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# Characterization of [M–H] cations, radicals and anions of glycine in the gas phase: a combined experimental and ab initio study

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## Abstract

The gas phase structures of the [M–H] cations and anions of glycine have been studied by using a combination of ab initio calculations (at the MP2(FC)/6–31+G\* level of theory) and tandem mass spectrometry (MS/MS). It was found that the ab initio stability order for the anions is  $[\text{H}_2\text{NCH}_2\text{CO}_2]^- > [\text{H}_2\text{NCHCO}_2\text{H}]^- > [\text{HNCH}_2\text{CO}_2\text{H}]^-$ . In contrast, the cations exhibit different behaviour, whereas  $[\text{H}_2\text{NCHCO}_2\text{H}]^+$  is predicted to be a stable structure,  $[\text{H}_2\text{NCH}_2\text{CO}_2]^+$  spontaneously fragments to the ion–molecule complex  $[\text{H}_2\text{NCH}_2^+ \cdots (\text{OCO})]$  and the singlet  $[\text{HNCH}_2\text{CO}_2\text{H}]^+$  isomer is predicted to undergo a skeletal rearrangement to form  $[\text{CH}_2\text{NHCO}_2\text{H}]^+$ . MS/MS spectra of  $[\text{M–H}]^+$  cations of various glycine isotopomers were obtained via: (i) collisional activation of electron impact generated cations and (ii) charge reversal of anions formed via  $\text{HO}^-$  negative ion chemical ionization. The resulting spectra were significantly different, suggesting different structures were involved. Neutralization–reionization experiments were performed on  $[\text{M–H}]^-$  anions in order to gain insights into the structures of the intermediate radicals. (Int J Mass Spectrom 182/183 (1999) 203–211) © 1999 Elsevier Science B.V.

**Keywords:** Glycine; Tandem mass spectrometry; Charge reversal; Neutralization-reionization; [M–H] anions, cations, neutrals; Ab initio calculations

## 1. Introduction

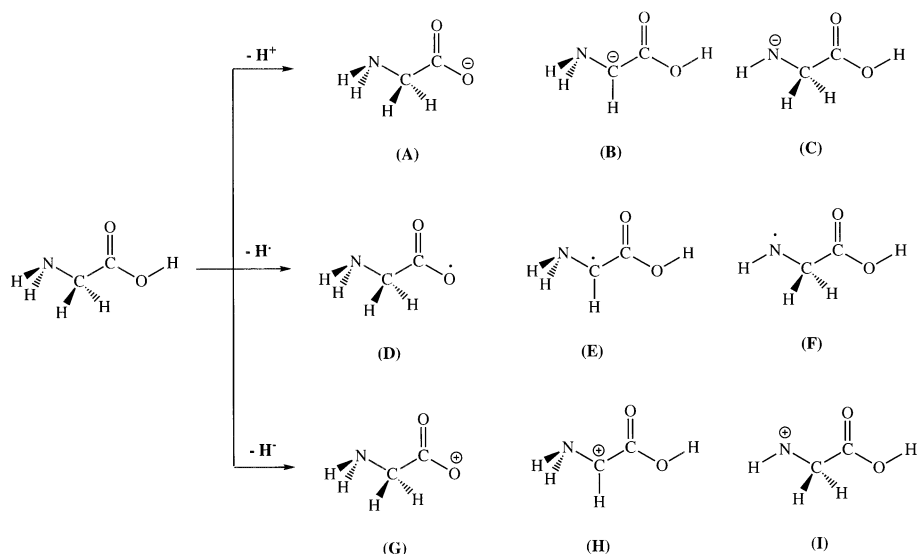
During a recent study on the gas phase reaction of  $\text{NO}^+$  with glycine, we discovered a novel mechanism involving regiospecific hydride abstraction from the methylene group to form the stabilized immonium ion  $[\text{H}_2\text{N}=\text{CHCO}_2\text{H}]^+$  [1]. This ion is also of interest because it is (i) formed via electron impact on glycine;

(ii) related structurally (i.e. in the connectivity of atoms) to: (a) the enolate anion of glycine [2] (via two electron reduction); (b) the captodative radical  $\text{H}_2\text{N}^-\text{CHCO}_2\text{H}$  (via one electron reduction) [3–8]. The latter species has attracted considerable attention as a model system for a backbone radical in protein systems [7,8].

