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Low energy collisionally activated dissociation of Cu^{2+}(glycine)(H_2O), Cu^{2+}(glycine)$(H_2O)_2$, Cu^{2+}(glycine)$_2$, and Cu^{2+}(glycylglycine)$_2$

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Abstract

Doubly charged complex ions of Cu(II) have been formed in an electrospray source from dilute aqueous solutions containing copper sulfate and glycine (G) or glycylglycine (glygly). The most prominent ions, $Cu^{2+}GW$, $Cu^{2+}GW_2$, $Cu^{2+}G_2$, and Cu^{2+}(glygly)$_2$ (W = water) were studied by low energy, collisionally activated dissociation (CAD) in a triple quadrupole instrument. At very low collision energy, background water adds to all ions, except Cu^{2+}(glygly)$_2$, with no charge reduction. Loss of the elements of water from $Cu^{2+}G_2$ and Cu^{2+}(glygly)$_2$ without loss of charge suggests the occurrence of intracomplex peptide bond formation. In charge reduction reactions, the two ions containing water as a ligand produce ${}^{+}H_3NCH_2^{\cdot}$ as the only significant organic product ion, together with several singly charged copper containing ions. By contrast, Cu^{2+}(glygly)$_2$ produces $CH_2NH_2^+$ and (glygly)H^+, and $Cu^{2+}G_2$ produces GH^+, G^+, $CH_2NH_2^+$, and ${}^{+}H_3NCH_2^{\cdot}$, the latter two becoming more important at high collision energy. The major product from both $Cu^{2+}GW$ and $Cu^{2+}GW_2$ is $[Cu,H_2,O]^+$, which is suggested to be $[Cu(H)OH]^+$ rather than $[Cu(H_2O)]^+$. From the same two parent ions a stripping reaction at centre of mass collision energies above ~3 eV leads to loss of water, but no charge reduction, in the formation of $Cu^{2+}G$. In all CAD spectra there is a large imbalance at low collision energy between the relative amounts of the singly charged copper-containing product ions and the expected organic ions originating from the ligands. It is suggested that the latter are discriminated against because of inefficient containment following coulomb explosion. (Int J Mass Spectrom 192 (1999) 289–302) © 1999 Elsevier Science B.V.

Keywords: Copper(II); CAD; Glycine; Glycylglycine; Charge reduction

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1. Introduction

The study of gas-phase metal cation/molecule complexes has received much attention since the introductions of fast atom bombardment (FAB), laser desorption, and electrospray ionization (ESI) as methods of introducing to, or forming such complexes in, the gas phase. Since metal cations play major roles in biological processes, there have been numerous studies of the formation and reactivities of complexes consisting of metal cations and biological molecules. Studies of the association of amino acids and metal cations are, however, few in number and the majority of the studies have dealt with singly charged com-

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plexes. $[\text{AA/metal}]^+$ ions (AA = amino acid) were formed by plasma desorption mass spectrometry of AA/MCl_2 mixtures (M = Co, Ni, Cu, Fe) and the accompanying fragment ions, showed losses of the elements CH_2O_2, CH_4O_2, and CH_6O_2 [1,2]. The formation of these fragments and the observed metastable ions was interpreted in terms of insertion of singly charged metal cations into the C–COOH bond of the amino acid. A study of unimolecular and collisionally activated dissociation (CAD) fragmentations of Cu^+-cationated α-amino acids concluded that such insertion leads to the loss of (H_2, C, O_2) in the major fragmentation reaction and that it occurs by three pathways. These are: direct elimination of CH_2O_2, elimination of H_2O followed by CO, and elimination of CO_2 followed by H_2 [3]. Similar reactions were observed in a study of the unimolecular and CAD fragmentations of $[\text{AA–Ni}]^+$ complexes for which there was also a reaction, not observed with the $[\text{AA–Cu}]^+$ complexes [4]. This was the formation of immonium ions, $RCH{=}NH_2^+$. FAB was used to form singly charged, alkaline earth cationized amino acids and high energy CAD showed that the predominant decompositions lead only to the losses of small neutral molecules and fragments—CO_2, H_2O, CH_2O, NH_3, etc. [5]. Lei and Amster used laser desorption–chemical ionization together with Fourier transform mass spectrometry for a systematic investigation of the gas-phase reactions of ground state Cu^+ and Fe^+ with 20 common amino acids [6]. It was shown by ^{13}C labeling that the main neutral loss of 46 u involved the carboxyl group. Ab initio calculations on $[\text{glycine-Cu}]^+$, $[\text{serine-Cu}]^+$, and $[\text{cysteine-Cu}]^+$ show that the preferred binding involves chelation with the carbonyl oxygen and the amino nitrogen [7].

The ESI spectra obtained from solutions containing cupric ions, amino acids, and 2,2′-bipyridyl (bpy) show ions of general formula $[\text{Cu(II)(AA–H)(bpy)}]^+$ for aliphatic amino acids [8]. Basic amino acids such as lysine were found to also form doubly charged ions, $[\text{Cu(II)(AA)(bpy)}]^{2+}$, since protonation occurred on the basic group [9]. The bidentate bipyridyl occupies two of the coordination sites of the metal cation and prevents the association of more than one deprotonated amino acid molecule which is, therefore, bidentate, the copper being presumed to be tetracoordinated. Collisionally activated dissociation at low energies of the aliphatic amino acid complexes resulted in decarboxylation to produce stable α-amino alkyl residues. Studies of the activated dissociation of the doubly charged complexes formed with the basic amino acids were not reported but their singly charged complexes were also decarboxylated [10]. This decarboxylation was followed by transfer of functional groups from the side chains to the metal.

The above FAB and ESI studies employed divalent metal salts to form the singly charged complexes. In some cases the amino acids in the complexes were in their anionic forms, that is the carboxyl groups was deprotonated, the copper remaining as Cu(II). In others it was presumed that Cu(I) was present in the initially formed complex. The mechanism of formation of the singly charged complexes from the divalent cation and the amino acids was not in general studied in any detail, although in a plasma desorption study it was suggested that charge reduction of an initially formed Cu^{2+}/glycine complex, leading to the reduced form, might occur by nondissociative electron capture in the selvedge region [2].

