA) of $[(Ac\text{-}Trp\text{-}OMe) - H]^{\bullet}$ (protonating on the indole nitrogen), at 244 kcal/mol, is larger than the PA of Ac-Trp-OMe (protonating on the amide oxygen), at 234 kcal/mol. By contrast, the PA of $[(Ac\text{-}Tyr\text{-}OMe) - H]^{\bullet}$ (protonating on the phenoxy oxygen), at 223 kcal/mol, is smaller than the PA of Ac-Tyr-OMe, at 229 kcal/mol. Accordingly, in the activated $M_2^{\bullet +}$, $[(Ac\text{-}Trp\text{-}OMe) - H]^{\bullet}$ is expected to bind the H^+ that bridges itself and Ac-Trp-OMe, and produces $[Ac\text{-}Trp\text{-}OMe]^{\bullet +}$ (reaction 7), while Ac-Tyr-OMe is expected to bind the H^+ that bridges itself and $[(Ac\text{-}Tyr\text{-}OMe) - H]^{\bullet}$, and produces $[(Ac\text{-}Tyr\text{-}OMe) + H]^+$ (reaction 8). In addition, the PA of $[(Ac\text{-}Trp\text{-}OMe) - H]^{\bullet}$ is larger than the PA of Ac-Tyr-OMe; as a consequence, CID of $[(Ac\text{-}Trp\text{-}OMe)(Ac\text{-}Tyr\text{-}OMe)]^{\bullet +}$ produces $[Ac\text{-}Trp\text{-}OMe]^{\bullet +}$ and Ac-Tyr-OMe (reaction 9). Alternatively, the IE of Ac-Trp-OMe is smaller than the IE of Ac-Tyr-OMe (Table 1); thus ionization of the former to produce $[Ac\text{-}Trp\text{-}OMe]^{\bullet +}$ is favored. Optimized structures of the neutral and protonated species are given in the Supporting Information.

TABLE 1: Calculated Proton Affinities (PAs) and Ionization Energies (IEs) of Amino Acid Derivatives[a]

compound	PA[b] (kcal mol^{-1})	PA[a] (kcal mol^{-1})	IE[a] (eV/kcal mol^{-1})
Ac-Trp-OMe	226	234	6.66/153
Ac-Tyr-OMe	213	229	7.41/171
$[(Ac\text{-}Trp\text{-}OMe) - H]^{\bullet}$	239	244	
$[(Ac\text{-}Tyr\text{-}OMe) - H]^{\bullet}$	205	223	

[a] Calculated using B3LYP/6-31G(d,p); the optimized structures are shown in the Supporting Information. [b] Calculated using HF/6-31G(d,p).

If hydrogen bonding plays a critical role within the $M_2^{\bullet +}$ complexes observed, then modification of the ligand that limits hydrogen bonding should deter formation of the dimeric radical cations. Experiments using Ac-Tyr(Me)-OMe, where Tyr(Me) is the O-methyl ether of Tyr, in Cu^{II}(dien) complexes of mixed M show the sensitivity of dimeric radical cation formation to intermolecular hydrogen bonding. The CID spectrum of $[Cu^{II}(dien)(Ac\text{-}Tyr\text{-}OMe)_2]^{\bullet 2+}$ (Figure 6a) shows that the abundance of $M_2^{\bullet +}$ is much larger than that of $M^{\bullet +}$. In the CID spectrum of $[Cu^{II}(dien)(Ac\text{-}Tyr(Me)\text{-}OMe)_2]^{\bullet 2+}$ (Figure 6b), this trend is reversed. In fact, no $M_2^{\bullet +}$ is evident. When complexes containing Ac-Tyr(Me)-OMe and a second amino acid derivative are subjected to CID, dimeric radical cations of mixed M, including $[(Ac\text{-}Trp\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +}$ (Figure 6c) and $[(Ac\text{-}Tyr\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +}$ (Figure 6d) are formed. Under