There are three isomers for each of the glycine [M–H] species giving a total of nine possible structures shown in Scheme 1 [corresponding to loss of either a proton, hydrogen atom, or hydride ion from either nitrogen, carbon, or oxygen to yield the anions (A)–(C), radicals (D)–(F), and cations (G)–(I)]. Of the species shown in Scheme 1, the carboxylate anion (A)

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Scheme 1

is thermodynamically most stable, although the isomers due to deprotonation at carbon (B) or nitrogen (C) have been postulated to be involved in the fragmentation reactions of the  $[M-H]^-$  anion [2,5]. Ab initio calculations have been carried out by various workers to characterize several of the species [1,5–8], including (A) [5,6], (D)–(F) [6], and (I) [1]. In this article we examine the gas phase structures of  $[M-H]$  anions, radicals, and cations of glycine by using a combination of ab initio calculations [9] and tandem mass spectrometry (MS/MS) [10]. The latter work involves the use of: (i) charge reversal (CR) [11–14] of precursor anions (formed via  $HO^-$  ion chemical ionization); (ii) collision induced dissociation (CID) of cations formed via electron impact (EI) [10], and (iii) neutralization–reionization [15–18] of precursor anions (formed via  $HO^-$  ion chemical ionization).

## 2. Computational methods

The structures of the ions of interest were initially optimized at the Hartree–Fock level by using the GAUSSIAN94 [19] system of programs with the standard 6–31+G\* basis set and then reoptimized at the Möller–Plesset (MP)2(FC)/6–31+G\* level of theory (FC = frozen core) [20–22]. All fully optimized

structures were then subjected to frequency calculations with the same basis set. Energies were corrected for zero-point vibrations scaled by 0.967 [23]. In each case, a set of possible rotamers was explored [24]. Complete structural details and lists of vibrational frequencies for all MP2(FC)/6–31+G\* optimized structures are available from the authors upon request.

The charge reversal process is a vertical ionization process in which an anion is stripped of two electrons to form a cation, which is energetically excited and either undergoes relaxation or reacts further by rearrangement or fragmentation. In order to model the charge reversal experiments, single point energy calculations were carried out on the cations at the MP2(FC)/6–31+G\* level of theory by using the optimized geometries of the anions. The cations were then allowed to relax to their preferred MP2(FC)/6–31+G\* geometries by using the standard GAUSSIAN94 optimization routines.

## 3. Experimental

Experiments were performed by using a VG ZAB-2HF reverse sector tandem mass spectrometer [2]. Hydroxide ions, generated in the chemical ionization

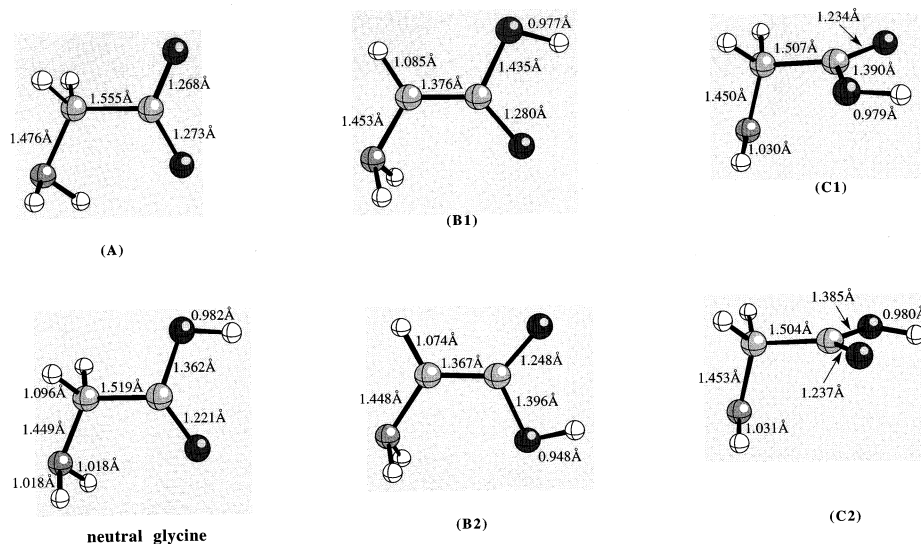


Fig. 1. MP2(FC)/6–31+G\* optimized structures of the most stable isomer of neutral glycine and various conformers of the [M–H] anions (A), (B), and (C).

