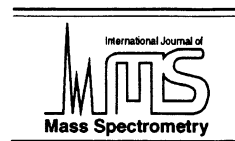




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Ligand-field photochemistry of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex in the gas phase

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Abstract

Photochemistry resulting from excitation of a $d-d$ transition at 575 nm between ligand-field states in the gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex generated by electrospray ionization is reported. These results are discussed in terms of a reaction scheme proposed by Tureček and co-workers [C.L. Gatlin, F. Tureček, T. Vaisar, *J. Mass Spectrom.* 30 (1995) 1617] for collisionally activated dissociation of this complex at low center-of-mass collision energies under multiple-collision conditions. Three photofragment ions are observed, corresponding to elimination of formaldehyde, stepwise loss of formaldehyde followed by OC(OH)CHNH_2 , and transfer of a hydroxyl group to the Cu^{II} ion accompanied by loss of $\text{H}_2\text{CCHNH}_2\text{CO}_2$. A fourth fragmentation channel reported by Tureček and co-workers corresponding to decarboxylation and associated dehydration is also observed at moderate center-of-mass collision energies under single-collision conditions but is absent in the laser photofragmentation difference mass spectrum resulting from excitation of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ at 575 nm. (*Int J Mass Spectrom* 177 (1998) 187–196) © 1998 Elsevier Science B.V.

Keywords: Electrospray ionization; Photochemistry; Transition-metal-ion complex

1. Introduction

Ternary complexes consisting of a transition-metal ion, a rigid aromatic bidentate ligand, and an aromatic or aliphatic amino-acid ligand have been studied in solution as models for hydrophobic and aromatic interactions that are relevant in biological systems [1,2]. Fisher and Sigel [1] investigated interactions between aromatic ligands ($L = 1,10$ -phenanthroline, 2,2'-bipyridine) and deprotonated amino acids (AA-H) having aliphatic side chains of different

lengths and branching bound to divalent transition-metal ions ($\text{M}^{\text{II}} = \text{Zn}^{\text{II}}$ and Cu^{II}) in aqueous solutions. Measurement of stability constants for these $[\text{M}^{\text{II}}(\text{L})(\text{AA-H})]^+$ complexes as a function of amino-acid side-chain length, aromatic ligand size, and transition-metal ion coordination geometry using potentiometric pH titrations revealed that the stability of the ternary complexes in solution increased with increasing length of the aliphatic side chain and size of the aromatic ligand [1]. In addition, Zn^{II} complexes were more stable than the analogous Cu^{II} complexes, suggesting that the octahedral geometry of the Zn^{II} complexes afforded a more effective binding interaction with the ligands than the square planar geometry of the Cu^{II} complexes. Fischer and Sigel [1] also used

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^1H NMR shift measurements to characterize the hydrophobic interactions within the ternary complexes. Yamauchi and Odani [2] subsequently measured stability constants for ternary complexes of $[\text{Cu}^{\text{II}}(\text{DA})(\text{AA}-\text{H})]^+$, where DA was an aromatic or nonaromatic bidentate diamine ligand; they also used circular dichroism to probe π -stacking between aromatic DA and AA ligands.

Recently, Tureček and co-workers [3–5] demonstrated that the ternary transition-metal complexes $[\text{Cu}^{\text{II}}(\text{bpy})(\text{AA}-\text{H})]^+$, where bpy = 2,2'-bipyridine, can be transferred to the gas phase using electrospray ionization, permitting investigation of ligand–ligand interactions, as well as amino acid/transition-metal ion binding, in the absence of solvent. In the gas-phase complexes, the amino acids coordinate to the Cu^{II} ion using lone pairs from the N atom of the amino group and one of the O atoms of the carboxylate formed by deprotonation of the carboxylic acid group. The methyl esters of amino acids do not bind strongly to Cu^{II} in the gas phase, providing evidence that deprotonation of the carboxylic acid group is important for formation of these complexes [5]. Tureček and coworkers [3] found that complexation of amino acids with $[\text{Cu}^{\text{II}}(\text{bpy})]^{2+}$ enhanced the detection sensitivity for these species in electrospray ionization mass spectrometry by up to 2 orders of magnitude relative to direct detection of protonated amino acids. Recently, complexation of a variety of pharmaceutical compounds with $[\text{Cu}^{\text{II}}(\text{bpy})]^{2+}$ has been employed to improve detection sensitivity in electrospray ionization mass spectrometry [6,7].

