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Probing the fragmentation reactions of protonated glycine oligomers via multistage mass spectrometry and gas phase ion molecule hydrogen/deuterium exchange†

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Abstract

An ion trap mass spectrometer equipped with electrospray ionization has been modified to study the structure of protonated polyglycyl peptides G_n (where n = 2–5 glycine residues) and their product ions formed by collision induced dissociation tandem mass spectrometry (CID MS/MS) via the novel application of gas phase ion–molecule hydrogen/deuterium (H/D) exchange reactions. In particular, the structures of the b_2, b_3, b_4, and b_5 ions formed via CID MS/MS from various protonated glycine oligomer precursors have been examined. The b_2 ions, formed from the protonated G_2 and G_3 precursor ions, the b_3 ion from the protonated G_3 precursor, and the b_4 ion from the protonated G_5 ion all undergo CID and gas phase H/D exchange consistent with formation of protonated oxazolone structures previously proposed for b_n-type ions. However, CID MS/MS, MS^3, and H/D exchange of the putative b_4 and b_5 arising from the protonated G_4 and G_5 precursor ions, respectively, as well as experiments with various methylated derivatives of G_4, suggest that the major portion of these ions are *not* b_n ions, but are instead formed via backbone–backbone neighboring group participation reactions remote to the C-terminal amino acid. Efforts to elucidate the mechanisms behind this loss of H_2O are described. (Int J Mass Spectrom 190/191 (1999) 209–230) © 1999 Elsevier Science B.V.

1. Introduction

1.1. Mass spectrometry as a tool for the structural analysis of biomolecules

Over the past decade mass spectrometry has emerged as an essential tool for the analysis of biologically important molecules. This renaissance in biological mass spectrometry (documented in numerous reviews and books) [1] has largely been fueled by the development of "new" soft ionization methods such as electrospray ionization (ESI) [2] and matrix assisted laser desorption ionization (MALDI) [3]. Primarily, the use of biological mass spectrometry has focused on two areas: (1) to "weigh" the mass of the biomolecule (i.e. to determine its molecular weight); and (2) to induce fragmentation of the molecular ion via tandem mass spectrometric (MS/MS) techniques [4] [e.g. collision induced dissociation (CID)],

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thereby providing primary sequence information. Indeed, the identification of proteins by interpretation of the CID product ion spectra of peptides generated by specific proteolytic enzymes is rapidly becoming routine, particularly when coupled with powerful database search algorithms [5]. With these successes, the mass spectrometry community has now turned its attention to the application of MS for the characterization of (1) noncovalent complexes, and (2) higher order structure such as the secondary, tertiary, or quaternary structures of proteins.

Additionally, several groups have focused their efforts on developing structurally diagnostic tools that are complementary to CID methods. These include (1) the measurement of gas phase thermochemical properties such as gas phase basicities and proton affinities [6], (2) alternate activation methods in tandem mass spectrometry, including surface induced dissociation (SID) [7], photofragmentation [8], electron induced dissociation [9], blackbody infrared dissociation (BIRD) [10], as well as probes of the structures of neutrals such as neutralization–reionization (NR) and neutral fragment reionization (NFR) mass spectrometry [11], (3) bimolecular reactions (which can be further classified into (a) ion–molecule reactions [12] and (b) ion–ion reactions [13]), (4) physical probes of the gas phase conformational shape of ions (examples include ion mobility [14] and surface impact mass spectrometry [15] that measures the defects of a surface bombarded by an ion) and (5) theoretical molecular modeling methods [16] (including semi-empirical, ab initio, or molecular dynamics techniques).

Ion–molecule reactions [12] are proving to be both popular and elegant probes of the structure of biomolecules since they (1) offer such a huge scope (the combination of different types of ions and neutrals is limitless), (2) may be more useful probes of higher order structure because they involve inherently less energetic reactions than CID (which neccesitates "heating up" the ion to induce fragmentation), and (3) are applicable to a wide range of existing mass spectrometers with little or no modification, thereby obviating the need to invest in new instrumentation. Additionally, many of these novel gas phase ion–molecule reactions are complementary to the more established MS/MS based methods and thus can be used in conjunction with CID in instruments such as ion traps to provide supplementary information. To date, the most extensively used gas phase ion–molecule reactions of biomolecules have been gas phase hydrogen-deuterium (H/D) exchange [6,17] and acid–base reactions involving proton transfer [6].

1.2. Role of the ion trap in biomolecular structure analysis

Critical to the development of techniques to probe the gas phase structure of biomolecules have been advances in the development of mass analyzers that are not only more versatile, but offer ever increasing levels of sensitivity (i.e. decreasing detection limits). Arguably, the most impressive recent advances have been those associated with time of flight [18], Fourier transform ion cyclotron (FTICR) [19] and ion trap [20] mass analyzers. The ion trap, largely because of its unique trapping capabilities that allow multistages of mass spectrometry (MS^n) to be performed on mass selected ions, has proved to be a particularly useful instrument. Since the successful coupling of ESI to ion traps [21], these analyzers have been used to study the structure of biomolecules through (1) the measurement of thermochemical quantities such as proton affinities [22], (2) MS/MS methods such as CID [23,24c], (3) gas phase ion–molecule reactions [24], (4) gas phase ion–ion reactions (i.e. reactions of multiply charged anions or cations with ions of opposite charge [13], and (5) the development of hyphenated systems such as ion trap–ion mobility mass spectrometry [25].