(CI) source by using H<sub>2</sub>O as the CI reagent, were allowed to react with glycine which was introduced via a direct insertion probe. Typical source conditions were source temperature, 250 °C; electron energy, 70 eV (tungsten filament); emission current, 200 mA; accelerating voltage –7 kV. The pressure in the source housing was typically  $7 \times 10^{-4}$  Torr, thus giving an estimated pressure inside the source of 0.1 Torr. The [M–H]<sup>+</sup> ions were generated by electron impact on glycine at pressures around  $7 \times 10^{-5}$  Torr with source parameters analogous to the negative ion conditions, save for a positive accelerating voltage. Collisional activated mass-analysed ion kinetic energy (CA-MIKE) spectra were carried out on the [M–H]<sup>+</sup> ions by using argon as the collision gas (at an indicated pressure of  $2 \times 10^{-6}$  Torr). Both charge reversal (CR) and neutralization–reionization (NR) of the [M–H]<sup>–</sup> ions was achieved by using oxygen gas in two adjacent collision cells each with a nominal pressure of  $5 \times 10^{-6}$  Torr, for the latter experiment, a deflection voltage (300 V) was applied between the cells to generate the pure neutral beam.

All compounds were of reagent grade obtained commercially and were used without further purification. H<sub>2</sub><sup>15</sup>NCH<sub>2</sub>CO<sub>2</sub>H (99% <sup>15</sup>N) and H<sub>2</sub>NCD<sub>2</sub>CO<sub>2</sub>H

(98% D) were obtained from Cambridge Isotope Laboratories.

## 4. Results and discussion

### 4.1. Ab initio studies of the structures and stabilities of [M–H] cations and anions

Given that previous mass spectrometry experiments have suggested the existence of various glycine [M–H] cation and anion structures, we turned to ab initio calculations to gain further insights into the structures and relative stabilities of the different isomers as the parent neutral shown in Scheme 1. In each case a number of different rotamers were examined in order to find the most stable conformation. Our results are shown in Fig. 1 (anions and parent neutral), Fig. 2 (cations), Fig. 3 (plot of the first ten steps of the CR cation relaxation starting from the optimized MP2(FC)/6–31+G\* anion geometries), and Fig. 4 [geometries of the CR cation structures after ten steps along the MP2(FC)/6–31+G\* potential energy surface (PES)] and Table 1.

According to our calculations, all proposed anion

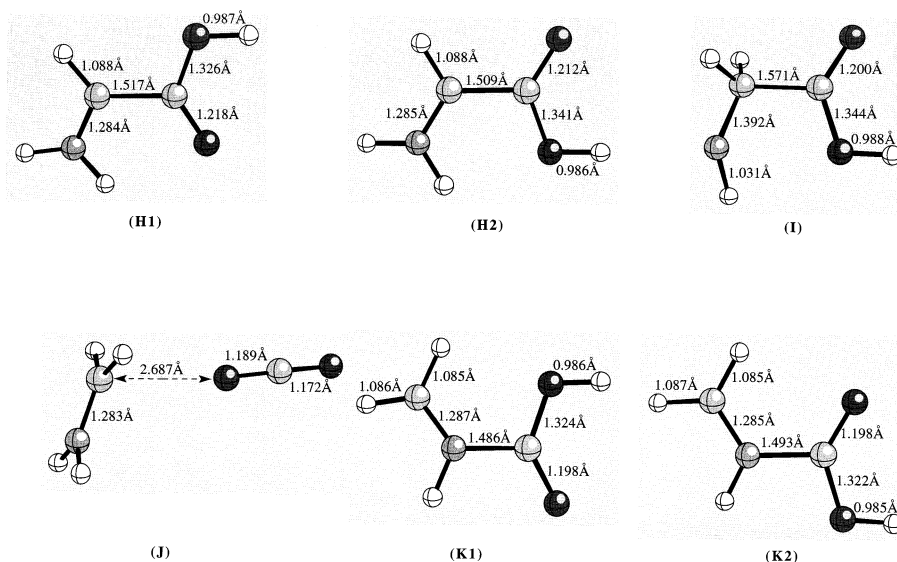


Fig. 2. MP2(FC)/6–31+G\* optimized structures of various conformers of the [M–H] cations (H), (I), (J), and (K).

structures are stable in the gas phase with the carboxylate anion (A) being the most stable and the amide anion (C) the least. Indeed, the carboxylate anion (A) is predicted to be 35.6 kcal/mol (MP2(FC)/6–