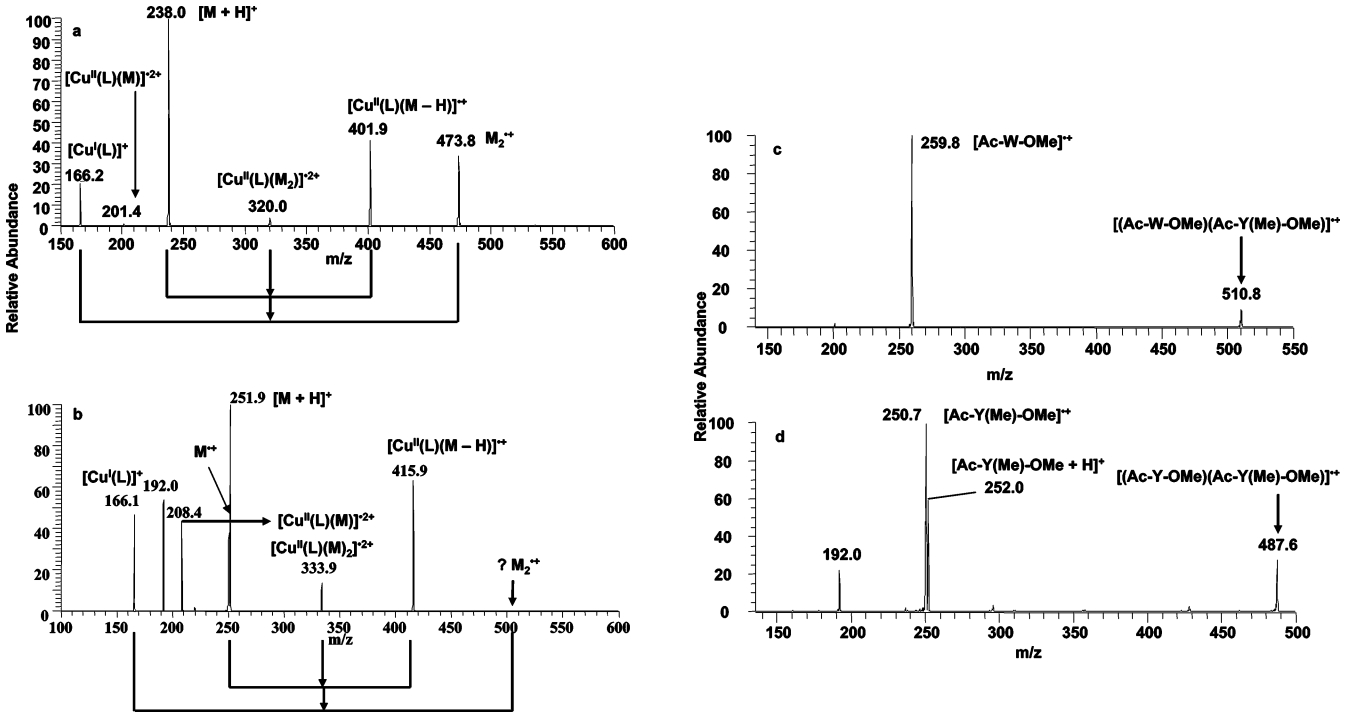


Figure 6. CID mass spectra of (a) $[Cu^{II}(dien)(Ac\text{-}Tyr\text{-}OMe)_2]^{\bullet 2+}$, (b) $[Cu^{II}(dien)(Ac\text{-}Tyr(Me)\text{-}OMe)_2]^{\bullet 2+}$, (c) $[(Ac\text{-}Trp\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +}$, and (d) $[(Ac\text{-}Tyr\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +}$ at relative collision energies of 7%, 9%, 9%, and 10%, respectively. W = Trp; Y = Tyr. The ions at =192 Th in parts b and d are the products of $[Ac\text{-}Tyr(Me)\text{-}OMe]^{\bullet +}$ after the loss of acetamide.

similar CID conditions, these mixed species yield a radical cation and a neutral fragment (reactions 10−12).

$$[(Ac\text{-}Trp\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +} \rightarrow [Ac\text{-}Trp\text{-}OMe]^{\bullet +} + Ac\text{-}Tyr(Me)\text{-}OMe \quad (10)$$

$$[(Ac\text{-}Tyr\text{-}OMe)(Ac\text{-}Tyr(Me)\text{-}OMe)]^{\bullet +} \rightarrow [Ac\text{-}Tyr(Me)\text{-}OMe]^{\bullet +} + Ac\text{-}Tyr\text{-}OMe \quad (11)$$

$$\rightarrow [Ac\text{-}Tyr(Me)\text{-}OMe + H]^{+} + [Ac\text{-}Tyr\text{-}OMe - H]^{\bullet} \quad (12)$$