If water, rather than a water/alcohol mixture is used as solvent in ESI then it is possible to observe doubly charged complexes of amino acids and divalent metal ions. Small yields of $Cu^{2+}GW$, $Cu^{2+}GW_2$, $Cu^{2+}G_2$, $Cu^{2+}(\text{glygly})_2$, and $Cu^{2+}(\text{glygly})G$ (G = glycine, glygly = glycylglycine, W = water) were observed in ESI spectra of aqueous solutions containing $CuSO_4$ (5×10^{-4} *M*), glycine (1×10^{-3} *M*) and/or glycylglycine (1×10^{-3} *M*) [11]. It was suggested that these ions were present in the bulk phase and entered the gas phase by a direct desorption process and that the singly charged, copper-containing ions resulted from gas-phase intramolecular charge transfer reactions. However this led to the conclusion that since the relative intensities of protonated glycine and the Cu^{2+}/glycine complexes were very different from calculated equilibrium concentration ratios [12], the spectra were not quantitative representations of bulk solution concentrations. Window factor analysis of visible spectra of aqueous solutions containing copper(II) and glycine give an equilibrium constant of 8 ± 2 for the association reaction between glycine and cupric ion [13],

$$Cu^{2+}(aq) + glycine(aq) = Cu^{2+}(glycine)(aq) \qquad (1)$$

The concentration of $Cu^{2+}G(aq)$ in solutions containing millimolar concentrations of the reactants is therefore in the micromolar range, confirming that such complexes cannot be major copper species in aqueous solutions.

Since the redox chemistry of copper is of extreme importance in biological systems it is useful to probe the differences between the chemistry of Cu(I) and Cu(II) in their associations with simple biological molecules. In this paper we describe a study of the CAD of doubly charged ions obtained from the electrospray ionization of aqueous solutions containing Cu^{2+} and glycine or glycylglycine. The objective was to examine the stabilities and reactivities of doubly charged Cu(II)/amino acid complexes in the gas phase for comparison with the reactivities of the singly charged complexes.

Although $Cu^{2+}G$ could not be obtained by electrospray ionization, the singly and doubly hydrated ions, $Cu^{2+}GW$ and $Cu^{2+}GW_2$, and $Cu^{2+}G_2$ were readily obtainable in sufficient yields to be studied by CAD. In addition, the complex formed between two molecules of glycylglycine and Cu^{2+}, $Cu^{2+}(glygly)_2$, was also readily produced for comparative study.

2. Experimental

The experimental method has been described in detail elsewhere [14]. Data were obtained using a Quattro quadrupole/hexapole/quadrupole instrument (VG, Fisons, Manchester, UK). Dry nitrogen was used as the nebulizing and drying gas in the low pressure region (~0.5 Torr) between the cone and the skimmer electrode. The positive potentials with respect to ground which were used were: capillary, 3 kV, HV lens (counter electrode) 0–0.1 kV, cone 0–12 V, skimmer lens 5 V and skimmer 0 V. Ions selected by the first quadrupole entered the hexapole collision cell at kinetic energies determined by the potential difference between the grounded skimmer and the collision cell (from 0 to −40 V) together with any axial energy gained in the extraction of ions from the source. Some ions still reached the detector after the second quadrupole when the collision cell was at ground potential. A stopping curve was obtained by biasing the collision cell at positive voltages with the ion source operating under the experimental conditions employed. Using the singly charged protonated glycylglycylglycine it was observed that ion transmission decreased by 80% when the cell potential was increased from 0 to +0.1 V and the decrease was 98% at +0.5 V when the intensity became too small to work with. A correction of +1 eV was therefore applied to the collision energy scale for the doubly charged ions. The pressure of the collision gas, argon, was monitored by a Baratron Type 128A capacitance manometer (MKS Instruments, Andover, MA), range 10^{-1}–10^{-5} Torr, directly attached to the collision cell.

Deionized water was the solvent for all samples, which were 1×10^{-3} *M* in each of $CuSO_4 \cdot 5H_2O$ and glycine or glycylglycine. The solutions were flowed through the capillary by means of a Harvard Model 11 syringe pump (Cambridge, MA). Both the position of the metal capillary tip with respect to the counter electrode and the potential of the cone were varied to optimize the signal for the study of each ion of interest. The cone potential was always low (<12 V) and the capillary tip–counter electrode distance was usually ~2 cm. A variation of the flow rate of the solution in the range 2–15 $\mu L\ min^{-1}$ had little effect on the mass spectral pattern observed and a flow rate of 5 $\mu L\ min^{-1}$ was used in all experiments.

The background pressure in the instrument, as measured on a Penning gauge situated on the vacuum housing adjacent to the first quadrupole, was 1×10^{-5} Torr with no collision gas present. When argon was present in the collision cell at a capacitance manometer pressure reading of 5×10^{-4} Torr, the reading of the Penning gauge was 1.5×10^{-5} Torr. The collision cell pressure held steady within ±5% of the set value. The only uncontrolled variable was the pressure of background water in the collision cell. The instrument is used in a multipurpose manner with several different ion sources and as such, of necessity, vacuum is often broken. The characteristics of CAD spectra, which are attributable to adventitious water, appear to depend only on how long the machine has been at vacuum and does not appear to be influenced by water from the electrospray source. As described in Sec. 3, the adventitious water is only of significance at very low collision energies.

3. Results

The copper-containing ions to be described are either singly or doubly charged. Any doubly charged ion will certainly contain Cu^{2+}, for example the ion formed by the association of two molecules of glycine with Cu^{2+}. This ion, and similar doubly charged ions, will be shown with the charge on copper, e.g. $Cu^{2+}G_2$. This does not imply that the ligands are present in the complex in a known form. For example, in $Cu^{2+}G_2$ the glycines may both be in the neutral state, one might be in the zwitterionic state and the other not, etc. Singly charged copper-containing product ions may also contain Cu(II) together with a negatively charged ligand or they may contain Cu(I). If the elemental composition of the solvation shell of a singly charged ion can be written in terms of known ligands then the ion will be written with the metal to the left and the charge to the right. For example, $CuGW^+$ is the ion with elemental composition $CuC_2H_7NO_3$ but there is no implication that one ligand is water and one is glycine. Ions having elemental compositions which cannot be written in terms of discrete ligands will be shown with square brackets.

Copper has two isotopes which differ in mass by 2 u with relative abundances, ^{63}Cu:^{65}Cu of 69:31. This distinctive isotopic pattern enabled a copper-containing ion to be readily identified, the peaks being two mass units apart for a singly charged ion and one mass unit apart for a doubly charged ion. Most CAD experiments were carried out with parent ions containing the more abundant ^{63}Cu isotope, which always gave better signal-to-noise ratios. Therefore, unless otherwise specified, all discussions of, and figures for, CAD spectra refer to results obtained using parent ions containing ^{63}Cu. CAD spectra were always also obtained for parent ions containing ^{65}Cu to definitively identify copper-containing product ions.