31+G\*) more stable in energy than the most stable enolate anion (B1) and 53.9 kcal/mol (MP2(FC)/6–31+G\*) more stable in energy than the most stable amide anion (C2). When these energies are combined with the energy of the most stable conformer of neutral glycine, they allow for the ab initio estimation of the gas phase acidity ( $\Delta H_{\text{acid}}^{\circ}$ ) (MP2(FC)/6–31+G\*) of glycine [via Eq. (1)] at the various sites:  $\Delta H_{\text{acid}}^{\circ}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 348.7$  kcal mol<sup>-1</sup>;  $\Delta H_{\text{acid}}^{\circ}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 384.2$  kcal mol<sup>-1</sup>;  $\Delta H_{\text{acid}}^{\circ}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 402.6$  kcal mol<sup>-1</sup>. An experimental comparison can only be made with the gas phase acidity for deprotonation at oxygen ( $\Delta H_{\text{acid}}^{\circ}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 342.0$  kcal mol<sup>-1</sup> [25,26]), which suggests theoretical predictions of the gas phase acidity of glycine at the MP2(FC) level of theory somewhat underestimates the experimentally derived value (note that ab initio calculations at the computationally more expensive G2 level of theory give a better agreement [6]). The ab initio estimates of the gas phase acidities for deprotonation at the other sites are in line with those that one might predict from a consideration of the experimentally known gas phase acidities of acetic acid (the differences in

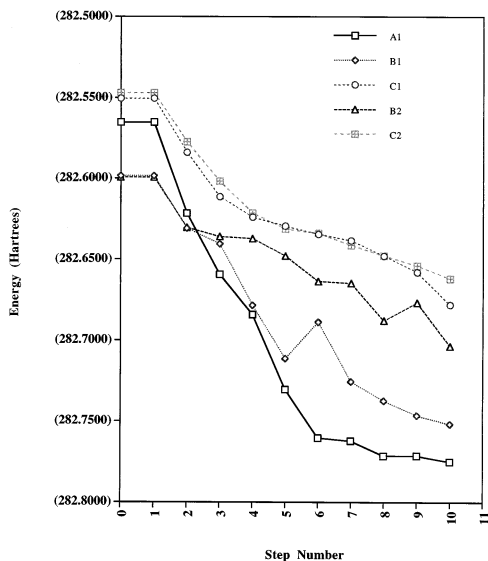


Fig. 3. Plot of the MP2(FC)/6–31+G\* energies for the initial energy and first ten steps for the “charge reversed” cation optimizations starting from the geometries of the most stable anion conformations (A1), (B1), (B2), (C1), and (C2).

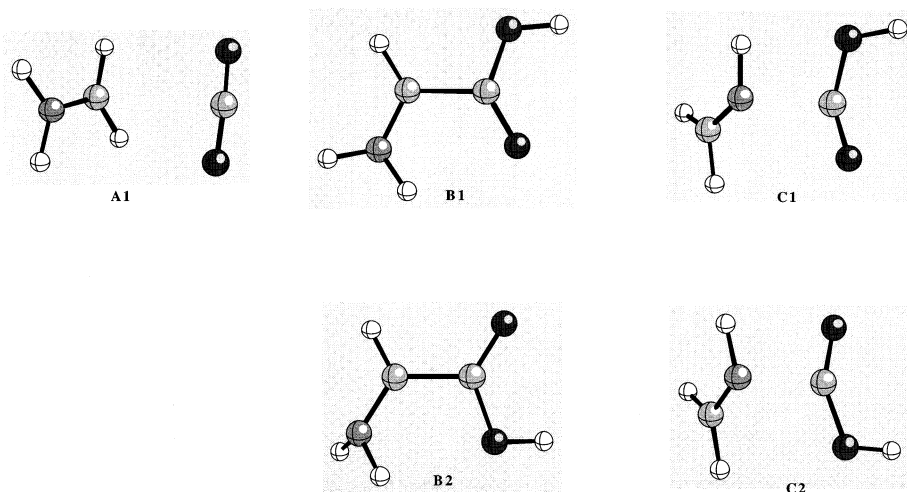


Fig. 4. MP2(FC)/6–31 + G\* structures after ten steps for the “charge reversed” cation optimizations starting from the geometries of the anion conformations (A1), (B1), (B2), (C1), and (C2).

energy between deprotonation at carbon versus oxygen is about  $22.0 \text{ kcal mol}^{-1}$  [27]) and methylamine (for deprotonation at nitrogen:  $\Delta H_{\text{acid}}^{\circ}(\text{CH}_3\text{NH}_2) = 403.0 \text{ kcal mol}^{-1}$  [26]).