Collisional activation of gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{AA}-\text{H})]^+$ ions at laboratory kinetic energies (E_{lab}) of 5–25 eV also produced amino-acid-specific decomposition products. Tureček and co-workers [4,5] proposed a series of reaction schemes to rationalize the observed fragments and the role of the copper ion in directing dissociation. Subsequently, they extended this work to $[\text{Cu}^{\text{II}}(\text{bpy})(\text{dipeptide}-\text{H})]^{2+}$ complexes, where they found that the C- and N-termini could be identified from the collisionally activated fragmentation patterns [8], suggesting another possible strategy for mass spectrometric sequencing [9] of biologically significant molecules.

The $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$ complex was one of the species examined by Tureček and co-workers. In 50/50 (v/v) aqueous methanol, this complex has a broad absorption band in the visible ($\lambda_{\text{max}} \cong 614$ nm, $\epsilon = 58$ L mol $^{-1}$ cm $^{-1}$), which has been assigned as a $d-d$ transition [2]. Similar absorption spectra were reported previously for an aqueous solution of the related $[\text{Cu}^{\text{II}}(1,10\text{-phenanthroline})(\text{glycine}-\text{H})]^+$ complex ($\lambda_{\text{max}} \cong 617$ nm, $\epsilon = 50$ L mol $^{-1}$) [10]. In this article, we report photodissociation of the serine moiety triggered by excitation of this $d-d$ transition in gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$ complexes. This is the first evidence for excitation of a ligand-field transition in a gas-phase complex containing a divalent transition metal ion. Although collisionally activated dissociation (CAD) and metastable decomposition measured in this work follow the three dissociation pathways previously reported by Tureček and co-workers [5] for $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$, one of these pathways is absent when energy is supplied to the complex by ligand-field excitation. The product branching ratios measured for CAD combined with metastable decomposition, as well as those measured for photodissociation will be compared with those previously reported by Tureček and co-workers [5].

2. Experimental

Following the procedure described by Tureček and co-workers [5], $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$ was prepared by combining methanolic stock solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Fisher, Certified A.C.S.) (1 equivalent) and 2,2'-bipyridine (Aldrich, 99+%) (1 equivalent) with an aqueous stock solution of DL-serine (Aldrich, 99%) (4 equivalents) to produce either 1.0×10^{-2} M or 1.5×10^{-4} M solutions of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$ in 50/50 (v/v) aqueous methanol. A UV-visible absorption spectrum of the 1.0×10^{-2} M solution was recorded using a Hitachi U-2000 spectrophotometer.

Gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^+$ ions were generated by electrospray ionization of the 1.5×10^{-4} M solution. The metal capillary [11] through which ions travel from the electrospray ionization region of the source (1 atm) to the first stage of differential pump-

ing (2 Torr) was heated to 80 °C. The potential difference between the metal capillary and the skimmer separating the first and second stages of differential pumping was maintained at 150–200 V to optimize the intensity of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions, which are susceptible to collisional dissociation in the high pressure region of the electrospray ionization source [4,5]. In cluster studies [11,12] using the same electrospray ionization source, comparable potential differences but even lower capillary temperatures were required to preserve weakly bound ion-solvent clusters formed directly by electrospray ionization [11,12].

Electrospray needle currents of 0.2–0.3 μA yielded typical signal intensities of $1.5\text{--}1.8 \times 10^5$ ion s^{-1} for the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex following primary mass selection with a Wien filter [13], 90° deflection by an electrostatic turning quadrupole [14], passage through the 106-cm laser interaction region that includes a 80-cm-long quadrupole ion guide, and secondary mass analysis by a quadrupole mass filter (QMF) [12]. Ions traveled approximately 2.0 m from the exit of electrospray ionization source to the detector. Ion currents on the order of 0.01 nA immediately following passage through the Wien filter and deflection by the electrostatic turning quadrupole were typical in this instrument. Kebarle and co-workers [15] reported similar ion currents at the exit of their electrospray ionization source for transition-metal ion salts dissolved in methanol and signals of 10^5 ions s^{-1} following mass analysis with a QMF.

Following primary mass-selection, $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions were irradiated at 575 nm with the output from a tunable dye laser (Coherent 899-01, typical bandwidth < 2 GHz) pumped by an argon-ion laser (Innova 420, Coherent, Santa Clara, CA). The low concentration of ionic chromophores in the laser interaction region necessitated using photodissociation to detect excitation of the $d\text{--}d$ transition. The excitation wavelength chosen represented a compromise between maximizing the photon energy and absorption cross section to enhance the probability of observing photodissociation. Moving the excitation wavelength from $\lambda_{\text{max}} = 614$ nm for the broad, visible $d\text{--}d$ transition observed in 50/50 (v/v) aqueous meth-

anol solution to 575 nm delivered an additional 0.14 eV per photon with an absorption cross section that was 85% of the maximum, assuming that the shift in absorption spectrum accompanying transfer to the gas phase was small. Collection of a wavelength-dependent photofragmentation action for the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex to determine the nature and magnitude of the spectral shift was beyond the scope of this work. Laser power was maintained between 0.50 and 0.80 W ($1.1\text{--}1.8 \times 10^2$ W cm^{-2}).