Proposed structures of the dimeric radical cations in the form of proton-bridged complexes are shown in Scheme 1. The most acidic hydrogen in the tryptophan derivatives is the indole hydrogen, while in the tyrosine derivatives it is the phenolic hydrogen. For the two $M_2^{\bullet +}$ involving identical ligands, $(Ac\text{-}Trp\text{-}OMe)_2^{\bullet +}$ and $(Ac\text{-}Tyr\text{-}OMe)_2^{\bullet +}$, the proposed proton bridges depicted in Scheme 1a and 1b are exaggerated to show differences in proton affinities of the components. For the $M_2^{\bullet +}$ involving Ac-Trp-OMe and Ac-Tyr-OMe, the structure shown in Scheme 1c is proposed, which reflects differences in PAs. CID of these dimeric radical cations results in the radical cation of tryptophan-containing derivatives and the protonated tyrosine-containing derivatives.

Conclusions

Dimeric radical cations of tryptophan and tyrosine derivatives can be generated by collision-induced dissociation of copper(II) complexes formed via electrospray. In specific cases, the presence of an auxiliary ligand in such complexes is not a prerequisite for the formation of the dimeric radical ions. Results of experiments with a derivative of the methyl ether of tyrosine, a derivative that deters hydrogen bonding, are in accordance with an interpretation of intermolecular hydrogen bonding (between ligands) within the dimeric radical cations. CID of the dimeric radical cations yields fragments that are consistent with results of intermolecular proton competition.

Acknowledgment. This research is supported by the Natural Sciences and Engineering Research Council of Canada, MDS Sciex, and York University. Y.K. acknowledges receipt of an Ontario Graduate Scholarship.

Supporting Information Available: Optimized structures and energies of the neutral and protonated species. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) *Copper-Containing Proteins*; Valentine, J. S., Gralla, E. B., Eds.; Advances in Protein Chemistry 60; Academic Press: San Diego, CA, 2002.
(2) Ferguson-Miller, S.; Babcock, G. T. Heme/Copper Terminal Oxidases. *Chem. Rev.* **1996**, *96*, 2289−2907.
(3) Solomon, E. I.; Chen, P.; Metz, M.; Lee, S.; Palmer, A. E. *Angew. Chem., Int. Ed.* **2001**, *40*, 4570−4590.
(4) Marko, I. E.; Tsukazaki, M.; Giles, P. R.; Brown, S. M.; Urch, C. J. *Angew. Chem., Int. Ed.* **1997**, *36*, 2208−2210.
(5) Fenn, J. B. *Angew. Chem., Int. Ed.* **2003**, *42*, 3871−3894.
(6) Tao, W. A.; Zhang, D.; Nikolaev, E. N.; Cooks, R. G. *J. Am. Chem. Soc.* **2000**, *122*, 10598−10609.
(7) Seymour, J. L.; Tureček, F. *J. Mass Spectrom.* **2000**, *35*, 566−571.
(8) Gatlin, C. L.; Tureček, F. *J. Mass Spectrom.* **2000**, *35*, 172−177.
(9) Lavanant, H.; Hecquet, E.; Hoppilliard, Y. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 11−23.
(10) Gatlin, C. L.; Rao, R. D.; Tureček, F.; Vaisar, T. *Anal. Chem.* **1996**, *68*, 263−270.
(11) Chu, I. K.; Rodriquez, C. F.; Lau, T.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2000**, *104*, 3393−3397.
(12) Seymour, J. L.; Tureček, F.; Malkov, A. V.; Kocovsky, P. *J. Mass Spectrom.* **2004**, *39*, 1044−1052.
(13) Chu, I. K.; Rodriquez, C. F.; Hopkinson, A. C.; Siu, K. W. M.; Lau, T. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1114−1119.
(14) Bagheri-Majdi, E.; Ke, Y.; Orlova, G.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2004**, *108*, 11170−11181.
(15) Biemann, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 3192−3193.
(16) Weinkauf, R.; Schanen, P.; Metsala, A.; Schlag, E. W.; Burgle, M.; Kessler, H. *J. Phys. Chem.* **1996**, *100*, 18567−18585.