Table 1  
Ab initio total energies and relative energies of the isomeric [M + H] cations and anions of glycine

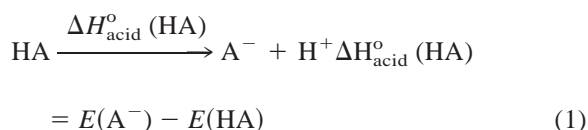
Species	Energies (Hartrees) MP2(FC)/6–31+G* + ZPVE <sup>a</sup>	Relative energies ( $\text{kcal mol}^{-1}$ ) MP2(FC) <sup>b</sup>
Neutral glycine	–283.701 44	...
Anions		
(A1)	–283.145 78	0.0
(B1)	–283.089 11	35.6
(B2)	–283.086 82	37.0
(C1)	–283.056 94	55.8
(C2)	–283.059 89	53.9
Cations		
(G) <sup>c</sup>	...	...
(H1)	–282.817 66	21.5
(H2)	–282.819 13	20.6
(I) <sup>d</sup>	–282.665 54	117.0
(J)	–282.851 98	0.0
(K1)	–282.816 74	22.1
(K2)	–282.819 13	20.6

<sup>a</sup>ZPVE is corrected by 0.967.

<sup>b</sup>At the MP2(FC)/6–31+G\* + 0.967 ZPVE level of theory.

<sup>c</sup>Isomer (G) is unstable and rearranges to the ion molecule complex (J).

<sup>d</sup>In the triple state.



The ab initio calculations reveal that not all of the [M–H] cations are stable. Two conformers of the immonium ion,  $[\text{H}_2\text{NCHCO}_2\text{H}]^{+}$  (H1) and (H2) were found to be stable species, which is consistent with our previous calculations (at a lower level of theory) [1]. Surprisingly, neither of the immonium ion conformers, (H1) and (H2), are the most stable species of those calculated. We predict the carboxylate cation  $[\text{H}_2\text{NCH}_2\text{CO}_2]^{+}$  (G) to spontaneously fragment to form a highly stable ion–molecule complex between an immonium ion  $\text{CH}_2=\text{NH}_2^{+}$  and  $\text{CO}_2$  (J). This ion–molecule complex is predicted to be  $20.6 \text{ kcal/mol}$  (MP2(FC)/6–31+G\*) more stable in energy than the most stable immonium cation (H2). The nitrogen cation  $[\text{HNCH}_2\text{CO}_2\text{H}]^{+}$ , (I), exhibits interesting behaviour: in the singlet state, one conformation rearranges without barrier (presumably via a 1,2 hydride transfer) to the immonium ion,  $[\text{H}_2\text{NCHCO}_2\text{H}]^{+}$  (H). Another conformation rearranges smoothly (via an ion–molecule complex between  $\text{HOCO}^{+}$  and  $\text{HN}=\text{CH}_2$ ) to form a new ion with a different connectivity:  $[\text{CH}_2\text{N}(\text{H})\text{CO}_2\text{H}]^{+}$  (K). Two stable conformers

of structure (K) were located. Interestingly, both of these conformers are predicted to have similar stabilities to the immonium cations, (H), with the most stable conformer, (K2), 20.6 kcal/mol (MP2(FC)/6–31+G\*) less stable than the ion–molecule complex (J). In contrast, structure (I) is predicted to be a stable, high energy structure in the triplet state at the MP2 level of theory. The ion–molecule complex (J) is predicted to be 116.9 kcal/mol (MP2(FC)/6–31+G\*) more stable in energy than the triplet nitrogen cation  $[\text{HNCH}_2\text{CO}_2\text{H}]^+$ , (I). These results suggest that if (G) and (I) were formed experimentally in the singlet state, they would spontaneously rearrange.