Laser-on–laser-off difference mass spectra were obtained while simultaneously chopping the laser beam delivered to the mass spectrometer at 30 Hz (SR540 chopper, Stanford Research Systems, Sunnyvale, CA) and rapidly sweeping the mass axis of the QMF used for secondary mass analysis through the mass range $m/z = 210\text{--}340$. Operation of the mechanical chopper at 30 Hz ensured that ions experienced only one laser-on or laser-off period as they traversed the laser-interaction region of the mass spectrometer. A Stanford Research Systems computer interface module (SR245) provided the external mass command to sweep the QMF; it also monitored the QMF mass command voltage, the number of ions scored by a gated photon counter (SR 400, Stanford Research Systems), and the laser sense (on or off). Complete details of this rapid-scan method for data acquisition, which reduces the susceptibility of photodissociation experiments to instability in the electrospray ionization source, are reported elsewhere [16].

3. Results and discussion

Fig. 1 shows the mass spectrum resulting from CAD and metastable decomposition of a mixture of $^{63,65}\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})^+$ isotopomers ($m/z = 323,325$), which were not separated by primary mass selection. The four fragment peaks below $m/z = 300$ are produced either by collisions of the mass-selected $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions with background gas or by metastable decomposition [12] of the gas-phase complex in the 1.5×10^2 μs required for the ions to travel from the exit of the Wien filter used for primary

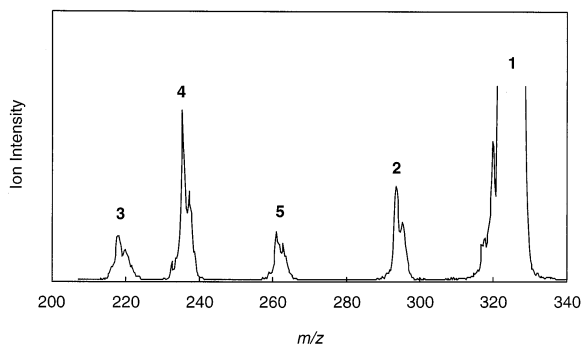


Fig. 1. Mass spectrum showing product ions arising from CAD under single-collision conditions (see text) and metastable decomposition of a mixture of mass-selected $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ isotopomers (1). The numbers that identify the peaks in the mass spectrum correspond to the structures in Fig. 2. The feature on the low-mass side of the truncated parent ion (1) peak at $m/z \approx 320$ is ascribed to splitting introduced by tuning of the mass-analyzing quadrupole mass filter based upon its resemblance to the profile of 4. Furthermore, the remaining lower intensity peaks 2, 3, and 5 have unresolved shoulders on their low-mass side.

mass selection to the entrance of the QMF used for secondary mass analysis. The peaks in the mass spectrum are identified using numbers that refer to structures in the reaction scheme proposed by Tureček and co-workers [5] (Fig. 2). The two naturally occurring isotopes of copper, ^{63}Cu (69.09%) and ^{65}Cu (30.91%) [17], and operation of the QMF under conditions of reduced resolution to improve ion throughput for detection of low intensity fragment channels both contribute to the broadness of peaks in the mass spectrum.

A rough estimate of the fraction of mass-selected ions that collide with background-gas molecules may be determined by calculating the number of molecules occupying the volume swept out by a $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ion as it travels between the two stages of mass selection. The effective radius and cross section of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex are estimated from a space-filling model generated by MacroModel (Columbia University, New York, NY) [18]. Although no crystallographic data exist for the $[\text{Cu}^{\text{II}}$