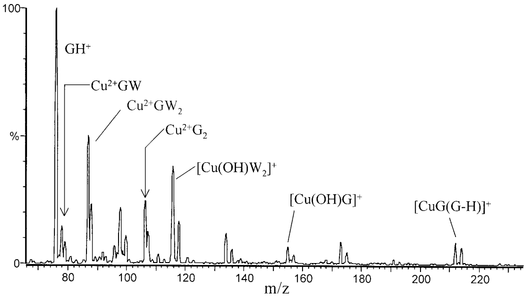


Fig. 1. Electrospray mass spectrum of an aqueous solution 1.0 × 10^{-3} *M* in both $CuSO_4$ and glycine (G = glycine, W = water).

Electrosprayed aqueous solutions of cupric sulfate, in the absence of amino acid or dipeptide, gave hydrated Cu(II) ions, both singly charged of general formula $[Cu(OH)W_n]^+$ $(0 < n < 10)$ and doubly charged of general formula $Cu^{2+}W_n$ $(4 < n < 14)$. The relative yields of the two types of ions and their degrees of solvation could be changed by varying the ion source potentials. Kebarle and co-workers [15] in an ESI study of the hydration of doubly charged alkaline earth and transition metal ions found that although most of the metal cations examined could be obtained with very small solvation shells, $Cu^{2+}W_n$ could not be observed. It was suggested that n would have to be greater than 15 for stability against charge reduction to $[Cu(OH)W_{n-x}]^+$ since the second ionization energy of Cu (20.29 eV) is much higher than the first (7.72 eV). In an earlier publication we concurred with that conclusion [14]. However earlier experiments had shown that it was possible to produce, by ESI, hydrated cupric ions in yields that can be, under carefully controlled ion source conditions, as great as, or greater than those of the singly charged ions [16] and we now agree with those findings [17]. The high second ionization energy of copper relative to those of other first row transition metals does not therefore result in instability with respect to intramolecular reduction of hydrated Cu^{2+} ions as was suggested [15].

Fig. 1 shows the ESI spectrum of an equimolar

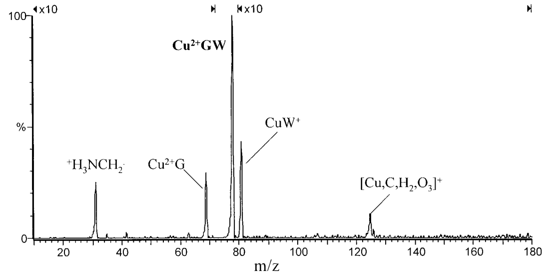


Fig. 2. CAD spectrum of $Cu^{2+}GW$ at a centre of mass collision energy of 4.9 eV. The intensities of all ions except the parent ion (in bold) have been multiplied by a factor of 10 (G = glycine, W = water).

(1.0×10^{-3} M) aqueous solution of glycine and $CuSO_4·5H_2O$. The ion source conditions were optimized to maximize the yields of the doubly charged ions containing glycine and under those conditions the $Cu^{2+}W_n$ peaks were negligibly small. The major ion in the spectrum is that of protonated glycine, GH^+, but $Cu^{2+}GW_2$ is the second most intense and is the highest peak for a copper-containing ion. The other two doubly charged ions observable in the spectrum are $Cu^{2+}GW$ and $Cu^{2+}G_2$. Cu^{2+} solvated solely by a single glycine molecule could not be produced in the source, but a doubly charged isobaric ion was observable in the CAD spectra of both $Cu^{2+}GW$ and $Cu^{2+}GW_2$. In Fig. 1 a series of ions with significant intensities and of general formula $[Cu(OH)W_n]^+$ has $n = 1, 2,$ and 3. Another series of copper-containing, singly charged ions with *m/z* values 155, 173, and 191 for the ^{63}Cu isotope could be either $[Cu(G–H)W_n]^+$, that is Cu(II) associated with the glycinate ion and 0, 1, or 2 water molecules, or $[Cu(OH)GW_n]^+$, Cu(II) with hydroxide, glycine and 0, 1 or 2 water molecules. No attempt was made to investigate the structures of these ions, which were the major copper-containing ions at high cone voltages. The highest mass ion in the spectrum is $[CuG(G–H)]^+$, Cu(II) with a glycine and a glycinate as ligands.

The spectrum in Fig. 1 is similar to a previously reported electrospray spectrum obtained with an aqueous solution containing 5.0×10^{-4} M Cu^{2+} and 1.0×10^{-3} M glycine [11]. The major difference is that in Fig. 1 the protonated glycine peak is much less dominant, as is expected for the lower glycine/Cu^{2+} ratio in the solution. The ESI mass spectrum of a solution 1×10^{-3} M in each of $CuSO_4·5H_2O$ and N-glycylglycine contained doubly charged, copper-containing ions analogous to those of Fig. 1, namely Cu^{2+}(glygly)W, Cu^{2+}(glygly)W_2, and Cu^{2+}(glygly)$_2$. Again, as for the system containing glycine, no cupric ion solvated by just a single dipeptide ligand could be produced.

CAD spectra for $Cu^{2+}GW$, $Cu^{2+}GW_2$, $Cu^{2+}G_2$, and Cu^{2+}(glygly)$_2$ were obtained with argon as collision gas at a pressure of 5×10^{-4} Torr over the nominal laboratory ion energy ranges of 1–41, 1–51, 2–51, and 2–43 eV, respectively. These laboratory energies correspond to the centre of mass collision energy ranges of 0.2–8.4, 0.2–9.5, 0.2–8.1, and 0.2–4.7 eV, respectively, for $Cu^{2+}GW$, $Cu^{2+}GW_2$, $Cu^{2+}G_2$, and Cu^{2+}(glygly)$_2$. The parent peak intensities decreased with increasing collision energy but, with the exception of $Cu^{2+}GW_2$ which readily loses H_2O, the decrease was never more than 15% at the highest energies. Under these experimental conditions multiple collisions occur, as discussed later. Figs. 2 and 3 show examples of CAD spectra and Figs. 4–7 show the normalized product ion intensities as functions of centre of mass (c.m.) collision energy. Each CAD spectrum was obtained by accumulating from 20 to 100 scans, the number depending on signal intensity. Baseline subtractions (50% noise level) were followed by two smoothings by the Savitzky Golay method. The relative intensities shown in the

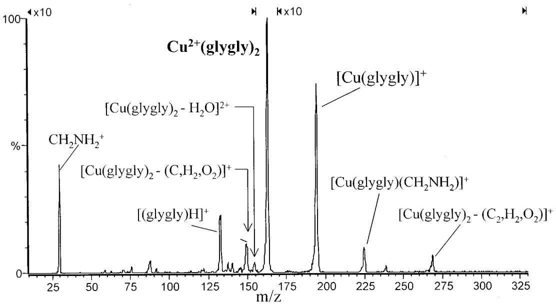


Fig. 3. CAD spectrum of Cu^{2+}(glygly)$_2$ at a centre of mass collision energy of 5.9 eV. The intensities of all ions except the parent ion (in bold) have been multiplied by a factor of 10 (glygly = glycylglycine).