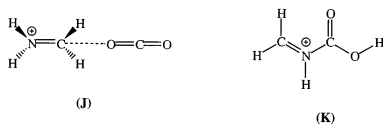


Diagram 1

It is interesting to compare the relative stabilities of the various structural isomers of the  $[\text{M}-\text{H}]$  anions and cations with those of the corresponding neutral radicals. All three of the isomeric  $[\text{M}-\text{H}]$  radicals available by X–H bond homolysis (where X = C, N, and O) in glycine have been previously predicted to be stable in the gas phase, following the stability order:  $\text{H}_2\text{NCHCO}_2\text{H} > \text{HNCH}_2\text{CO}_2\text{H} > \text{H}_2\text{NCH}_2\text{CO}_2$  (G2(MP2)) [6]. These results can be quantified by calculating the following bond dissociation energies:  $\text{BDE}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 79.1 \text{ kcal mol}^{-1}$ ;  $\text{BDE}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 102.5 \text{ kcal mol}^{-1}$ ;  $\text{BDE}(\text{H}_2\text{NCH}_2\text{CO}_2\text{H}) = 112.8 \text{ kcal mol}^{-1}$ .

Finally, for the charge reversal experiment, it is useful to know the energy content of the cation with the same geometry as the anion, and, starting from that geometry, to predict what geometry the cation will relax to (Figs. 3 and 4). Thus, we have used ab initio calculations to gain insights into: (i) the energetics of the initial cation geometry formed by the CR experiment (i.e. all fully optimised anion structures were taken and used to calculate single point cation energies); (ii) what structures the initial cation geometries would relax to. Our results are shown in Fig. 3 (a plot of energy versus step number) and Fig. 4 (the partially optimised geometries of each cation structure after ten steps along the  $[\text{M}-\text{H}]^+$  MP2(FC)/6–31+G\*

potential energy surface). Each of the structures depicted in Fig. 4 were then allowed to fully optimise on the MP2(FC)/6–31+G\* cation potential energy surface (via the standard GAUSSIAN94 optimization procedure).

Our calculations reveal that the initial “charge reversed” structures (B1) and (B2) are more stable than structures (A1) or (C1) and (C2) on the MP2(FC)/6–31+G\* cation potential energy surface (Fig. 3). Each of the structures then undergoes quite different behaviour when allowed to relax (Fig. 4). After ten steps both (C1) and (C2) begin to dissociate to an ion molecule complex (between  $\text{HOCO}^+$  and  $\text{HN}=\text{CH}_2$ , see Fig. 4) and these systems ultimately optimize to form covalent structures with different connectivities (K2) and (K1), respectively. (A1) also begins to dissociate to form the ion–molecule complex between  $\text{CH}_2=\text{NH}_2^+$  and  $\text{CO}_2$  (Fig. 4), ultimately forming the stable ion–molecule complex, (J). In contrast, (B1) and (B2) are the only species to relax without dissociation or rearrangement (see Fig. 4) to form the immonium cations (H1) and (H2), respectively.

#### 4.2. MS/MS studies on the structures of $[\text{M}-\text{H}]$ cations

The MS/MS reactions of various ions related to glycine have been examined previously. In particular, Bowie et al. have examined the high energy collisional activation of glycine  $[\text{M}-\text{H}]^-$  ions [2], whereas several groups have examined the fragmentation reactions of the  $[\text{M} + \text{H}]^+$  ions [28,29]. The fragmentation reactions of glycine  $[\text{M}-\text{H}]^+$  ions have, however, not been reported previously. Such reactions could be examined via two different types of experiments: (i) collisional activation (CA) of  $[\text{M}-\text{H}]^+$  ions [10]; or (ii) charge reversal (CR) of  $[\text{M}-\text{H}]^-$  ions [11–14].

The CR MS/MS spectra of  $[\text{M}-\text{H}]^-$  ions yield product ions that are significantly different from the CA MS/MS spectra of  $[\text{M}-\text{H}]^+$  ions (Table 2), suggesting that different structures are probed by the different methods. Note that based upon thermochemical considerations (with respect to proton trans-