Of particular relevance to the work presented here is the ability to combine a number of different events in an ion trap. Because ion trap events (i.e. MS^n experiments involving ion isolation, activation, and detection) are tandem-in-time and not tandem-in-space, this instrumentation is particularly suited to performing gas phase ion–molecule chemistry. Thus, it is possible to carry out ion–molecule or ion–ion reactions on mass selected ions either before or after CID reactions [26]. For example, the reaction of

multiply charged oligonucleotide anions with trimethylsilylchloride results in the formation of trimethylsilylated anions, whose structure can then be interrogated via CID [24d]. Additionally, ion–molecule proton transfer reactions [24c] and ion–ion reactions [27] on CID product ions derived from multiply charged cations have been used to reduce charge states and thereby simplify the product ion spectra.

1.3. Previous MS studies on the gas phase ion chemistry of protonated glycine oligomers

Aspects of the gas phase ion chemistry of protonated glycine oligomers previously studied by others include (1) determination of proton affinities [28] and gas phase basicities (GB) [29] (which show an increase in GB as the size of the oligomer increases), (2) examination of the H/D exchange reactions of their $[M + H]^+$ ions [30], (3) a study of the reaction between the $[M + H]^+$ ion of diglycine and acetonylacetone [31], (4) ion–mobility mass spectrometry [32], (5) molecular modeling [29b,c,3,32], and (6) CID [33]. Of most relevance to the work described here are the previous studies on the gas phase H/D exchange [30] and CID reactions of protonated glycine oligomers [33].

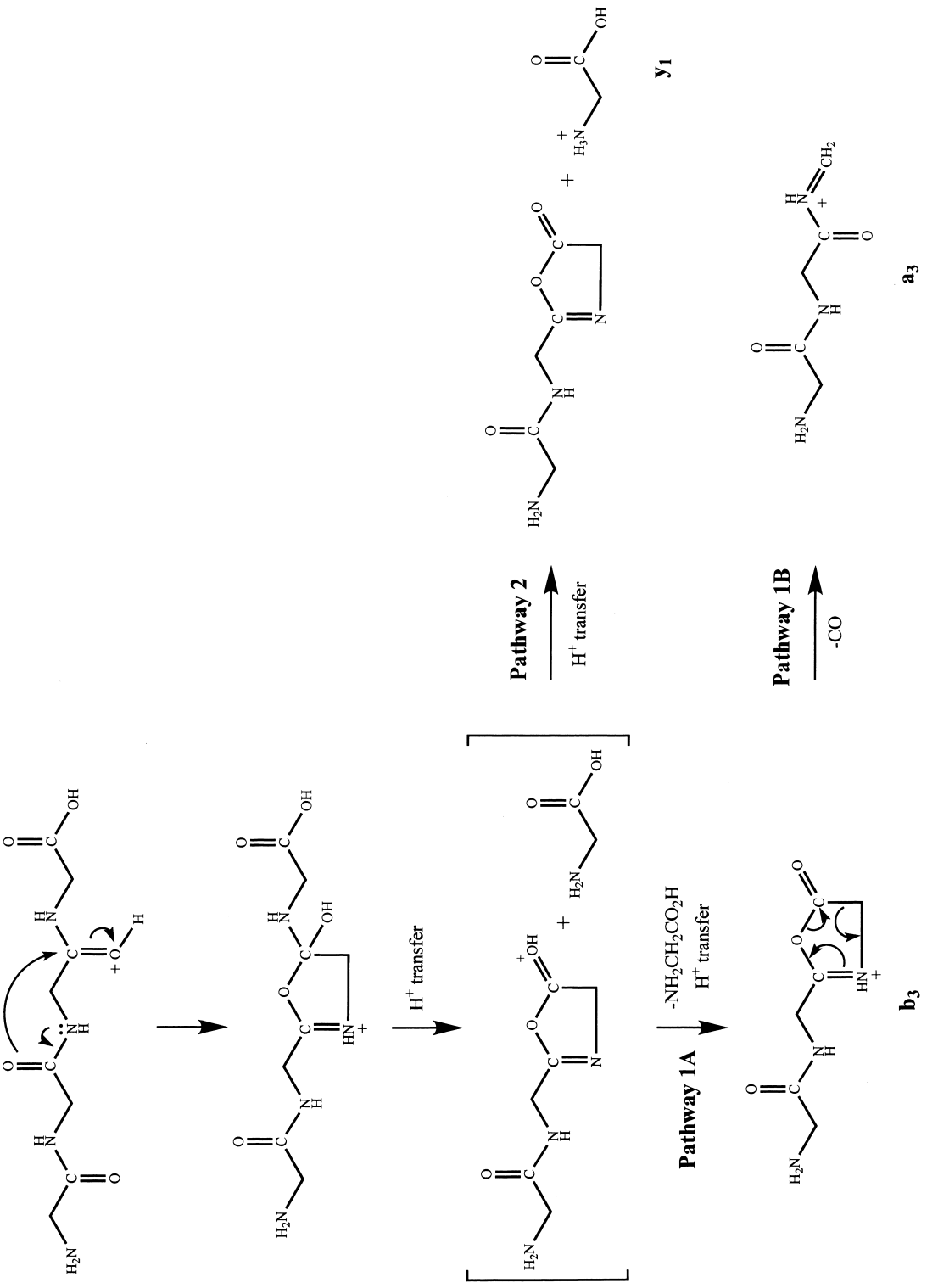
Previously, gas phase H/D exchange experiments using FTICR MS have shown that in addition to differences in the GB [34], (i.e. as the difference in GB between the protonated peptide and D_2O increases, the rate and extent of H/D exchange decreases), the formation of multiple hydrogen bonds between the $[M + H]^+$ ion and D_2O, particularly for G_2 and G_3, is an important factor that can effect the extent of H/D exchange. The importance of hydrogen bonding on H/D exchange is exemplified by previous studies that reveal poor H/D exchange yields for protonated glycine, which is unable to form the multiple hydrogen bonds described [30].