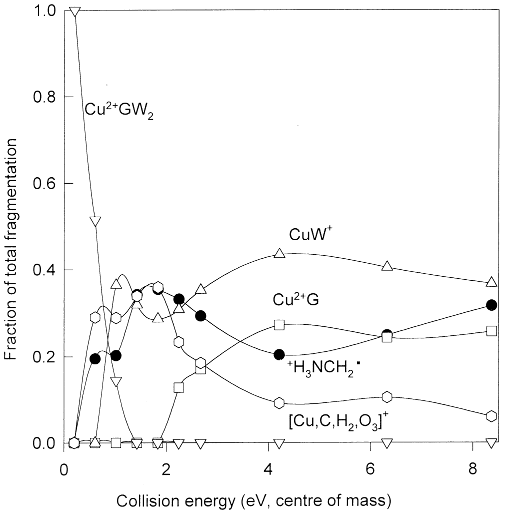


Fig. 4. Relative product ion intensities from the collisionally activated dissociation of $Cu^{2+}GW$ as functions of centre of mass collision energies. Argon collision gas at a pressure of 5 × 10^{-4} Torr (G = glycine, W = water).

figures were computed from the heights of the resulting peaks using all product ions in the spectra with intensities greater than 0.5% total product ion abundance. The relative intensities of the product ions were reproducible to within ±15%.

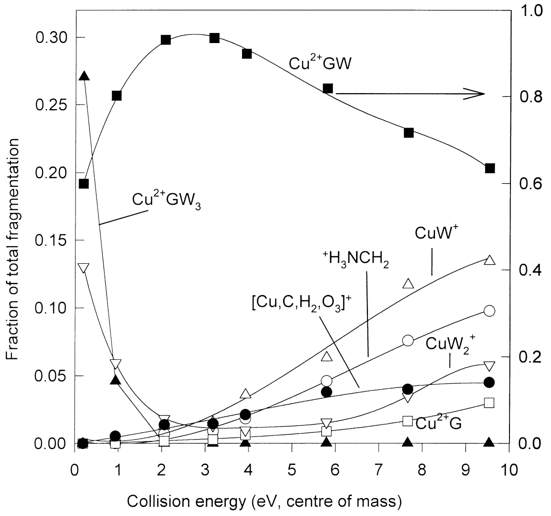


Fig. 5. Relative product ion intensities from the collisionally activated dissociation of $Cu^{2+}GW_2$ as functions of centre of mass collision energies. Argon collision gas at a pressure of 5 × 10^{-4} Torr (G = glycine, W = water).

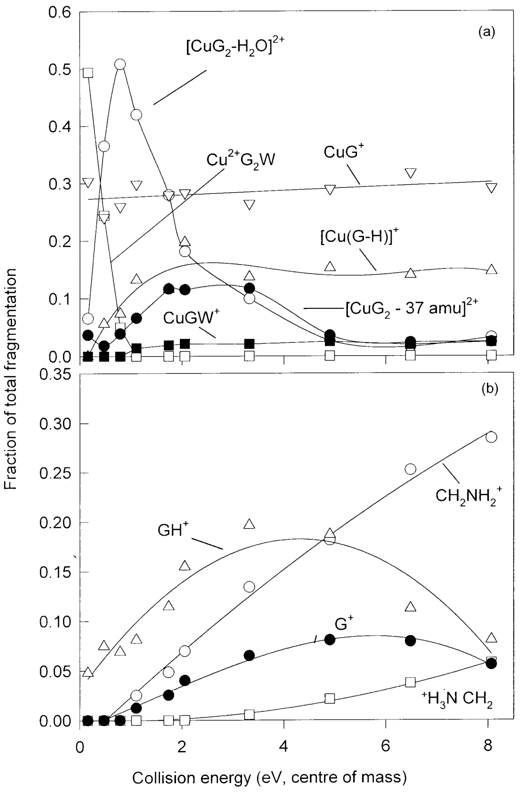


Fig. 6. Relative product ion intensities from the collisionally activated dissociation of $Cu^{2+}G_2$ as functions of centre of mass collision energies. (a) Copper containing product ions, (b) organic ions. Argon collision gas at a pressure of 5 × 10^{-4} Torr (G = glycine, W = water).

4. Discussion

The conformation and even the structure of a glycine molecule associated with Cu^{2+} in the gas phase is not known. Ab initio calculations by Hoyau and Ohanessian on CuG^+, the complex formed between the cuprous ion and neutral glycine, at the MP2/6-31G* level show that the most stable structure has glycine in its nonzwitterionic form [7]. The complex is a five-membered ring in which Cu^+ chelates with both the carbonyl oxygen and the amino nitrogen, the best estimate of the complexation, energy at 0 K being 269 kJ mol^{-1}. The conformation of glycine is little changed in forming the complex, indicating that its interaction with Cu^+ is probably

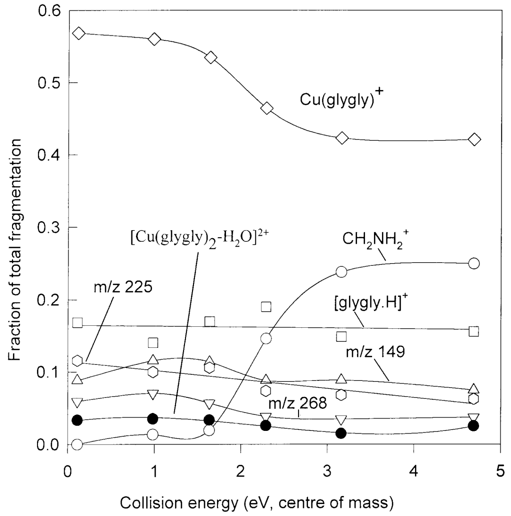


Fig. 7. Relative product ion intensities from the collisionally activated dissociation of $Cu^{2+}(glygly)_2$ as functions of centre of mass collision energies. Argon collision gas at a pressure of 5×10^{-4} Torr (glygly = glycylglycine).

mainly electrostatic, although the nonlinear $Cu \cdots O{=}C$ is suggestive of a Lewis acid–base interaction between the cation and an electron lone pair on oxygen. The next lowest conformation has the zwitterionic form of glycine in which the metal cation interacts only with the two oxygens of the carboxylate group, one at 1.94 Å and the second at 2.74 Å, this difference is due to the interaction of the furthest oxygen with a hydrogen on nitrogen. The difference in energy between the zwitterionic and nonzwitterionic complexes is computed to be 38 kJ mol^{-1}, which is significantly lower than the 107.4 kJ mol^{-1} for glycine alone in the gas phase [18].

