Electrospray Ionization of Copper–Glycine Solutions

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We have characterized the electrospray ionization (ESI) spectra of mixtures of the 3d transition metal ion Cu^{2+} with both a model amino acid (glycine) and a simple peptide system (glycylglycine). We found what appear to be two major classes of ions. We suggest that these apparent classes may offer some further insight into the nature of the ion formation process in ESI. Specifically, we argue that one group of ions is a consequence of gas-phase reactions of the intramolecular charge transfer type. We also argue that the second group of ions has a close association with species that have been identified in the bulk phase, and which therefore may arise in ESI by a direct desorption process. *(J Am Soc Mass Spectrom 1996, 7, 25–29)*

Complexes of metal ions with proteins play a vital physiological role in numerous biochemical processes [1, 2] that involve transport and catalysis. These intensively investigated systems have been studied via a variety of analytical techniques, which include polarimetry [3], potentiometry [4], and proton nuclear magnetic resonance [5] as well as electrophoresis, chromatography, and, more recently, mass spectrometry [6–9].

Since the first application of electrospray principles to chemical analysis by Dole et al. [10] in 1968 and its initial application to mass spectrometry by Fenn and co-workers [11, 12] 20 years later, this technique rapidly has become widely recognized as an important tool in chemical characterization, particularly for labile, nonvolatile large polar bimolecules [13–21]. In addition, there recently have been a number of reports of electrospray ionization mass spectra of transition metal-containing species, either as solvated complexes [22–26] or as complexes with peptides—proteins [27, 28].

A number of important questions arise in the use of electrospray ionization mass spectrometry to study transition metal ion complexes with model ligands. The first is, could this approach be used to improve the general understanding of the basic processes involved with electrospray ionization? Specifically, can ions be observed that are unlikely to have an origin other than desorption of species that exist in the bulk phase? Second, could this approach increase the extent of knowledge about the type of metal–ligand complex that is present in the solution phase?

To address these questions, we investigated very simple systems of copper ions with glycine and its dimer glycylglycine. We chose this simple system initially to serve as a model for more complex interactions of metals in proteins, but found that even such an apparently simple system gave rise to rather complicated spectra in our instrumentation. The interactions of copper with a simple amino acid and its dimer were chosen because they have been reported in the solution chemistry literature, which we felt might serve as a basis for the present investigations [29–31].

Experimental

Experiments were carried out with the apparatus shown in Figure 1 that consists of a home-built electrospray ionization source interfaced to a component-based quadrupole mass analyzer (Waters-Extrel, Pittsburgh, PA) with control and data collection that uses a Teknivent Vector One data system (Teknivent, St. Louis, MO). The source and interface were a heated metal capillary electrospray source [32] and employed a 500-μm-i.d., 26-cm-long stainless steel capillary that was resistively heated. The heating temperature was monitored by a thermocouple attached to the outer wall of the capillary exit end. Multiply charged droplets were produced from solutions that flowed at 1.8 μL/min from a syringe pump (model 341B, Sage Instruments, Boston, MA) through the discharge needle held at +2.6 kV. The capillary was maintained at the lowest temperature possible consistent with detection of ions—typically 60 °C. The discharge needle was separated from the capillary by about 6.0 mm. The 0.5-mm aperture skimmer was 3.5 mm away from the capillary end; that is, seven capillary diameters down-

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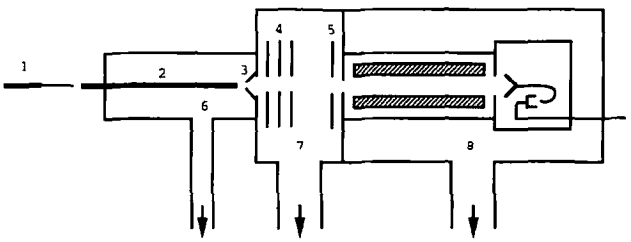

Figure 1. Schematic of the apparatus used to study transition metal ion complexes: (1) electrospray needle; (2) heated stainless steel capillary; (3) skimmer; (4) einzel lens; (5) entrance plate for quadrupole; (6) first stage pumping; (7) second stage pumping; (8) third stage pumping.

stream and thus within the Mach disk formed at the capillary exit. The 1.2-torr average pressure in the region between the capillary and skimmer was maintained by a 600-L/min mechanical pump (model 2033, Alcatel, France). The ion beam exiting the skimmer passed through an einzel lens held in a differentially pumped chamber evacuated by a 4-in., 1200-L/s oil diffusion pump (model VHS4, Varian Vacuum Division, Lexington, MA) backed by another 600-L/min rotary pump. The einzel lens was operated with +20 V on the outer two elements and the center element at ground. The ion beam passed through a 5-mm-diameter aperture into the quadrupole analyzer. Operating pressure in the latter was 1×10^{-6} torr. A model 4752-G (Galileo, Sturbridge, MA) continuous dynode electron multiplier was used for ion detection and was shielded in a separate container to prevent stray photons and/or ions from reaching the detector.