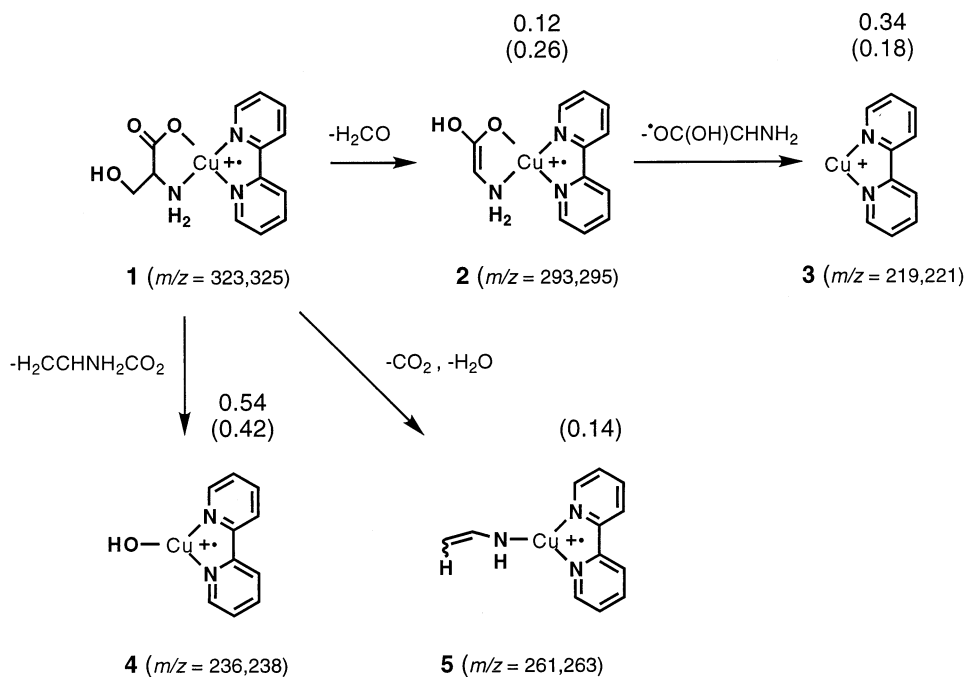


Fig. 2. Reaction scheme proposed by Tureček and co-workers [5] for CAD of gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions. The branching ratios for the photofragment ions at 575 nm are shown above each structure, and the combined branching ratios for CAD and metastable decomposition in the present study are given in parentheses directly below the photofragmentation branching ratios.

(bpy)(serine-H)⁺ complex itself, the crystal structure [10] of the related compound glycyglycinato(1,10,-phenanthroline)copper(II) trihydrate provides a guide to the metal–ligand bond lengths. Glycyglycinato(1,10,-phenanthroline)copper(II) has a distorted square pyramidal geometry with one nitrogen of the 1,10-phenanthroline ligand coordinated at the apex of the square pyramid and the other in the basal plane. The respective Cu–N bond distances are 2.275 and 2.009 Å. The 5-coordinate [Cu^{II}(bpy)₃]²⁺ complex isolated as the perchlorate salt also assumes a square pyramidal geometry with comparable Cu–N bond distances, 2.22 Å for the apical nitrogen and an average of 2.026 ± 0.009 Å for the four basal nitrogens [19]. In glycyglycinato(1,10,-phenanthroline)copper(II) the distances from the copper atom to the bonding carboxylate oxygen and the amide nitrogen are 2.01 and 1.90 Å, respectively [10].

The space-filling model produced by using the metal–ligand bond distances from related complexes, an energy-minimized structure for (serine-H)⁻, and crystallographic data for coordinated 2,2'-bipyridine [20] while constraining the coordination geometry to square planar yields a volume of 202 Å³ and an average radius of 3.64 Å, assuming that the complex is spherical. The 124-cm ion flight path between primary mass selection and secondary mass analysis passes through regions having ambient pressures of ~1 × 10⁻⁶ Torr (35 cm) and ~1 × 10⁻⁷ Torr (89 cm). With an estimated average cross sections of 42 Å², only 0.6% of the mass-selected [Cu^{II}(bpy)(serine-H)]⁺ ions will collide with background gas; however, fragment ions accounted for 2.9% of the integrated ion signal, suggesting that metastable decomposition was a major contributor.

The contribution of metastable decomposition to the dissociation that occurs in the ~150 μs between primary mass selection and secondary mass analysis is consistent with the temperatures typical of ions generated by electrospray ionization and the heat capacity of an ion having 96 vibrational degrees of freedom. We found previously that application of the evaporative ensemble [21,22] to model the metastable decay fractions observed for loss of a weakly bound methanol molecule from [Fe(bpy)₃·(CH₃OH)_n]²⁺ clus-