The interaction of glycine with the harder Cu^{2+} is expected to be even more electrostatic in nature than with Cu^+, which should then be more favourable toward the zwitterionic form. In this case, association would be only with the carboxylate group, the ammonium group being repelled from the metal centre. The preferred sterochemistry of Cu(II) when complexing with biological molecules is described as tetragonal, five-coordination or tetrahedral [19]. Octahedral coordination of Cu(II) is also possible with, however, elongation of the two bonds along one fourfold axis [20–23]. Evidence has been presented for the particular stability of $Cu^{2+}(H_2O)_8$ in the gas phase [16,17] although the structure of such an ion must involve a second solvation shell. How glycine coordinates with Cu(II) in the gas phase will almost certainly depend on the presence, or absence, and identities of other ligands present and cannot be known without very high level calculations since several structures for each complex will probably be very close in energy.

The second ionization energy of copper, 20.29 eV, is considerably higher than the first ionization energy, 7.72 eV, which leads to a great tendency to charge reduction either by interligand proton transfer

$$CuL_n^{2+} = Cu(L–H)L_{n-2} + LH^+ \tag{2}$$

or by electron transfer from one of the ligands

$$CuL_n^{2+} = CuL_{n-1}^{+} + L^+ \tag{3}$$

both followed by dissociation [24]. In either case the result is a singly charged copper complex.

Charge reduction of doubly charged Cu(II) complexes was found to occur by collisional activation in the pressure transition region of the electrospray source which accounted for the very limited, and very low range of cone voltage that was available for observation of such complexes. It was found that collisionally activated dissociation in the collision cell may occur with and without charge reduction. The product ions observed in the CAD spectra of $Cu^{2+}GW$, $Cu^{2+}GW_2$, $Cu^{2+}G_2$, and $Cu^{2+}(glygly)_2$ will therefore be discussed under two headings, according to whether charge reduction does or does not occur.

5. CAD products formed with no charge reduction

The observation that no product ions were formed when $Cu^{2+}GW$, $Cu^{2+}G_2$, and $Cu^{2+}(glygly)_2$ traversed the collision cell held at −20 V in the absence of argon collision gas means that under these conditions there was neither unimolecular decomposition nor any collisionally activated dissociation due to the presence of background gas molecules in the collision cell. These ions are therefore very stable on the time scale of the experiment and were produced in the electrospray process with little excitation energy. $Cu^{2+}GW_2$ did show a very small peak, due to an ion formed by the loss of one water molecule with no accompanying reduction in charge

In CAD experiments with 5×10^{-4} Torr argon in the collision cell, water was found to add to $Cu^{2+}GW$, $Cu^{2+}GW_2$, and $Cu^{2+}G_2$ at the very lowest collision energies without charge reduction (Figs. 4–6). This water may have been from the background or have been introduced with the argon. Such association reactions can occur only at, or very close to, thermal energies, and the presence of argon is necessary to reduce the translational energies of the ions. This shows that multiple collisions of the ions with gas molecules are occurring in the collision cell since the water will be at an unknown, but considerably lower partial pressure than is the argon. As expected for association reactions, both the relative and the absolute yields of the water adducts have their maximum values at the lowest collision energies. The yields decrease rapidly with increasing collision energy and these adducts are absent at $E_{\text{c.m.}} > 1$ eV. The relative and absolute intensities of these water adducts varied somewhat from day to day, according to the background water content of the instrument. If the instrument had recently been opened to the atmosphere then the background water content was "high" and the intensities were high. In fact if experiments were attempted immediately after pumpdown, the additions of a further one and sometimes two water molecules, in particular to $Cu^{2+}GW$, was observed. No CAD data were recorded until the instrument had been at vacuum for at least 24 h.

The addition of one molecule of water to $Cu^{2+}GW$ would form $Cu^{2+}GW_2$ where the copper is probably tetracoordinated, through four oxygens, two from glycine and two from the water molecules, if the glycine is in the zwitterionic form and through three oxygens and one nitrogen if the glycine is bidentate but is not in the zwitterionic form. The addition of water to $Cu^{2+}GW_2$ and $Cu^{2+}G_2$ could be either on the metal or at an acidic hydrogen of one of the ligands. It is to be noted that $Cu^{2+}(glygly)_2$ did not add adventitious water under the same conditions as the other ions. A water adduct of this ion was however observable immediately after pump-down when background water levels were high. There are several ways in which glycylglycine can bind to a metal cation. Calculation shows that in the complex formed between Na^+ and a single glycylglycine molecule, a bidentate (carboxyl and amide oxygens) or a tridentate (amine nitrogen and carboxyl and amide oxygens) coordination leads to the most stable structures, which have essentially identical energies [25]. Such structures should also be possible for the Cu(II) complex. The fact that $Cu^{2+}GW_2$, where the metal coordination number must be at least four, readily adds water but $Cu^{2+}(glygly)_2$ does not suggests that in the latter ion Cu^{2+} is probably penta- or hexacoordinated to the glycylglycines. If, however, both glycylglycines are behaving in a bidentate manner, then either any other metal site(s) is (are) sterically hindered or the enthalpy of binding at an available site is small. It also follows that the Cu^{2+} does not activate the hydroxyl hydrogen of the carboxyl groups sufficiently to engender the formation of strong hydrogen bonds to additional water molecules. Further hindrance to hydration may also be the presence of intra- and/or interligand hydrogen bonding.