The CID reactions of protonated glycine oligomers have also been studied by a number of workers using different types of mass analyzers [33]. Of most relevance are the studies by the groups of Bursey [33a,b,c,e] and Harrison [35c,d] using low energy CID in hybrid mass spectrometers and the measurement of activation energies for dissociation by Kebarle [33f] using energy resolved CID in a triple quadrupole.

1.4. Probing the mechanisms of CID reactions of $[M + H]^+$ peptide ions

The structures of peptides can be probed by studying the CID reactions of their $[M + nH]^{n+}$ [36] or $[M - H]^-$ ions [37]. Generally, the major "sequence" ions formed upon CID of a protonated peptide ion are the N-terminally truncated y_n type and complementary C-terminally truncated b_n type ions [36]. Mechanisms for the formation of these structurally relevant "sequence" ions (i.e. b- and y-type ions) [36] from singly or multiply protonated peptides have been proposed [35,38], including the structures of the neutral products [39], using the concept of the "mobile" proton [38]. Harrison et al. [35c] have shown that the protonated N-benzoyl-glycyl-glycine peptide ion fragments via loss of the C-terminal glycine residue to form a 2-phenyl-5-oxazolone product ion. They proposed that the mechanism for the formation of this product ion, by nucleophilic attack at the protonated carbonyl of the second amide bond by the preceding carbonyl followed by proton transfer and bond cleavage, could be a general one that operates for other protonated peptide ions. This general mechanism is illustrated for the formation of the b_3 product ion from a protonated tetraglycine peptide (Scheme 1, Pathway 1A). Others have also proposed similar mechanisms [35e]. According to the mechanism shown in Scheme 1, a truncated peptide is formed as the complementary neutral to this protonated oxazolone. However, if proton transfer occurs within the ion–molecule pair, a neutral oxazolone and an N-terminally truncated molecular ion corresponding to a y_{n-3} ion would be formed (Scheme 1, Pathway 2). B_n-type ions can fragment further by loss of CO to produce a_n-type immonium ions (Scheme 1, Pathway 1B) or by direct fragmentation to a_{n-1} type, or smaller b-type ions [35c,d,f,h]. A-type ions may also be formed directly by fragmentation of the intact peptide ion [35f].

Four different structures have been considered for


Pathway 2
H+ transfer
y1
Pathway 1B
-CO
a3
H+ transfer
-NH2CH2CO2H
H+ transfer
Pathway 1A
b3

Scheme 1.

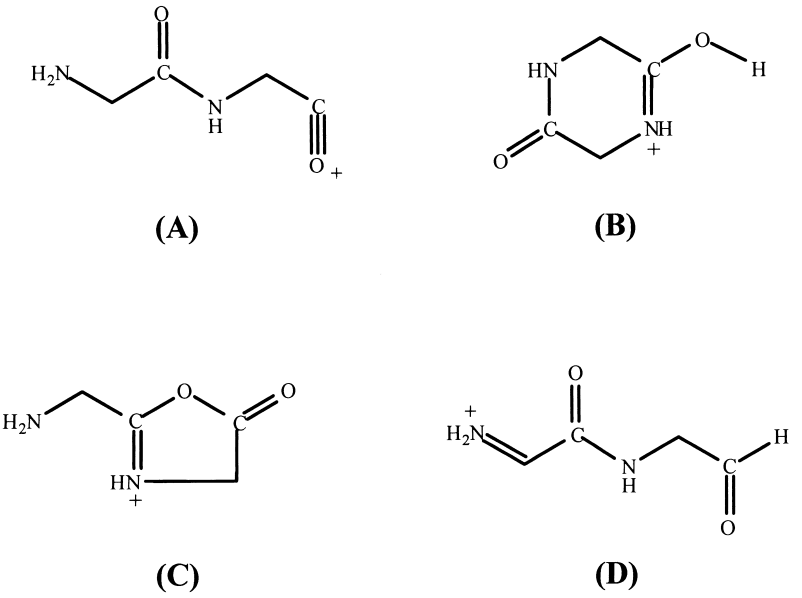
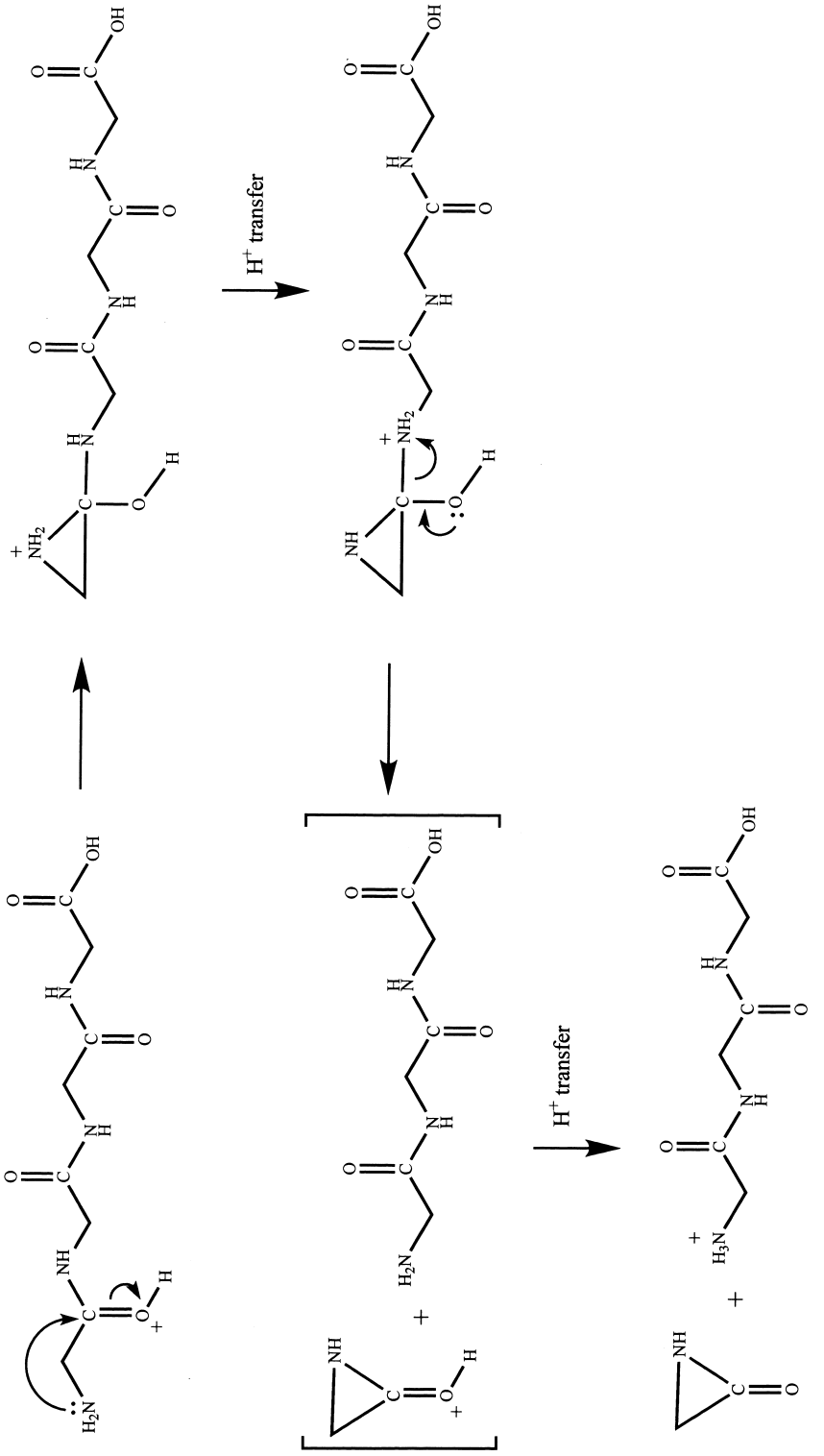