ters (*n* = 2–6) yielded temperatures ranging from 321 K for *n* = 2 to 239 K for *n* = 6. Although the principle of equipartition of energy significantly overestimates the contribution of vibrational degrees of freedom to a molecule's heat capacity and internal energy [23], Tureček [24] has found that the ratio (*H_T*–*H₀*)/(3*N*–6)*RT*, where *H_T* is the molar enthalpy and *H₀* is the zero-point vibrational energy, is nearly independent of the number of vibrational degrees of freedom for related organic compounds at a specific temperature. This observation held for experimentally determined values of *H_T*–*H₀*, as well as for values determined using either Benson's empirical method [25] or vibrational frequencies derived from ab initio calculations. For the three classes of compounds studied, *n*-alkanols, polyethylene glycols, and phenylalanine oligomers, (*H_T*–*H₀*)/(3*N*–6)*RT* was approximately equal to 0.2 at 350 K. This ratio yields a value for (*H_T*–*H₀*) of 56 kJ mol⁻¹. After conversion from enthalpy to internal energy and subtraction of 3*RT* for the contributions of translation and rotation, which can be treated classically at 350 K, the vibrational contribution to the average internal energy in excess of the zero-point energy of a [Cu^{II}(bpy)(serine-H)]⁺ ion is estimated to be ~0.5 eV.

The average laboratory kinetic energy, *E*_{lab}, of the [Cu^{II}(bpy)(serine-H)]⁺ ions in this study is 108 eV, yielding an average center-of-mass collision energy, *E*_{com}, at 298 K ranging from 5.7 eV for collisions with H₂O to 8.6 eV for collisions with N₂ target molecules, which establishes an upper limit on the average energy transferred in the collisions [26]. Tureček and co-workers [5] observed the same CAD fragments after passing [Cu^{II}(bpy)(serine-H)]⁺ ions through a hexapole collision cell containing Ar. In their experiments, the laboratory kinetic energy of the [Cu^{II}(bpy)(serine-H)]⁺ ions entering the cell was only 5 eV, resulting in *E*_{com} = 0.55 eV. The pressure of Ar in the collision cell was adjusted to achieve 30% transmission of the parent ion beam [5]. Although neither the pressure of Ar employed in CAD studies nor the length of the collision cell were reported, 30% transmittance should yield a broad Poisson distribution of multiple collisions. Tureček and co-workers [5] reported that fragment ions accounted for 39% of the

total observed ion signal under these conditions. Although the center-of-mass collision energies employed by Tureček and co-workers were significantly lower than those used in the present study, the average number of collisions experienced by $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ parent ions and collision products traversing the Ar collision cell was not reported. As a result, energy in excess of 0.55 eV may have been imparted to gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions under the CAD conditions reported by Tureček and co-workers [5].

The branching ratios for fragment ions produced by CAD and metastable decomposition in this study are given in parentheses above the structures in the reaction scheme proposed by Tureček and co-workers (Fig. 2) [5]. Despite the eight- to tenfold increase in collision energies used in the current study, more of the two largest fragments, **2** ($m/z = 293, 295$) and **5** ($m/z = 261, 263$), are observed in the current study than reported by Tureček and co-workers [5]. The $[\text{M-H}_2\text{CO}]^+$ fragmentation product (**2**), corresponding to elimination of formaldehyde from the complex, accounts for 26% of the total fragment ion intensity in the current study; it contributed only 8% of the total fragment ion intensity in the previous study. Stepwise elimination of formaldehyde followed by the remainder of the deprotonated amino acid to produce the $[\text{M-H}_2\text{CO}, -\text{OC}(\text{OH})\text{CHNH}_2]^+$ fragment (**3**) comprises 18% of the total fragment ion yield in the current study versus 41% when $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions underwent multiple low energy collisions with Ar in the previous work. It is likely that the increased yield of the smaller fragment (**3**) reported by Tureček and co-workers resulted from subsequent collisions of $[\text{M-H}_2\text{CO}]^+$ product ions with Ar in the collision cell, because a single collision depositing 0.55 eV at most is probably insufficient to drive sequential loss of H_2CO followed by the remainder of the deprotonated serine, $-\text{OC}(\text{OH})\text{CHNH}_2$.

Two additional reaction pathways are observed, transfer of the hydroxyl group from serine to the Cu^{II} ion with elimination of the rest of the amino acid, $\text{H}_2\text{CCHNH}_2\text{CO}_2$, to form **4** and decarboxylation accompanied by elimination of water to form **5**. If the $[\text{Cu}^{\text{I}}(\text{bpy})]^+$ product (**3**) arises solely from fragmen-

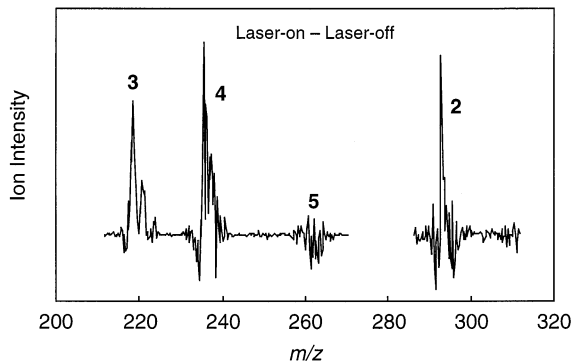


Fig. 3. Fragmentation region of the laser-on-laser-off difference mass spectrum resulting from excitation of mass-selected $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions at 575 nm. The labels for the peaks correspond to the numbered structures in Fig. 2.