The mass scale of the quadrupole analyzer was calibrated by using a solution that contained a mixture of quarternary ammonium salts: $(NH_4)_2SO_4$, $(CH_3)_4NI$, $(C_2H_5)_4NBr$, and $(C_5H_{11})_4NCl$. All samples were prepared in deionized water (Hydroservices, Durham, NC) to which no organic solvent was added for electrospraying. Glycine and glycylglycine were obtained for Sigma Chemical Co. (St. Louis, MO), $CuSO_4 \cdot 5H_2O$ was purchased from Baker Chemical Co. (Phillipsburg, NJ). All material were used as received, with no further purification. Solutions for the complex experiments contained 5.0×10^{-4}-M metal ions and 1.0×10^{-3}-M gly and/or gly-gly. To make sure that all sample solutions had the identical concentration of gly and gly-gly, stock solutions that contained 1.0×10^{-2}-M gly (0.75 mg/mL) and gly-gly (1.32 mg/mL), respectively, were prepared in deionized water. Stock solutions of 5.0×10^{-3}-M Cu(II) also were prepared. The solutions of metal and ligand that were sprayed were held at pH 3.9–4.3 as determined via a calibrated model 340 pH meter (Corning Inc., Corning, NY). The pH of the solutions is set by the concentrations of the solutes alone. In this pH range the predominant metal and ligand species in solution were the cupric ion Cu(II) and the zwitterionic form of glycine, respectively.

The quadrupole mass analyzer was operated at nominal unit mass resolution. The natural abundance distribution of the copper isotope cluster was used to verify ionic compositions.

Results and Discussion

The electrospray mass spectrum of a Cu(II)/gly solution is shown in Figure 2. The base peak of the spectrum, m/z 76 u, is taken to be the protonated ligand gly · H^+. We have identified the pairs of ions at m/z 138-140, 173-175, 212-214, and 287-289 u as the singly charged complex ions $[Cu(I) \cdot gly]^+$, $[Cu(II) \cdot (H_2O)_2 \cdot (gly^-)]^+$ or $[Cu(II) \cdot H_2O \cdot gly \cdot (OH^-)]^+$, $[Cu(II) \cdot gly \cdot (gly^-)]^+$, and $[Cu(II) \cdot (gly)_2 \cdot (gly)^- -]^+$, respectively. The mass assignments of the last two ions listed were unambiguous, and thus the only composition consistent with these mass-to-charge ratios are ions that contain a glycine that has presumably lost a proton. In addition, we have identified the pairs of peaks at m/z 87-88 and 106.5-107.5 μ as the doubly charged ions $[Cu(II) \cdot (H_2O)_2 \cdot gly]^{2+}$ and $[Cu(II) \cdot (gly)_2]^{2+}$, respectively. The fractional abundances of ^{63}Cu and ^{65}Cu at 0.692 and 0.308 were reflected to an acceptable extent in the ion intensities observed for all six of these species. Replacement of gly with gly-gly led to analogous singly and doubly charged ions. When a mixture of the solutions that contained Cu(II) and equal amounts of both ligands was introduced to the system, the singly and doubly charged ions of both ligands as well as mixtures were observed. That is, singly charged ions at m/z 269-271 correspond to either $[Cu(II) \cdot gly\text{-}gly \cdot (gly^-)]^+$ or $[Cu(II) \cdot gly \cdot (gly\text{-}gly^-)]^+$, singly charged ions at m/z 344-346 are associated with either $[Cu(II) \cdot gly \cdot gly\text{-}gly \cdot (gly^-)]^+$ or $[Cu(II) \cdot (gly)_2 \cdot (gly\text{-}$