Diagram 1.

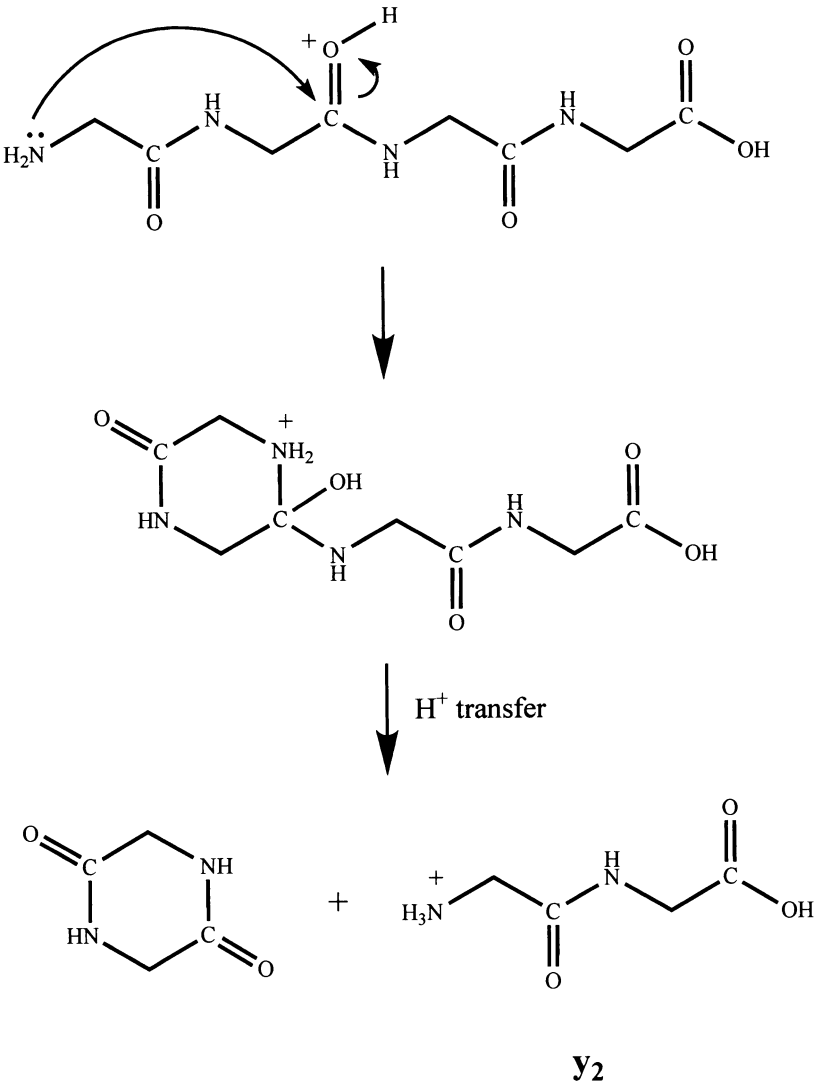
the b_2 ions of simple peptides [structures (**A**)–(**D**) are shown for the b_2 ion of G_2 in Diagram 1] [33]. One of the challenges to unequivocally assigning any one of these structures to the b_2 ion is the independent "synthesis" of each of these structures in order to compare the MS/MS spectra to that of the b_2 ion formed in the fragmentation of a peptide $[M + H]^+$ ion. Many have ruled out the open chain acylium ion (**A**) as the b_2 product ion structure, because b_1 ions are not stable for simple aliphatic amino acids [33i]. The diketopiperazine structure (**B**) has been ruled out on the basis that it exhibits a different CID MS/MS product ion spectrum to that of an equivalent b_2 ion [33g,h,39a]. Until recently, this had left the oxazolone (**C**) as the default structure. However, based solely upon ab initio calculations, Eckart et al. have suggested an immonium ion (**D**) as yet another possible structure [33h].