tation of the $[\text{M-H}_2\text{CO}]^+$ intermediate (**2**), the branching ratios observed in this study for the three reaction paths are 0.44 for the reaction path that proceeds through the $[\text{M-H}_2\text{CO}]^+$ intermediate, 0.14 for the reaction path producing $[\text{M-CO}_2, -\text{H}_2\text{O}]^+$, and 0.42 for the reaction path yielding $[\text{M-H}_2\text{CCHNH}_2\text{CO}_2]^+$. The branching ratios observed by Tureček and co-workers [5] were 0.49, 0.07, and 0.43 for these three reaction paths, respectively. Under the higher center-of-mass collision energy, single collision conditions of this study, there is a twofold increase in the $[\text{M-CO}_2, -\text{H}_2\text{O}]^+$ reaction product (**5**), with concomitant 10% and 5% decreases in the $[\text{M-H}_2\text{CO}]^+$ and $[\text{M-H}_2\text{CCHNH}_2\text{CO}_2]^+$ products, respectively.

Several of these decomposition reactions involving the serine moiety may be induced in gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions through excitation of the $d-d$ ligand-field transition of the Cu^{II} ion. Fig. 3 shows the laser-on-laser-off difference mass spectrum in the fragment ion region for excitation of mass-selected $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions at 575 nm. This spectrum is an average of 2250 laser-on-laser-off mass spectra with ion counts acquired for 10 ms at each point in the individual mass spectra. Positive-going peaks reflect production of charged photodissociation fragments. The parent ion region of the mass spectrum is not shown, because the low absorption cross section for $d-d$ excitation of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions impedes direct measurement of parent ion

beam depletion. Three of the four fragments observed in CAD of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions are clearly produced upon absorption of a 2.16 eV photon. The $[\text{M-CO}_2, -\text{H}_2\text{O}]^+$ dissociation product (**5**) does not appear significantly in the laser-on–laser-off difference mass spectrum. Unlike CAD where the center-of-mass collision energy sets an upper limit on the energy supplied to promote dissociation of the ion, laser excitation delivers the entire photon energy to the ion, affording greater control albeit with the inherent constraint that the energies available are limited by the ion's absorption spectrum. In both cases, the actual energy available to break bonds is limited by the kinetic energy release accompanying dissociation [27].

Approximately 0.8% of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ parent ions photodissociate when excited at 575 nm with a laser fluence of $1.2 \times 10^2 \text{ W cm}^{-2}$ (0.55 W, 0.75 mm beam diameter). The efficiency of this photoinitiated fragmentation process may be examined quantitatively by calculating the fraction of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions that absorb a photon in the laser interaction region of the tandem mass spectrometer. Using the absorption cross section for the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ion in a 50/50 (v/v) aqueous methanol solution and assuming complete overlap between the ion and laser beams, 1.0% of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions are predicted to absorb a 575 nm photon at this laser fluence while in the laser interaction region. This result indicates that the photofragmentation process occurs with a quantum efficiency of at least 0.8. This value may be viewed as a lower limit, because the overlap between the laser and the ion beam may be less than 100%.

Ligand-field excitation of the Cu^{II} ion in the gas-phase $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ complex triggers reactions along two pathways. The first pathway is initiated by elimination of formaldehyde to produce $[\text{M-H}_2\text{CO}]^+$ followed by release of the remainder of the serine ligand from a fraction of the primary product ions to form $[\text{M-H}_2\text{CO}, -\text{OC}(\text{OH})\text{CHNH}_2]^+$. In reactions proceeding along the second path, the serine hydroxyl group transfers to the Cu^{II} ion accompanied by loss of the rest of the serine ligand to yield $[\text{M-H}_2\text{CCHNH}_2\text{CO}_2]^+$. The decarboxylation/dehy-