(17) Schröder, D.; Schwarz, H.; Wu, J.; Wesdemiotis, C. *Chem. Phys. Lett.* **2001**, *343*, 258−264.
(18) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3265−3266.
(19) Masterson, D. S.; Yin, H.; Chacon, A.; Hachey, D. L.; Norris, J. L.; Porter, N. A. *J Am. Chem. Soc.* **2004**, *126*, 720−721.
(20) Hodyss, R.; Cox, H. A.; Beauchamp, J. L. *J. Am. Chem. Soc.* **2005**, *127*, 12436−12437.
(21) Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1791−1805.
(22) Chu, I. K.; Siu, S. O.; Lam, C. N. W.; Chan, J. C. Y.; Rodriquez, C. F. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1798−1802.
(23) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11; Gaussian, Inc.: Pittsburgh, PA, 1998.
(24) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098−3100.
(25) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(26) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789.
(27) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257−2261.
(28) Hariharan, P. C.; Pople, J. A. *Chem. Phys. Lett.* **1972**, *16*, 217−219.
(29) Chu, I. K.; Lau, T.-C.; Siu, K. W. M. *J. Mass Spectrom.* **1998**, *33*, 811−818.
(30) Bertrán, J.; Rodriguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. B* **1999**, *103*, 2310−2317.
(31) Hu, P.; Loo, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 11314−11319.
(32) Gatlin, C. L.; Tureček, F.; Vaisar, T. *J. Am. Chem. Soc.* **1995**, *117*, 3637−3638.
(33) Barlow, C. K.; Wee, S.; McFadyen, W. D.; O'Hair, R. A. J. *J. Chem. Soc., Dalton Trans.* **2004**, 3199−3204.
(34) Wee, S.; O'Hair, R. A. J.; McFadyen, W. D. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1221−1226.
(35) Berces, A.; Nukada, T.; Margle, O.; Ziegler, T. *J. Phys. Chem. A* **1999**, *103*, 9693−9701.
(36) Wright, R. R.; Walker, N. R.; Firth, S.; Stace, A. *J. Phys. Chem. A* **2001**, *105*, 54−64.
(37) Walker, N. R.; Wright, R. R.; Barran, P. E.; Murell, J. N.; Stace, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 4223−4227.
(38) Seymour, J. L.; Tureček, F. *J. Mass Spectrom.* **2002**, *37*, 533−540.
(39) Combariza, M. Y.; Vachet, R. W. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 813−825.
(40) Vachet, R. W.; Hartman, J. A. R.; Callahan, J. H. *J. Mass Spectrom.* **1998**, *33*, 1209−1225.
(41) Chaparro, A. L.; Vachet, R. W. *J. Mass Spectrom.* **2003**, *38*, 333−342.
(42) Henke, W.; Kremer, S.; Reinen, D. *Inorg. Chem.* **1983**, *22*, 2858−2863.
(43) Sigel, H.; Martin, R. M. *Chem. Rev.* **1982**, *82*, 385−426.
(44) Shvartsburg, A. A.; Siu, K. W. M. *J. Am. Chem. Soc.* **2001**, *123*, 10071−10075.
(45) Schröder, D.; Schwarz, H. *J. Phys. Chem. A* **1999**, *103*, 7385−7394.
(46) Jayaweera, P.; Blades, A. T.; Ikonomou, M. G.; Kebarle, P. *J. Am. Chem. Soc.* **1990**, *112*, 2452−2454.
(47) Blades, A. T.; Jayaweera, P.; Ikonomou, M. G.; Kebarle, P. *J. Chem. Phys.* **1990**, *92*, 5900−5906.
(48) Stone, J. A.; Vukomanovic, D. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 227−229.
(49) Rannulu, N. S.; Rodgers, M. T. *Phys. Chem. Chem. Phys.* **2005**, *7*, 1014−1025.
(50) Gerlach, A.; Unterberg, C.; Fricke, H.; Gerhards, M. *Mol. Phys.* **2005**, *103*, 1521−1529.
(51) Gerhards, M.; Unterberg, C. *Phys. Chem. Chem. Phys.* **2002**, *4*, 1760−1765.
(52) Gerhards, M.; Unterberg, C.; Gerlach, A. *Phys. Chem. Chem. Phys.* **2002**, *4*, 5563−5565.
(53) Chu, I. K.; Lam, C. N. W.; Siu, S. O. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 752−762.