Alternate mechanisms have also been proposed for the formation of y_{n-1} and y_{n-2} product ions (Schemes 2 and 3, respectively). Both product ions may potentially be formed via nucleophilic attack at either the protonated first or second carbonyl by the N-terminal amino group, followed by elimination of a neutral aziridinone [35a,b] (Scheme 2) or diketopiperazine (Scheme 3), respectively. Evidence for the diketopiperazine neutral loss upon formation of the y_{n-2} ion has been provided using NFR mass spectrometry [39].

The losses of small neutrals such as H_2O, NH_3, and CO from the protonated precursor ion have also been the subject of some recent interest. The product ions corresponding to these "nonsequence" losses do not lead to structurally relevant information regarding the primary sequence and have therefore generally been ignored. However, further interrogation of these product ions might provide useful additional structural information. Recent results suggest that competing neighboring group participation reactions [40] involving side chain–backbone interactions [41], side chain–side chain interactions [42], as well as the effect of secondary and tertiary structures [43] may have a significant effect on the formation of "sequence" versus "nonsequence" ions. Thus, in light of our previous results with cysteine containing peptides, (which suggested that the loss of H_2O may be in competition with the formation of sequence ions via neighboring group side chain–backbone participation reactions [41b,c]), we were interested in determining whether the "nonsequence" loss of H_2O could compete with "sequence" ion formation in the absence of any side chain interactions. Simple polyglycyl pep-



Scheme 2.



Scheme 3.

tides are particularly suited to such studies in that complications arising from side chain reactivities are removed and thus only the key backbone chemistry is probed. Potentially, bimolecular ion–molecule reactions could be employed to study the structures of the product ions formed via CID and could provide valuable additional mechanistic insights into CID fragmentation processes. Therefore, in this article we have employed gas phase H/D exchange ion–molecule reactions in a modified ion trap in combination with collision induced dissociation to study the structure of protonated glycine containing peptides and their product ions.

2. Experimental

2.1. Materials

The glycine oligomers, G_n (n = 1–5), were purchased from Sigma (St. Louis, MI) and used without

further purification. N,N dimethylglycine was obtained from Aldrich (Castle Hill, NSW, Australia). t-butyloxycarbonyl (Boc)-N-methyl-glycine (Boc-Sarcosine) and Boc-Alanine were purchased from Auspep (Melbourne, Victoria, Australia). Various methylated peptide derivatives were synthesized using automated rapid solid phase peptide synthesis as previously described [44]. O-methyl ester derivatives were formed via standard procedures [41b]. Deuterium oxide (D_2O, 99.9%) was obtained from Cambridge Isotope Laboratories (Woburn, MA). CH_3OH was purchased from Ajax Chemical (Auburn, NSW, Australia). Glacial acetic acid (Analar grade) was obtained from BDH Laboratories (Poole, England).

2.2. Ion trap methods

All experiments were performed using a commercially available quadrupole ion trap mass spectrometer (Finnigan-MAT model LCQ, San Jose, CA) equipped with ESI. The ion trap was modified by the addition of two 3-way valves in the helium background gas inlet line, forming a split flow system, to allow the introduction of neutral reagents [26,45]. Under normal operating conditions, the gas flow was plumbed directly to the fused silica restriction capillary within the instrument where an external regulator maintained at 3 lb/in^2 allowed a flow of approximately 1 mL/min of helium to enter the trap in accordance with the manufacturers specifications. Samples (0.1 mg/mL), dissolved in 1:1 CH_3OH/H_2O containing 0.1% acetic acid, were introduced to the mass spectrometer at 2.5 μL/min via the ESI source. The ESI conditions used, particularly the auxiliary gas pressure, dictates the amount of H_2O present in the trap that is able to participate in isotopic back exchange reactions during subsequent H/D exchange experiments. Thus, to minimize the level of H_2O present, the ESI conditions were optimized as follows: spray voltage, 4.5–5.5 kV, capillary temperature, 200 °C, nitrogen sheath pressure and auxiliary gas flow rates, 30 psi and 20 (arbitrary units), respectively, capillary voltage, 10 V, tube lens offset voltage, −25 V. No attempt was made to dry either the sheath or auxiliary nitrogen gases (obtained from a boiling liquid nitrogen source). Ions were accumulated for a fixed period of 100 ms to maintain a constant reaction time for subsequent H/D exchange.

CID and H/D exchange experiments were performed utilizing the advanced scan functions of the LCQ instrument. In order to scan for low mass ions following CID MS/MS and MS^3 experiments (ions were mass selected using a 2 u window), the activation Q value was changed from 0.25 to a setting of 0.175. For H/D exchange experiments, deuterium oxide (D_2O) (5 μL/min) was introduced continuously into the helium background gas line through a gas tight septum via the second arm of the split flow system using a syringe drive (Harvard, St. Natick, MA). Approximately 99.9% of the gas flow was diverted to waste via a flowmeter, whereas approximately 0.1% was allowed into the ion trap at a flow rate of approximately 1 mL/min. The external regulator was maintained at 3 lb/in^2 throughout. For H/D exchange on both intact precursor and CID product ions, a 5 u window was used to isolate the ion of interest, which was allowed to undergo H/D exchange for 10 s, prior to being ejected from the trap and detected.