Loss of one molecule of water from $Cu^{2+}GW_2$ without charge reduction is facile (Fig. 5), the product ion presumably being identical to $Cu^{2+}GW$. As noted previously, this product ion was present in low yield in the absence of argon, implying that the second water molecule on $Cu^{2+}GW_2$ is relatively loosely bound and is probably situated on a less favourable site than is the first. When $Cu^{2+}GW$ is formed in the electrospray source and then subjected to collisional activation it can also lose water without charge reduction (Fig. 4). There is a steep threshold for this production of $Cu^{2+}G$, after which the relative yield becomes essentially constant. Attempts to produce $Cu^{2+}G$ directly in the ion source by varying the cone voltage were unsuccessful, probably because of the ion's high affinity for the water present in the desolvation region. $Cu^{2+}G$ is also a product in the CAD spectrum of $Cu^{2+}GW_2$ (Fig. 5) but the shape of the yield curve at threshold is very different to that for its production from $Cu^{2+}GW$. However, the slowly rising curve has a threshold not dissimilar to that seen in Fig. 4. The threshold of ~1.8 eV (174 kJ mol^{-1}) for the loss of water from $Cu^{2+}GW$ to produce $Cu^{2+}G$ can be taken as an approximation to the energy to dissociate the water to molecule. The value of course does not take into account the thermal distribution of the ions or kinetic shifts. The preference for loss of neutral water rather than neutral glycine is consistent with the expectation of the latter having the higher enthalpy of association. The measured complexation enthalpy of Cu^{+} and water is -157 kJ mol^{-1} [26] and the calculated smaller $Cu \cdots O$ distance in Cu(II) hydrates compared with Cu(I) hydrates [22], implying higher binding energies, is consistent with the observed high threshold energy. For comparison, the computed values for complexation with Cu^{+} are -153 kJ mol^{-1} for water and -269 kJ mol^{-1} for glycine [7]. No $Cu^{2+}W$ was observed in any of the CAD spectra and this ion has not been described in the literature. The lowest hydrated forms of Cu^{2+} in the gas phase which have been reported so far are $Cu^{2+}W_2$, which was formed by the collisionally activated dissociation of $Cu^{2+}W_4$ [17], and $Cu^{2+}W_3$, formed by the electron impact ionization of Cu/W/Ar clusters, and in the collisionally activated dissociation of higher Cu(II) hydrates [27].

A very interesting product ion in the CAD spectrum of $Cu^{2+}G_2$ is $[CuG_2–H_2O]^{2+}$. This ion has a maximum yield at ~0.8 eV center of mass energy which then declines to a very low value at high energy. The most logical mechanism for the formation of this ion is the elimination of a molecule of water in an intracomplex condensation reaction, leading to the formation of a peptide bond between the two glycines. The dipeptide remains attached to the metal. The reaction is analogous to one proposed to occur in aqueous solutions containing glycine, which has been put forward as an explanation for the formation of peptides on prebiotic earth. Peptides were formed from their amino acid constituents in aqueous solution in the presence of 3–5 *M* NaCl and 0.4–0.8 *M* Cu(II) [28,29]. The proposed intermediate structure leading to glycylglycine has the cupric ion chelated to one deprotonated glycine via O and N and to a protonated glycine via the carbonyl oxygen. Also in the coordination shell are a chloride ion and two water molecules, the latter at a significantly larger distance than the other ligands [28]. Loss of water, resulting in peptide bond formation, is proposed to be due to nucleophilic attack of the deprotonated amino group on the OH of the protonated glycine. *Ab initio* calculations show that the function of chloride is to stabilize the complex.

The structure of $Cu^{2+}G_2$ in the gas phase is not known. It may be asymmetric in the sense of proton transfer having already occurred prior to collisional activation, or proton transfer could occur as the result of activation. It can be calculated from the proton affinity (887 kJ mol^{-1}) [30] and acidity (1429 kJ mol^{-1}) [31] of glycine, that the bimolecular self protonation of glycine in the gas phase

$$2H_2NCH_2COOH \rightarrow H_2NCH_2COO^- + HOOCCH_2NH_3^+ \qquad (4)$$

is endothermic by 546 kJ mol^{-1}. If proton transfer has already occurred in the Cu(II) complex then association with the metal cation has lowered this energy requirement to a very small value since the threshold for the apparent formation of the peptide bond, which occurs without the aid of charge reduction, is very low.

It was of interest to compare the energy dependence of water loss from $Cu^{2+}G_2$ with that from the proton bound dimer GHG^{+}. The major product at 4.3 eV center of mass collision energy from collisional activation of the latter ion was protonated glycine but there was a small peak due to the loss of water with an intensity less than 4% of total dissociation. At 2.3 eV only the water loss peak was present, but in extremely low yield, and below 2.0 eV, no decomposition was detectable. There is therefore a similarity between the collisionally activated dehydrations of the diglycine complexes of the proton and the cupric ion. Both are favoured over other processes at low energy, consistent with them being rearrangements and not simple bond cleavage. However, the dehydration is far more facile in the presence of the cupric ion.

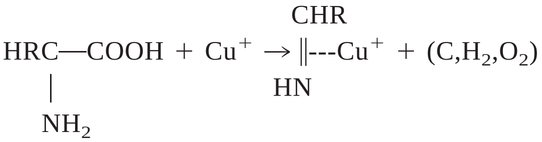
There is a very small peak at *m/z* 154.5 in the CAD spectrum of $Cu^{2+}(glygly)_2$ (Fig. 3), which corresponds to loss of water and possible formation of a Cu(II)/tetrapeptide complex, $[Cu(glygly)_2–H_2O]^{2+}$. The intensity varies little with collision energy (Fig. 7) unlike that of the analogous ion from $Cu^{2+}G_2$, and has a finite yield at close to zero centre of mass collision energy.

A very small yield of the doubly charged product ion of *m/z* 88 is formed by loss of 37 amu from $Cu^{2+}G_2$. The yield maximizes at ~2 eV with a threshold at 0 eV. 37 u corresponds to both H_5O_2 ($2H_2O + H$) and NH_5O ($NH_3 + H_2O$) and the production of either would imply that extensive rearrangement has occurred in forming the observed ion.

6. Product ions from charge reduction reactions

As shown in Figs. 2 and 4, $Cu^{2+}GW$ produces only two singly charged, copper-containing ions, *m/z* 81 and *m/z* 125. The first corresponds to Cu^+ plus the elements of water and results from the loss of ($C_2H_5NO_2$), the elements of glycine. Simple electron transfer from ligand to copper would be energetically much more favourable toward loss of ionized glycine (I.E. = 8.8 eV) over loss of ionized water (I.E. = 12.61 eV) though both processes would be highly exothermic because of the large difference between the first and second ionization energies of the copper atom. However, the threshold for the appearance of *m/z* 81 is very small (~0.3 eV), which suggests that its formation may not simply be a direct electron transfer. The very low threshold could imply a rearrangement rather than a simple loss of glycine and the ion might be due to the reductive loss of the glycine cation concurrent with the insertion of the resulting Cu^+ into the O–H bond of water to give $[HO–Cu–H]^+$. The glycine cation is not an observed product and neither is $CH_2NH_2^+$, which is the expected product of its further decomposition since this ion is the base peak in the electron-impact mass spectrum of glycine.