dration reaction observed to generate $[\text{M-CO}_2, -\text{H}_2\text{O}]^+$ via CAD and metastable decomposition is absent. The branching ratios in the photoinitiated process are 0.12 for the $[\text{M-H}_2\text{CO}]^+$ product (**2**), 0.34 for the $[\text{M-H}_2\text{CO}, -\text{OC}(\text{OH})\text{CHNH}_2]^+$ product (**3**), and 0.54 for the $[\text{M-H}_2\text{CCHNH}_2\text{CO}_2]^+$ product (**4**). The bonds between the amino N of serine and Cu^{II} are broken in 88% of the $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine-H})]^+$ ions, which photodissociate, forming products **3** and **4**. Products **2** and **3** share loss of H_2CO as a common step in their formation. The sum of the branching ratios for formation of these products is 0.46, which is comparable to that observed for CAD and metastable decomposition in this study (0.44) and CAD in the previous study (0.49) [5]. The nearly equal branching between the two reaction paths initiated by laser excitation suggests that the barriers to elimination of H_2CO and transfer of OH accompanied by loss of $\text{H}_2\text{CCHNH}_2\text{CO}_2$ are comparable and well below the photon energy of 2.16 eV.

The branching ratios for the $[\text{M-H}_2\text{CO}]^+$ (**2**) and $[\text{M-H}_2\text{CO}, -\text{OC}(\text{OH})\text{CHNH}_2]^+$ (**3**) ions produced by photofragmentation most closely resemble those observed by Tureček and co-workers [5] for CAD at low collision energies under multiple collision conditions. Sufficient energy remains in the $[\text{M-H}_2\text{CO}]^+$ fragment ions (**2**) from the initial excitation with a 2.16 eV photon that, following elimination of formaldehyde, nearly 74% of these fragment ions lose the remaining portion of the serine ligand on the $1.5 \times 10^2 \mu\text{s}$ timescale of our experiment. In contrast to the isomeric $[\text{Cu}^{\text{II}}(\text{bpy})(\text{glycine-H})]^+$ complex [4], the $[\text{M-H}_2\text{CO}]^+$ dissociation product generated by CAD and photodissociation does not undergo decarboxylation, which is consistent with formation of an enol ligand rather than glycinate [5]. Delivery of energy through excitation of the $d-d$ electronic transition in Cu^{II} promotes complete elimination of deprotonated serine from the complex through the two-step mechanism more efficiently than a single collision that carries over twice as much energy in the center-of-mass frame.

Loss of H_2CO from the serine α -side chain, which is the initial step in formation of roughly half of the photoproduct ions, also is observed in CAD and

metastable decomposition of anionic complexes formed by alkaline earth metal ions [28–30] and several divalent transition-metal ions [31] with serine-containing peptides. The binding of peptides to the alkaline-earth-metal ions is presumed to occur via the deprotonated terminal carboxylate group and a deprotonated amide nitrogen from one of the peptides. Adams and co-workers [30] have proposed a mechanism analogous to the thermal retro-aldol condensation reaction for the observed elimination of H_2CO from $[\text{M} + \text{Ca}^{2+} - 3\text{H}]^-$ ions generated by fast atom bombardment (FAB), where M is a peptide containing serine. Singly deprotonated dipeptide ions containing serine $[\text{M}-\text{H}]^-$ also predominantly lose H_2CO from the side chain irrespective of whether the serine is the C- or N-terminal amino acid [32]. Observation of the same dissociation channel involving fragmentation of the deprotonated serine ligand in photoexcited $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ suggests that the initial electronic excitation of the Cu^{II} ion is converted to vibrational excitation of the complex before dissociation.

The absence of the $[\text{M}-\text{CO}_2, -\text{H}_2\text{O}]^{+\cdot}$ product (**5**) distinguishes photodissociation of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ from collisionally activated dissociation and metastable decomposition. The yield of **5** from CAD and metastable decomposition is relatively small, accounting for only 14% of the dissociation products in the present study and 7% in the previous study [5]. This contrasts with the behavior of ternary $[\text{Cu}^{\text{II}}(\text{bpy})(\text{AA}-\text{H})]^{+\cdot}$ complexes in which deprotonated amino acids with aliphatic and aromatic α -side chains coordinate to Cu^{II} via a carboxylate oxygen and the amino nitrogen. Low energy CAD of these complexes and the resulting fragment ions supports a scheme that has decarboxylation as the initial dissociation step for all channels observed [4], reflecting weaker binding of the carboxylate oxygen to the metal ion than the amino nitrogen [31].