A reviewer has asked us to estimate the pressure of D_2O in the trap during the H/D exchange experiments. The difficulties associated with measuring pressures of added neutral reagent gases in the volume of the ring and endcap electrodes in the LCQ have previously been discussed by Callahan and coworkers [26b]. Given the above flow rates for both the D_2O and the helium, and assuming that the pressure inside the trap is 1×10^{-3} Torr, a crude estimate of the added D_2O is $\approx 3 \times 10^{-5}$ Torr. This is about the same order of magnitude as the added reagents in Callahan's experiments (they typically added $1–2 \times 10^{-5}$ Torr [26b]). Note that we are interested in using H/D exchange to make comparisons of ion structures and thus qualitative experiments will suffice. To this end, all H/D experiments were performed on the same day to ensure that minor variations in the pressure of the trap (and therefore concentration of D_2O) were minimized because the extent of H/D exchange is a function of both the rate constants as well as D_2O concentration.

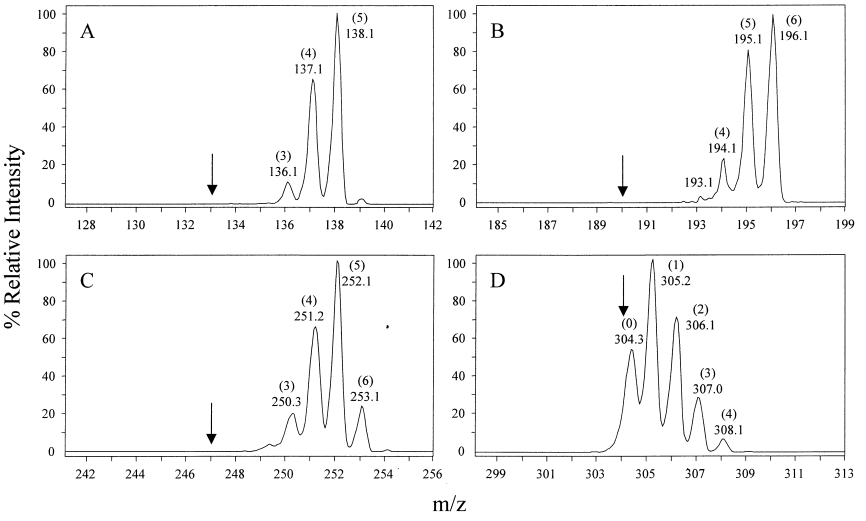


Fig. 1. Gas phase H/D exchange (reaction time = 10 s) of mass selected $[M + H]^+$ ions of glycine oligomers: (A) G_2, (B) G_3, (C) G_4, and (D) G_5. The mass of the ion selected for H/D exchange in each spectra is indicated by an arrow. The number of exchanges for each product ion is given in parentheses.

3. Results and discussion

3.1. Gas phase H/D exchange reactions of protonated glycine oligomers with D_2O

In order to establish the gas phase ion molecule H/D exchange technique in the modified ion trap and to provide a reference for later comparing the structures of y-type product ions formed via CID, the reactivity of the protonated glycine oligomers G_2–G_5 was examined (Fig. 1(A)–(D), respectively). The extent of gas phase H/D exchange reactions of protonated peptides is sensitive to the concentration of D_2O, the level of H_2O present (either from the electrospray solvent or gases, or as impurities in the D_2O), as well as the reaction time. Therefore, we have examined the H/D exchange reactions of protonated glycine oligomers under identical conditions [i.e. D_2O concentration, ion injection (accumulation) time and reaction time (10 s)]. Conditions were chosen to maximize the extent of H/D exchange without observing significant losses in sensitivity.

The results shown in Fig. 1 are consistent with previous studies obtained under FTICR mass spectrometric conditions [30]. Both the maximum number of exchanges and the base peak number of exchanges observed in the ion trap for each of the protonated precursor ion species G_2–G_5 (5,6,6, and 4, respectively, for the maximum and 5,6,5, and 1, respectively, for the base peak), compared with the number of possible exchangeable hydrogen atoms (N- and O-hydrogen atoms)(6,7,8, and 9, respectively) give an indication of the reactivity of each of the $[M + H]^+$ ions. Only minor decreases in the total ion currents of each were observed after 10 s, indicating that longer reaction times between ions and neutral reagents should be possible.

3.2. ESI/MS/MS of protonated glycine oligomers

The MS/MS spectra of the $[M + H]^+$ ions of the protonated G_2, G_3, G_4, and G_5 oligomers are shown in Fig. 2(A)–(D), respectively. Examination of the G_2 oligomer CID MS/MS spectra [Fig. 2(A)] reveals the neutral losses of H_2O, CO, and (CO + NH_3) (see Scheme 4 for a possible mechanism to explain the loss of CO and CO + NH_3 [46]), as well as the formation of the y_1 sequence ion as the only product ions. The b_2 sequence ion was the major product ion observed for the G_3 oligomer [Fig. 2(B)]. These results are in