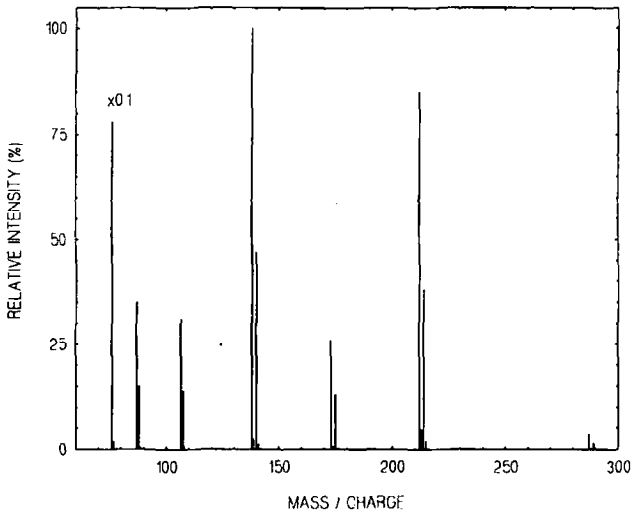

Figure 2. Electrospray ionization mass spectrum of 5.0×10^{-4}-M Cu^{2+} and 1.0×10^{-3}-M glycine in deionized water, pH = 3.92, flow rate = 1.8 μL/min. The capillary was heated to 60 °C.

$gly^-)]^+$, and singly charged ions at m/z 401-403 correspond to either $[Cu(II) \cdot gly \cdot gly\text{-}gly \cdot (gly\text{-}gly^-)]^+$ or $[Cu(II) \cdot (gly\text{-}gly)_2 \cdot (gly^-)]^+$. The copper isotope distribution is seen plainly in all the spectra. Our proposed identification of all of these ions is summarized in Table 1.

The ions of Cu · gly and Cu · gly-gly complexes that we have observed can be divided into two principal groups that are felt to differ in their basic formation mechanisms. Ions formed by these two different mechanisms are differentiated by superscripts b and c in the table, where b is an ion formed in the bulk phase and desorbed directly into the gas phase by the mechanism developed by Iribarne and Thomson [33]. Type c ions are those that we argue are formed by a gas-phase ion–molecule interaction of two possible types described in subsequent text. Throughout this discussion we define formation in the bulk phase to mean ions that have been identified in solution by other methods as well as ions formed in the electrospray process. At this point in time these two mechanisms cannot be distinguished.

Table 1 shows that three of the doubly charged ions are denoted as formed by either or both b and c processes. None of these three ions contains water as a ligand. A bulk phase process that could lead to their formation is protonation at a surface in the electrospray system of species that exist in solution. The doubly charged ion would then desorb from the surface by an Iribarne–Thomson mechanism. For example, the ions at m/z 106.5-107.5 could be formed by protonation of the neutral molecule $[Cu(II) \cdot (gly^-)_2]^0$ in the electrospray process. The presence of this neutral complex in aqueous solution under conditions similar to those used in the present experiments is well documented [29–31]. An alternative way in which these three doubly charged ions could be formed is via a gas-phase ligand replacement reaction. Such a process for the same ion is shown in eq. 1:

$$[Cu(II) \cdot (H_2O)_2 \cdot gly]^{2+} + gly = [Cu(II) \cdot (gly)_2]^{2+} + 2H_2O \quad (1)$$

This reaction is very dependent upon the presence of gas-phase neutral glycine. The zwitterionic form of glycine is predominant in the solutions used in these studies and one might anticipate that a free solvated glycine molecule could exist in the gas phase at the terminal phases of electrospray droplet decomposition. Desolvation of this neutral cluster to yield a gas-phase neutral glycine would simply require sufficient thermal energy to remove the clustered water input in excess of the ΔH_{solv}(glycine), that is, about 3 kcal/mol [34]. As a comparison, an estimate of $\Delta H_{solv}(H^+) > 260$ kcal/mol can be made from the literature of equilibrium ion–molecule chemistry. There is certainly sufficient thermal energy available in the ion source region to accomplish this.

At this juncture we are unable to argue convincingly for either the bulk phase ionization or the ligand transfer mechanisms to generate these three doubly charged ions. On the other hand, the hydrated doubly charged species seen at m/z 87-88 are not likely to be consequences of ligand transfer reactions, and therefore may be products of direct desorption. It is interesting to note that hydrated doubly charged ions were not detected in the Cu/gly-gly system.