The importance of decarboxylation when deprotonated amino acids coordinate to $[\text{Cu}^{\text{II}}(\text{bpy})]^{2+}$ using the carboxylate group requires consideration of the presence of a dominant isomer of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ that absorbs at 575 nm but does not coordinate to the Cu^{II} ion via the carboxylate group as a possible explanation for the absence of $[\text{M}-\text{CO}_2, -\text{H}_2\text{O}]^{+\cdot}$ in

photodissociation. This scenario also requires that the minor isomer coordinated by the carboxylate group absorbs very weakly at 575 nm or not at all. However, when deprotonation of the carboxylic acid group of an amino acid is blocked by formation of its methyl ester, the ternary $[\text{Cu}^{\text{II}}(\text{bpy})(\text{AA}-\text{OCH}_3-\text{H})]^{+\cdot}$ complex is barely detectable, if at all, in the electrospray ionization mass spectrum [4,5]. Even $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{OCH}_3-\text{H})]^{+\cdot}$ is a minor species despite the presence of the weakly acidic alcohol group of serine- OCH_3 that can coordinate to Cu^{II} following deprotonation. Furthermore, although coordination of deprotonated serine by the alkoxide rather than the carboxylate group removes decarboxylation as a potential decomposition channel, it also eliminates formation of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{OH})]^{+\cdot}$ (**4**) [5], which is inconsistent with the observed photoproduct branching ratio of 0.54 for this channel. Consequently, formation of a $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ isomer with coordination of the alkoxide group to Cu^{II} as the major component in the ion beam at $m/z = 323, 325$ is eliminated from consideration.

A second possible scenario involves the presence of a minor $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ isomer that yields $[\text{M}-\text{CO}_2, -\text{H}_2\text{O}]^{+\cdot}$ with collisional activation but does not absorb at 575 nm. The estimated quantum yield of 0.8 for photodissociation of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$, which represents a lower limit for the reasons discussed above, would be consistent with the presence of such an isomer. Interestingly, the $[\text{M}-\text{H}_2\text{CO}]^{+\cdot}$ CAD product of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ is thought to be the enol tautomer of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{glycine}-\text{H})]^{+\cdot}$ precisely because it does not exhibit decarboxylation upon subsequent collision activation [5]. This suggests that the species that photodissociates at 575 nm to form $[\text{M}-\text{H}_2\text{CO}]^{+\cdot}$, $[\text{M}-\text{H}_2\text{CO}, -\text{OC}(\text{O}-\text{H})\text{CHNH}_2]^{+\cdot}$, and $[\text{M}-\text{H}_2\text{CCHNH}_2\text{CO}_2]^{+\cdot}$ is possibly the enol tautomer.

Finally, if no isomers are present, it is necessary to examine energetic and kinetic explanations for the absence of $[\text{M}-\text{CO}_2, -\text{H}_2\text{O}]^{+\cdot}$ photoproducts when $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ absorbs a 2.16 eV photon. Although decarboxylation occurs with low energy collisional activation, loss of H_2CO dominates whenever serine or serine-containing peptides are coordi-

nated to transition-metal ions [5,31]. Because the observation window for photodissociation is limited to $1.5 \times 10^2 \mu\text{s}$ at most, failure to observe $[\text{M}-\text{CO}_2, -\text{H}_2\text{O}]^{+\cdot}$ as a photodissociation product of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$, which has 96 vibrational degrees of freedom, does not necessarily indicate that the barrier for this channel is greater than 2.16 eV. Lower barriers for elimination of H_2CO and $\text{H}_2\text{CCHNH}_2\text{CO}_2$ and/or inefficient coupling of the electronic excitation to the internal modes of the complex corresponding to the decarboxylation reaction coordinate as the system relaxes may simply prevent decarboxylation from competing favorably kinetically with the observed photodissociation channels.

4. Conclusions

This study illustrates that despite both the low absorption cross section for the transition between ligand-field states in the Cu^{II} ion and the low gas-phase concentration of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ ions generated by electrospray ionization, photofragments can be detected when these ions are irradiated at moderate laser fluences. Furthermore, phase-sensitive detection of the laser-on and laser-off ion signals while rapidly sweeping the mass-analyzing QMF permits the photoproduct ions to be distinguished from the background arising from CAD and metastable decomposition. This background is as much as eight times larger than the photoproduct ion signal under the conditions used in this work.

Excitation of the $d-d$ transition in the Cu^{II} ion yielded two of the three reaction pathways observed in CAD of this complex with a total photodissociation quantum yield in excess of 0.8. This work is the first report of photodissociation triggered by excitation of a $d-d$ transition in a gas-phase coordination complex containing a transition-metal ion in the +2 formal oxidation state. The photochemistry resulting from ligand-field excitation of $[\text{Cu}^{\text{II}}(\text{bpy})(\text{serine}-\text{H})]^{+\cdot}$ shows a selectivity not observed for CAD at low center-of-mass collision energies (0.55 eV) under multiple-collision conditions or at moderate center-of-mass collision energies (≥ 5.7 eV) under single-collision conditions.

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