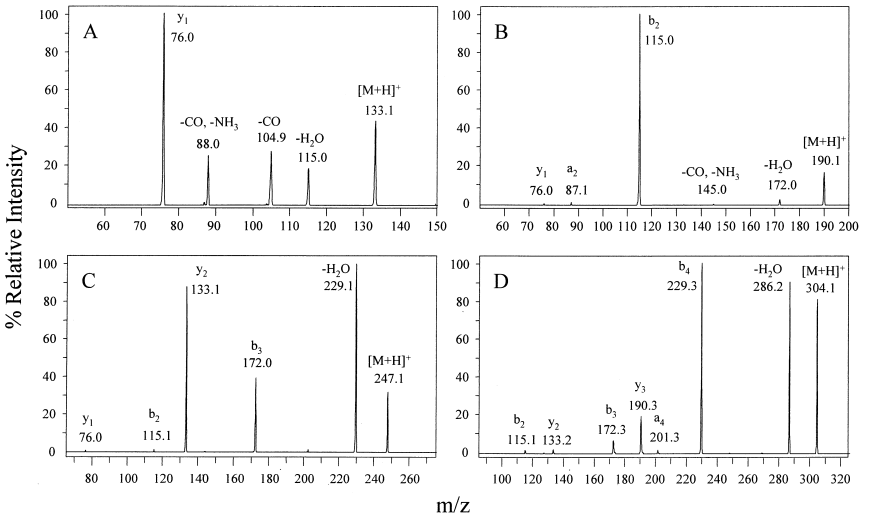
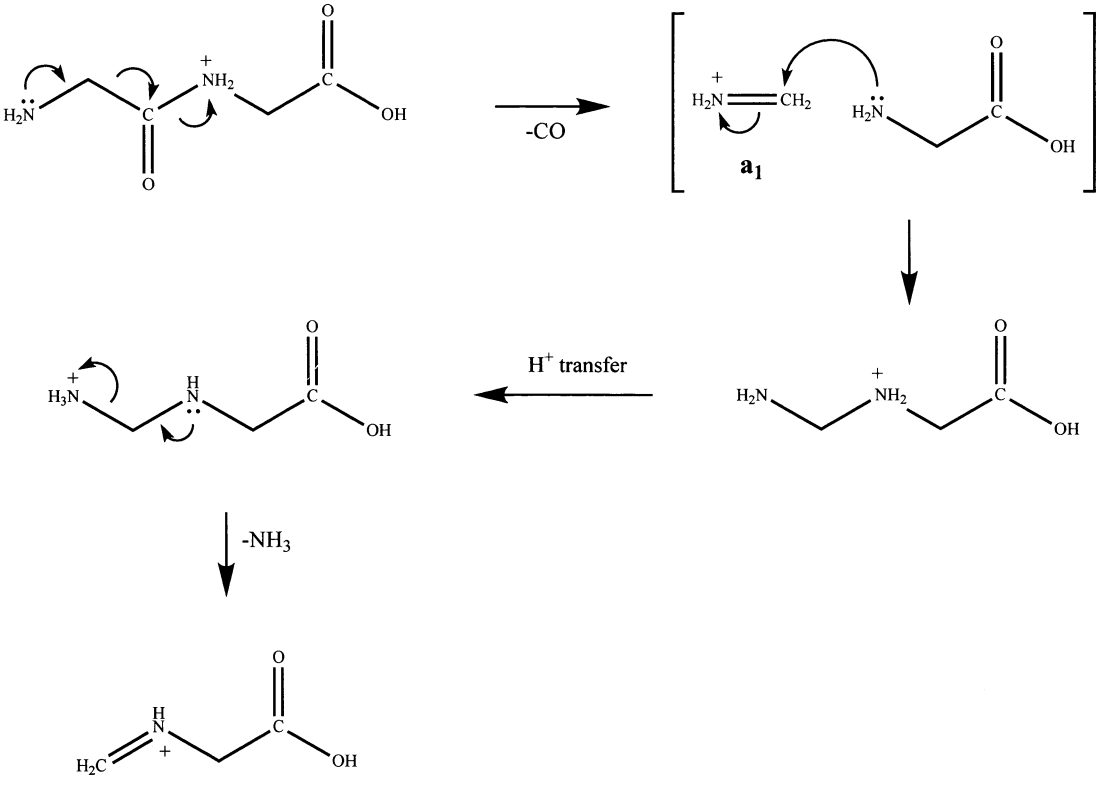


Fig. 2. MS/MS spectra of the $[M + H]^+$ ions of glycine oligomers: (A) G_2, (B) G_3, (C) G_4, and (D) G_5.



Scheme 4.

accord with previous work predicting the yields of the various product ions based on the proton affinity of the fragments [33]. In contrast, the spectra obtained by CID of the $[M + H]^+$ ions of G_4 [Fig. 2(C)] and G_5 [Fig. 2(D)] reveal major neutral losses of H_2O in both cases with poor yields of the b_2, and the b_2 and b_3 ions for the G_4 and G_5 oligomers, respectively.

3.3. Probing the structures of y_n product ions via MS^3 experiments as well as gas phase H/D exchange reactions with D_2O

The structure of product ions formed via CID may be probed in the ion trap using multistage CID reactions [24c] (i.e. MS^n). Alternatively, CID reactions combined with gas phase ion–molecule H/D exchange reactions could be used to examine aspects of product ion structure. In order to demonstrate the efficacy of each of these approaches, and to confirm that y_n-type ions exhibit identical behavior to that of truncated molecular ions [35a], the y_3 product ion (*m/z* 190) from the CID MS/MS of protonated G_5 [Fig. 2(D)] was isolated and subjected to further fragmentation in an MS^3 experiment. The resultant spectrum, shown in Fig. 3(A) is identical to the product ion spectrum obtained from CID MS/MS of the $[M + H]^+$ ion of the G_3 oligomer shown in Fig. 2(B). Likewise, the resultant gas phase ion–molecule H/D exchange profile [Fig. 3(B)] of the y_3 product ion (*m/z* 190) from the CID MS/MS of protonated G_5 [Fig. 2(D)] is also quite similar to that observed for the H/D exchange of the protonated G_3 peptide ion shown in Fig. 1(B). Both these results therefore confirm previously established findings regarding the structure of y-type ions (i.e. as truncated peptide molecular ions) [35a].