Table 1 identifies the origin of most of the singly charged ions as the bulk phase or type b. Consider as an example of this type of ion the one observed at m/z 212-214. This ion could originate from the same neutral solution species mentioned before, $[Cu(II) \cdot (gly^-)_2]^0$ simply by being singly rather than doubly protonated

Table 1. Copper ion complexes with amino acids and peptides

Ligand	Coordin. no.	Complex ion	Peak	Intensity (%)[a]
Gly	4[b,c]	$[Cu(II) \cdot (gly)_2]^{2+}$	106.5, 107.5	31
	2[c]	$[Cu(I) \cdot gly]^+$	138, 140	100
	4[b]	$[Cu(II) \cdot gly \cdot (gly^-)]^+$	212, 214	85
	6[b]	$[Cu(II) \cdot (gly)_2 \cdot (gly^-)]^+$	287, 289	3.5
Gly, H_2O	4[b]	$[Cu(II) \cdot (H_2O)_2 \cdot gly]^{2+}$	87, 88	35
	4[b]	$[Cu(II) \cdot (H_2O)_2 \cdot (gly^-)]^+$	173, 175	26
Gly-gly	4[b,c]	$[Cu(II) \cdot (gly\text{-}gly)_2]^{2+}$	163.5, 164.5	23
	2[c]	$[Cu(I) \cdot gly\text{-}gly]^+$	195, 197	100
	4[b]	$[Cu(II) \cdot gly\text{-}gly \cdot (gly\text{-}gly^-)]^+$	326, 328	73
	6[b]	$[Cu(II) \cdot (gly\text{-}gly)_2 \cdot (gly\text{-}gly^-)]^+$	458, 460	4.6
Gly, gly-gly[d]	4[b,c]	$[Cu(II) \cdot gly \cdot gly\text{-}gly]^{2+}$	135, 136	36
	4[b]	$[Cu(II) \cdot gly \cdot (gly\text{-}gly^-)]^+$	269, 271	100
	6[b]	$[Cu(II) \cdot (gly)_2 \cdot (gly\text{-}gly^-)]^+$	344, 346	12
	6[b]	$[Cu(II) \cdot gly \cdot gly\text{-}gly \cdot (gly\text{-}gly^-)]^+$	401, 403	6.0

[a]Relative intensity of primary ^{63}Cu ions.
[b]Complex ions from the bulk phase.
[c]Complex ions from gas-phase reactions.
[d]Species same as above are not listed.

before ejection from the bulk. A difficult to distinguish alternative origin could be the desorption of the well known solution species $[Cu(II) \cdot gly \cdot (gly^-)]^+$. This ion exists in the same systems as the previously mentioned neutral, albeit at lower concentrations. We suggest that origins analogous to the m/z 212-214 ions are reasonable to assume for the ions in Table 1 at m/z 287-289, 326-328, 458-460, 269-271, 344-346, and 401-403. Structures for gas-phase ions that are consistent with structures expected in solution have been reported previously [35].

We suggest that the origin of the doubly hydrated, singly charged ion at m/z 173-175 is similar to the other singly charged species just discussed. On the other hand, it has been suggested that these ions could arise from an intramolecular charge transfer reaction, vide infra, that has been described by Kebarle and co-workers [23, 24] to yield a cuprous ion with the composition $[Cu(I) \cdot (OH^{\cdot}) \cdot H_2O \cdot gly]^+$. Such reactions appear to be observed always in conjunction with a precursor doubly charged species. None of the ions in Table 1 can serve as such a precursor, and so the charge transfer mechanism is ruled out in this case.

The presence of ions that result from an intramolecular charge transfer process is proposed, however, as the origin of the type c ions seen at m/z 138-140 and 195-197. By using the case of the smaller ion as an example, the intramolecular charge transfer process is illustrated in eq. 2:

$$[Cu(II) \cdot (gly)_2]^{2+} = [Cu(I) \cdot gly]^+ + gly^+ \quad (2)$$

where the doubly charged species at m/z 106.5-107.5 serves as the precursor. Although eq. 2 requires that an ion that corresponds to gly^+ be observed at m/z 75, we are unable to detect it. This is undoubtedly due to the interference on the low mass edge of the very large base peak—the $glyH^+$ ion—in the system. At the present time the process proposed in eq. 2 must be speculation on our part although it is partially supported by evidence of similar processes reported previously [23, 24]. Access to a tandem mass spectrometer system would permit verification of this reaction. Similarly, the expected $gly\text{-}gly^+$ ion at m/z 132 is not detected due to overlap from the protonated form of dipeptide.