Table 2  
MS/MS spectra of [M–H] ions of glycine and its isotopomers

Ionic species, $m/z$ , type of MS/MS experiment <sup>a</sup>	Daughter ions
[M–H] <sup>−</sup> of H <sub>2</sub> NCH <sub>2</sub> CO <sub>2</sub> H, 74, CR	74 <sup>b</sup> ; 57 (2); 44 (91); 42 (13); 30 (50); 28 (100); 17 (1); 16 (2); 15 (1); 14 (2); 13 (1); 12 <sup>b</sup> .
[M–H] <sup>−</sup> of H <sub>2</sub> <sup>5</sup> NCH <sub>2</sub> CO <sub>2</sub> H, 75, CR	75 <sup>b</sup> ; 58 (1); 44 (88); 42 (18); 31 (61); 29 (100).
[M–D] <sup>−</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 75, CR	75 <sup>b</sup> ; 58 (7); 44 (88); 42 (13); 31 (65); 28 (100).
[M–H] <sup>−</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 76, CR	44 (100); 42 (10); 32 (68); 29 (79); 16 (4); 14 (2); 12 (1).
[M–H] <sup>+</sup> of H <sub>2</sub> NCH <sub>2</sub> CO <sub>2</sub> H, 74, CA	72 (6); 58 (6); 57 (12); 56 (20); 46 (100); 45 (9); 30 (59); 28 (36).
[M–H] <sup>+</sup> of H <sub>2</sub> <sup>5</sup> NCH <sub>2</sub> CO <sub>2</sub> H, 75, CA	73 (34); 58 (7); 57 (6); 47 (100); 45 (6); 30 (5); 29 (6); 28 (4).
[M–D] <sup>+</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 75, CA	73 (95); 58 (47); 57 (12); 47 (100); 45 (38); 30 (73); 29 (16); 28 (16).
[M–H] <sup>+</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 76, CA	74 (67); 59 (83); 48 (22); 45 (58); 31 (100); 30 (13); 29 (14); 28 (11).
[M–H] <sup>−</sup> of H <sub>2</sub> NCH <sub>2</sub> CO <sub>2</sub> H, 74, NR	74 (16); 57 (26); 44 (94); 30 (58); 29 (73); 28 (100).
[M–H] <sup>−</sup> of H <sub>2</sub> <sup>5</sup> NCH <sub>2</sub> CO <sub>2</sub> H, 75, NR	75 (47); 58 (3); 44 (96); 32 (76); 31 (90); 29 (83); 28 (100).
[M–D] <sup>−</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 75, NR	75 (32); 57 (6); 44 (74); 31 (77); 28 (100).
[M–H] <sup>−</sup> of H <sub>2</sub> NCD <sub>2</sub> CO <sub>2</sub> H, 76, NR	44 (94); 32 (76); 31 (85); 28 (100).

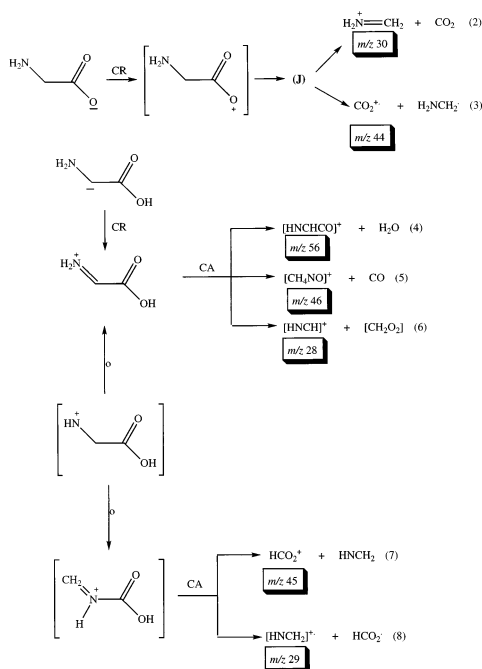
<sup>a</sup>CA refers to collisional activation; CR refers to charge reversal; NR refers to neutralization–reionization.

<sup>b</sup>Designates an ion with an intensity of less than 1.

fer to the HO<sup>−</sup> CI reagent ion that has  $\Delta H_{\text{acid}}^{\circ}$  (H<sub>2</sub>O) = 390.8 kcal mol<sup>−1</sup>), the likely anionic precursors in the CR experiments are the carboxylate anion, (A), to a larger extent and the enolate anion, (B), to a lesser extent. The amide anion precursors, (C), would not be expected to be formed as the required proton transfer would be significantly endothermic. The major fragment ions in the CR MS/MS spectra of [M–H]<sup>−</sup> ions derived from glycine are  $m/z$  28,  $m/z$  30, and  $m/z$  44, whereas some of the minor ions observed include  $m/z$  74 (CR parent ion) and 57. The isotopically labeled glycine MS/MS spectra are useful because they not only confirm these assignments, they also allow us to gain insights into the anionic precursors. In particular, when D2 glycine (H<sub>2</sub>NCD<sub>2</sub>CO<sub>2</sub>H) is used, both an [M–H]<sup>−</sup> and an [M–D]<sup>−</sup> ion are observed, which are likely to correspond to (A) and (B), respectively. As these are the precursors to different cations [the carboxylate anion (A) is formally the precursor to (H) although our ab initio results in Sec. 4.1 suggest that this spontaneously rearranges to the ion–molecule complex (J); (B) is the precursor to (H)], they should exhibit differences in their CR MS/MS spectra. This is indeed the case (Table 2). Thus, no parent charge reversed signal is observed for the [M–H]<sup>−</sup> ion, whereas one is observed for a [M–D]<sup>−</sup> ion. Furthermore, two major fragment ions in the [M–H]<sup>−</sup>CR MS/MS spectrum