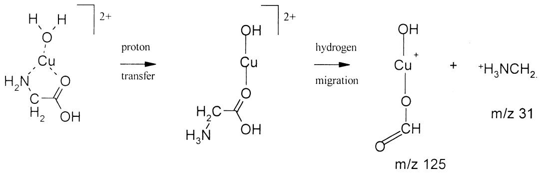
m/z 125 is a copper-containing product ion formed by loss of 31 mass units from $Cu^{2+}GW$, and a matching organic ion is observed at *m/z* 31, with presumed elemental composition CH_5N. The latter could be either the ylid, $^+H_3NCH_2^{\cdot}$ or $NH_2CH_3^{+\cdot}$, which have almost identical computed enthalpies of formation (862 and 866 kJ mol^{-1} respectively) [32,33]. An ion of identical *m/z* was a minor high energy product, which was identified in the CAD spectra of protonated glycine as the distonic ion $^+H_3NCH_2^{\cdot}$ [34] based on earlier neutralization/reionization experiments. However, the results of those experiments were not conclusive in distinguishing between the two isomers [35]. There is no mass balance between the intensities of *m/z* 31 and 125, an observation that will be discussed later. Taking CH_5N^+ as being indeed the complementary ion to *m/z* 125, the latter is then $[Cu, C, H_2, O_3]^+$ which means that the CO_2 of the carboxyl is retained in the copper-containing ion after charge separation. By contrast, in the CAD spectra of singly charged copper/alkyl-amino acid complexes, a major neutral loss is that of (C, H_2, O_2) leaving an RC(H)=NH associated with Cu^+ [1,3,10]. The accepted explanation for the formation of this complex is that the metal inserts into the C–COOH bond, leading to the elimination of (C, H_2, O_2) as in



$$\underset{NH_2}{\underset{|}{HRC}}\text{—}COOH + Cu^+ \rightarrow \underset{HN}{\overset{CHR}{\|}}\text{---}Cu^+ + (C,H_2,O_2) \qquad (5)$$

A suggestion that there is also a competitive reaction channel for the insertion complex leading to the formation of the immonium ion [1] has not been substantiated [3].

Neither a Cu^+/immine complex nor an immonium ion is a charge reduction product from the collisionally activated dissociation of $Cu^{2+}GW$. This implies that charge reduction followed by insertion of Cu^+ into the C–CO bond of glycine does not occur. An excited glycine molecular ion cannot be formed since this would lead to an immonium ion. A possible route to the observed formation of $[Cu, C, H_2, O_3]^+$ and CH_5N is shown in Scheme 1 where intracomplex



Scheme 1.

proton transfer as the initial step is followed by H migration and dissociation. This leads to *m/z* 31 being initially $^+H_3NCH_2^{\cdot}$ rather than $CH_3NH_2^{+\cdot}$.

It would be of interest to see whether the CAD spectra of the Cu(I) complexes of amino acids would also dissociate to give $^+H_3NCH_2^{\cdot}$ if a water ligand were also present. We have so far been unable to generate complexes such as $CuGW^+$ by ESI since, as seen in Fig. 1, $[Cu(OH)G]^+$ is preferentially formed.

Fig. 5 shows that the CAD spectrum of $Cu^{2+}GW_2$ has several singly charged ions in common with those from $Cu^{2+}GW$. The loss of glycine and one molecule of water in a charge reduction reaction again gives *m/z* 81 as the major ion at high energy. *m/z* 125 is also present. Once again the only ion arising solely from the ligands is *m/z* 31. Since a single water ligand is lost very readily from $Cu^{2+}GW_2$, the similarity of its CAD spectrum with that of $Cu^{2+}GW$ is not unexpected. Scheme 1 is therefore also an appropriate mechanism for the formations of *m/z* 31 and *m/z* 125 from $Cu^{2+}GW_2$ after the loss of one molecule of water. A product ion at *m/z* 99, corresponding to copper plus two water molecules, which has a maximum relative intensity at the lowest energy, attains a minimum value and then increases slowly at the higher energies. The logical composition for this ion is $[HCu(OH)W]^+$ corresponding to the loss of the glycine ligand in a manner analogous to the formation of *m/z* 81 from $Cu^{2+}GW$. Insertion of Cu^+ into the O–H bond of one water molecule to produce a strong Cu–O bond is an acceptable process but the simultaneous retention of the second, weakly bound water molecule is doubtful. The shape of the curve for *m/z* 99 at low energy in Fig. 6 is suggestive of that of the water adduct of the parent ion, $Cu^{2+}GW_2$, which points to *m/z* 99 occurring at low collision energy as a result of the addition of adventitious water to an initially formed $[HCuOH]^+$. There is however no support for this proposal in the CAD spectrum of $Cu^{2+}GW$, which shows no *m/z* 99 at any collision energy even though the relative yield of $[HCuOH]^+$ is high. The relative intensity of *m/z* 99 from $Cu^{2+}GW_2$ reaches a minimum value at ~2.5 eV and then increases at higher energy. This behavior must be because of a reaction different to that for the low energy region although the mechanism is unknown. A simple stripping reaction with accompanying charge separation may be occurring, however it is difficult to see why there would be preferential retention of two water molecules when the loss of one water molecule is by far the most favoured decomposition path.

The CAD spectra of $Cu^{2+}G_2$ contain many singly charged ions but, as seen in Fig. 6, the nature of the major ions is not unusual. The major copper-containing ion is CuG^+ with a relative yield having little dependence on collision energy. A simple loss of the glycine cation, G^+, from the parent ion would explain the appearance of CuG^+, however, this matching product ion does not appear until the collision energy is ~1 eV. The yield of G^+ increases from this threshold, attains a maximum value at 4 eV and then declines with increasing energy. The immonium ion, $CH_2{=}NH_2^+$, has the same threshold as G^+ and is the dominant organic product ion at high energy [Fig. 6(b)]. This ion is the base peak in the electron impact mass spectrum of molecular glycine [36] with an appearance energy of 10.27 eV, 2.06 eV higher than the ionization energy of glycine [37]. The formation of all three product ions, CuG^+, G^+, and $CH_2{=}NH_2^+$, is therefore consistent with the collisionally activated dissociation of $Cu^{2+}G_2$ to produce a glycine cation which has insufficient internal energy to decompose at low collision energy, but does have sufficient energy to decompose at the higher collision energies

$$Cu^{2+}G_2^* \rightarrow CuG^+ + G^{+*} \qquad (6)$$

$$G^{+*} \rightarrow NH_2CH_2^+ + (C, O_2, H) \qquad (7)$$

The decomposition of the glycine cation formed in the reductive dissociation of the complex [Eq. (6)] could also be due in part to its activation by subsequent collision(s) after formation. An inability to operate under single collision conditions, requiring a maximum pressure of 1×10^{-4} Torr, did not allow this point to be investigated.