It is curious to note that no copper-containing product ions of an intramolecular charge transfer process that involves the hydrated ion seen at m/z 87-88 are detected. Such ions would be expected to be at m/z 99-101, which corresponds to $[Cu(I) \cdot (H_2O)_2]^+$, or at m/z 155-157, which corresponds to $[Cu(I) \cdot (OH^{\cdot}) \cdot gly]^+$. The absence of such decomposition ions in our system is consistent with the absence of any hydrated, doubly charged precursor for the ion at m/z 173-175.

The very sensitive dependence of the charge transfer product ions on gas-phase reaction conditions was shown by decreasing the potential difference between the skimmer and the einzel lens. Gradual reduction of this difference from the original 25 V to 10 V resulted in the reduction and eventual disappearance of 138-140 peaks, but had little effect on the other species normally seen in the simpler system of Cu(II) and gly. Figure 3 shows the relationship of the relative intensities of 138-140 peaks versus skimmer voltage. Observation of the continuing presence of the doubly charged ions in the voltage reduction studies suggests they are formed mostly in the evaporating droplets and desorb into the gas phase. However, we cannot rule out the formation of at least some portion of these ions by gas-phase reactions despite the low gas-phase partial pressure of glycine

To what extent does the spectrum of Figure 2 reflect the bulk phase ionic composition? If the ions of clear gas-phase origin are eliminated, then relative gas-phase ion intensities might be compared to the best data for the bulk phase, although we recognize that the comparison is fraught with potential problems. Connolly and Orth [36] calculated equilibrium concentrations for the copper–glycine system based on experimental data [37, 38]. Their results are consistent with other reported values [29–31]. First, those results show that in the pH range of the present studies, the relative amount of $gly \cdot H^+$ should be much less than the sum of the concentration of all copper complexes. Because the $gly \cdot H^+$ intensity in Figure 2 is twice as great as the sum of all copper complex peaks, we conclude that the electrospray spectrum is not a quantitative representation of the aqueous solution for these species. On the other hand, it appears that the electrospray spectrum is a qualitative representation of the bulk phase compositions and, in the case of the multiligand complexes at least, show the existence of hitherto unrecognized species.

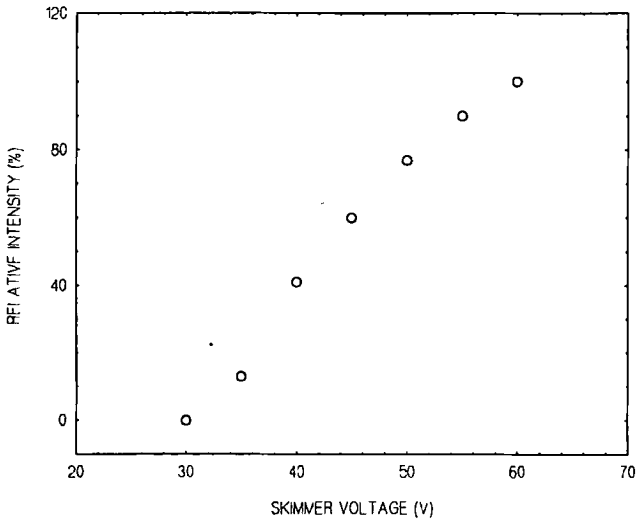

Figure 3. Relative intensities of peaks 138-149 as a function of skimmer voltage for a copper and glycine system. The einzel lens voltages are fixed: +20 V on the outer two elements and ground on the center element.

Conclusion

We have observed 3d transition metal ion complexes generated by electrospray ionization. We believe that a substantial fraction of the ions observed are associated with the species that exist in the bulk phase. Furthermore, it is possible that some of the ions deduced to form as a consequence of gas-phase charge transfer decompositions have precursors that are desorbed from the bulk phase. Finally, we believe that ions taken to originate in the bulk phase are a qualitative reflection of those species that exist in solution. As such, therefore, electrospray ionization mass spectrometry may offer some possibility to characterize the bulk phase interactions between metal ions and amino acids, small peptides, and potentially larger biological polymers. It is also clear that care should be taken in the interpretation of any spectral results of such studies.

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