are the ions at  $m/z$  44 and  $m/z$  30, which can be readily rationalized as being the dissociation products of (J) [Eqs. (2) and (3) in Scheme 2]. These experimental results are consistent with the theoretical calculations on the structures and stabilities of [M–H]<sup>+</sup> ions formed via charge reversal of the precursor anions as described in Sec. 4.1.

The major fragment ions in the CA MS/MS spectra of [M–H]<sup>+</sup> ions derived from electron impact (EI) on glycine are  $m/z$  28,  $m/z$  46, and  $m/z$  56, which correspond to losses of [H<sub>2</sub>O + CO], CO, and H<sub>2</sub>O. Once again, the isotopically labeled glycine CA MS/MS spectra confirm these assignments. These losses, together with the fact that the product ion due to loss of CO exhibits a dish shaped peak, suggest similarities to the CA MS/MS fragmentation behaviour of protonated glycine, which has been comprehensively discussed [28]. By using the analogy of the gas phase behaviour of the [M + H]<sup>+</sup> ion of glycine [28,29], we have assigned these losses as characteristic of the structure [H<sub>2</sub>NCHCO<sub>2</sub>H]<sup>+</sup> [Eqs. (4)–(6)] for the [M–H]<sup>+</sup> ion of glycine. Interestingly, the CA MS/MS spectra of the [M–D]<sup>+</sup> and [M–H]<sup>+</sup> cations of D2 glycine are significantly different, suggesting the involvement of different ion structures. The [M–D]<sup>+</sup> cation is likely to have a structure of (H), whereas potential candidates for the [M–H]<sup>+</sup> cations



include (J), (K), and (H), which could yield the fragment ions shown in Eqs. (2)–(8) in Scheme 2.

#### 4.3. MS/MS studies on the structures of $[M-H]$ neutrals

The neutralization-reionization MS/MS experiments were performed on  $[M-H]^-$  anions to gain some insights into the related radical structures. Recovery signals were observed for each species except for the  $[M-H]^-$  ion of  $H_2NCD_2CO_2H$ . If we compare the various MS/MS spectra with each other (Table 2) we note that the neutralization–reionization mass spectrometry (NRMS) spectra have more in common with the CR MS/MS spectra than the CA MS/MS spectra of the  $[M-H]^+$  ions formed via EI. This should not be surprising given that both types of experiments involve stripping electrons from anions that are formed from the same reagent ion ( $HO^-$ ). The main difference (apart from the energy transfer differences) is that in the NRMS experiments [15–18] the anion is stripped of its two electrons in a stepwise

process (i.e. one electron is removed to form the radical, which is subsequently stripped of another electron to form the cation), whereas in the CR process both electrons can be removed at the same time, as well as in a stepwise process [11–14].

Recalling our previous discussion on the rearrangement of the cation structures starting from the anion precursors [i.e. (C1) rearranges to (K2) and (A1) dissociates to (J)], it becomes apparent that similar rearrangements may be occurring in the NRMS experiments. Unfortunately, we cannot tell whether these rearrangements occur prior to forming the cations (i.e. it is the radical species which are undergoing rearrangement) or after the cations are formed [30]. We prefer the latter scenario given that previous ab initio calculations have shown that (D), (E), and (F) are all stable [6].

## 5. Conclusions

MS/MS experiments of various isotopomers in conjunction with ab initio calculations have been used to provide insights into the structures and rearrangement reactions of  $[M-H]^+$  ions derived from glycine. Ion-molecule reactions offer an alternative to these methods of structural characterization. For example, the anions (A)–(C) and the cations (H)–(K) are expected to exhibit quite different reactivity (e.g. in gas phase H/D exchange reactions). We have recently modified a commercial ion trap (LCQ) to allow the examination of gas phase ion-molecule reactions [31] of ions formed via electrospray ionization, and will report our results on the reactivity of ions derived from biomolecules in due course.

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