The only other major, singly charged copper-containing ion in the CAD spectrum of $Cu^{2+}G_2$ is $[Cu(G–H)]^+$. The matching organic ion, protonated glycine, is seen in Fig. 6(b) to have a similarly shaped yield curve at low collision energy, suggesting dissociation from an activated $Cu^{2+}G_2$ in competition with the reaction of Eq. (6)

$$Cu^{2+}G_2^* \rightarrow Cu(G–H)^+ + GH^+ \tag{8}$$

The relative yield of $[Cu(G–H)]^+$, unlike that of CuG^+, is not independent of collision energy but increases from zero to a constant value above 1 eV. The small decline in the yield of GH^+ at the higher collision energies appears to be due to further dissociation to either, or more probably both, $CH_2{=}NH_2^+$ and $^+H_3NCH_2^{\cdot}$. The former ion is the major product in the low energy collisionally activated dissociation of protonated glycine and the latter ion appears in low yield from the same ion but has a higher appearance threshold [34].

One other singly charged copper-containing ion is formed from $Cu^{2+}G_2$, but in very low yield. *m/z* 155 is formed by loss of 57 u from the parent ion and is designated in Fig. 6(a) as $CuGW^+$. There is no matching ion to confirm the elemental composition or the assigned structure.

The major, singly charged, copper-containing ion at all energies seen in Fig. 7 in the CAD spectrum of $Cu^{2+}(glygly)_2$ is $[Cu(glygly)]^+$. This implies simple dissociation, analogous to that for $Cu^{2+}G_2$

$$Cu^{2+}(glygly)_2 \rightarrow [Cu(glygly)]^+ + [glygly]^+ \tag{9}$$

The accompanying $[glygly]^+$ is expected to be shown by the appearance of $NH_2CH_2^+$

$$[glygly]^{+*} \rightarrow NH_2CH_2^+ + [C_3H_4NO_3] \tag{10}$$

which is the base peak in the electron impact mass spectrum of glygly.

The yield of $[Cu(glygly)]^+$ is fairly constant at low collision energy and then decreases at ~1.5 eV to a lower constant value at higher energies. Surprisingly, this latter energy range sees the first appearance of the immonium ion, $NH_2CH_2^+$. Two other organic ions, *m/z* 76 and 87, which may be, respectively, protonated glycine and $[glygly–HCO_2]^+$, are present but with intensities too low to be shown in Fig. 7.

Protonated glycylglycine (*m/z* 133) is the only organic ion at low collision energy and it has a relative yield that is invariant with energy. The only copper-containing ion having a sufficiently low mass to be associated with its formation is *m/z* 149, which corresponds to $[Cu(glygly)–(CO_2H_2)]^+$. Interligand proton transfer

$$Cu^{2+}(glygly)_2 \rightarrow [Cu(glygly–H)(glygly + H)]^+ \tag{11}$$

followed by loss of protonated glycylglycine

$$[Cu(glygly–H)(glygly + H)]^+ \rightarrow [Cu(glygly)–H)]^+ + glygly{\cdot}H^+ \tag{12}$$

would lead to $[Cu(glygly)–H]^+$. Subsequent loss of CO_2 and H

$$[Cu(glygly–H)]^+ \rightarrow [H_2NCH_2CON{=}CH_2{\cdot}Cu]^+ + CO_2 + H \tag{13}$$

would give the observed product ion, a Cu^+/immine complex, which has a similar relative yield as a function of collision energy as does the product, protonated glycylglycine.

The only other singly charged copper-containing product ions with significant intensities are *m/z* 225 and *m/z* 268. The first corresponds to the copper/imine complex, $[Cu(glygly){\cdot}CH_2NH_2]^+$, which has no counterpart in the CAD spectrum of $Cu^{2+}G_2$, even though that might be expected since $[Cu{\cdot}NH_2CH_2]^+$ is a major product in the CAD spectra of Cu^+-cationated amino acids [1–3]. *m/z* 268 shows charge reduction together with loss of 59 u from the doubly parent ion. The mass deficit could be either $C_2H_3O_2$ from the O end of the dipeptide or C_2H_5NO from the N end.

It is noticeable that all the copper-containing products in Fig. 7 appear with finite yields at close to the nominal zero of collision energy and that all their yields appear to decline, some more than others, with increasing energy. These declines may simply be a reflection of the appearance of $CH_2NH_2^+$ at the higher collision energies and its large contribution to the total ion intensity. There is no balance at low centre of mass collision energies between copper-containing product ions and other ions in the CAD spectra. This could in part be due to mass discrimination in the analysing quadrupole for which no correction was made. However, the balance does improve at higher collision energies so such an effect, which in any case usually favours the detection of the lighter ions, cannot provide the full explanation. It is more likely that the lighter ions are especially discriminated against at low collision energies because they are formed with high kinetic energies in a coulombic explosion and are not contained by the rf field of the collision cell. At higher kinetic energies, their greater initial forward momentum and the apparent increasing preference for stripping reactions leads to an increase in detection of the lighter products.

7. Conclusions

The collisionally activated dissociations of doubly charged Cu(II) complexes containing glycine, glycine, and water or glycylglycine show a large variety of products. Product ions may be formed both with and without charge reduction. Retention of charge and generation of $Cu^{2+}G$ occurs only when water is one of the ligands, that is with the two ions $Cu^{2+}GW$ and $Cu^{2+}GW_2$. Loss of one molecule of water from the latter ion, without charge reduction, is facile. The only product ion formed without charge reduction from Cu(II) complexed with either two glycine molecules or two glycylglycine molecules has a composition showing loss of the elements of one molecule of water. It is suggested that a condensation reaction has occurred, resulting in the formation of a peptide bond. Background water adds to $Cu^{2+}GW$, $Cu^{2+}GW_2$, and $Cu^{2+}G_2$ at very low collision energies but not to $Cu^{2+}(glygly)_2$ under the same conditions, showing that the latter probably has no accessible vacant metal binding site.

At low collision energies there is a deficit of low *m/z* ions to match the observed singly charged, copper-containing product ions. This is attributed to a failure to contain and transmit the light ions from the collision cell to the analysing quadrupole. The presence of water as one of the ligands has a profound effect on the nature of the organic product ions observed. The only organic ion observed when water is one of the ligands, from $Cu^{2+}GW$ and $Cu^{2+}GW_2$ as parent ions, is *m/z* 31, which may be the distonic ion, $^+H_3NCH_2^{\cdot}$. No ions derived mainly from water such as H_2O^+ or H_3O^+ are observed. $Cu^{2+}G_2$ yields both $CH_2NH_2^+$ and $^+H_3NCH_2^{\cdot}$ but $Cu^{2+}(glygly)_2$ yields only $CH_2NH_2^+$.

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