It is important to note that the events performed in the ion trap are in the following sequence: $[M + H]^+$ ion accumulation and isolation, followed by collisional activation and isolation of the selected product ion that is finally allowed to react with the D_2O. As the neutral reagent, D_2O, was in the trap throughout the entire experiment, both precursor and CID product ions subjected to H/D exchange contained a specific number of deuterons were found to undergo further exchange (both forward and back) during the time

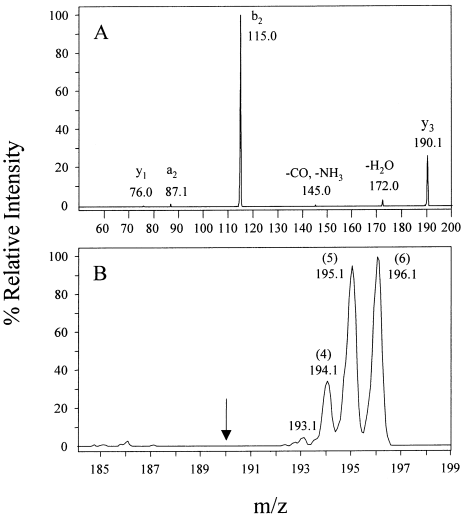


Fig. 3. Probing the structure of the y_3 CID MS/MS product ion (*m/z* 190) of G_5 via MS^3 CID and gas phase H/D exchange: (A) MS^3 CID spectrum of the y_3 ion at *m/z* 190, and (B) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the y_3 product ion. The mass of the ion selected for H/D exchange in each spectra is indicated by an arrow. The number of exchanges for each product ion is given in parentheses.

(~150 ms) required to isolate the ion of interest. Thus, CID of these ions following H/D exchange, to probe the specific location of individual deuterons, was not possible. For this reason, and for those discussed above concerning the need to account for each of the factors influencing H/D exchange rates, detailed kinetic studies, to determine the rate of incorporation of individual deuterons in either the precursor or CID product ions, were not performed at this time. The pulsed introduction of the reagent gas in future experiments may allow studies designed to probe the precise sites of exchange within the peptide ions. Not withstanding these limitations, the novel use of gas phase H/D exchange in the ion trap, to examine the structure of CID product ions, represents a useful and simple qualitative probe.

3.4. Probing the structures of b_n product ions via MS^3 experiments as well as gas phase H/D exchange reactions with D_2O

The same rationale applied to probing the structure of the y_n-type ions described above has also been used

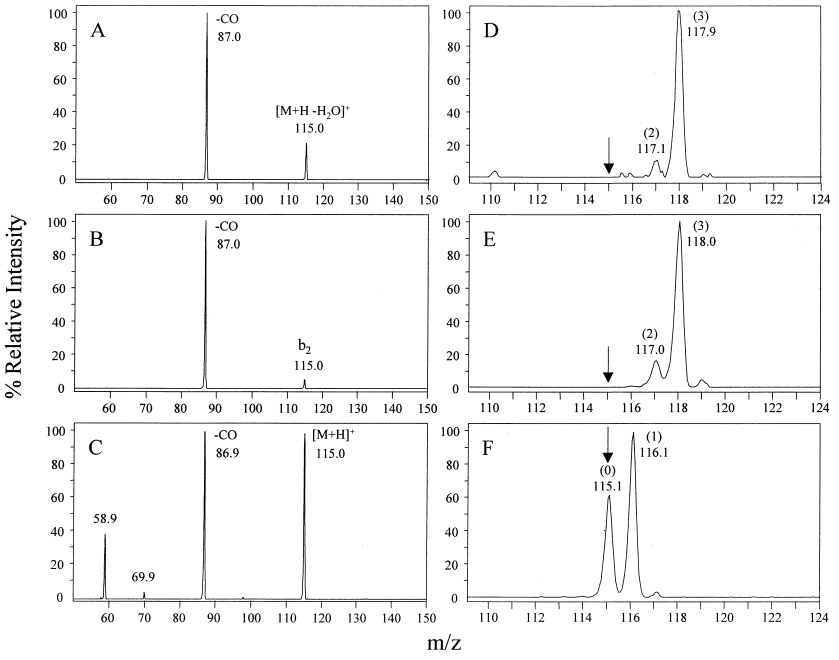


Fig. 4. Probing the structure of the b_2 product ions of G_2 and G_3 via MS^3 CID and gas phase H/D exchange: (A) MS^3 CID spectrum of the b_2 product ion (*m/z* 115) from G_2; (B) MS^3 CID spectrum of the b_2 product ion (*m/z* 115) from G_3; (C) CID MS/MS spectrum of the $[M + H]^+$ ion (*m/z* 115) of diketopiperazine; (D) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the b_2 product ion (*m/z* 115) from G_2; (E) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the b_2 product ion (*m/z* 115) from G_3; (F) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the $[M + H]^+$ ion (*m/z* 115) of diketopiperazine. The mass of the ion selected for H/D exchange in each spectra is indicated by an arrow. The number of exchanges for each product ion is given in parentheses.

to examine the fragmentation reactions and structure of b_n-type ions. As noted above, several different types of b_2 ion structures [(**A**)–(**D**)] have been proposed by others. Although we are unable to independently synthesize the oxazolone structures proposed for b_n-type ions [35c,d], we have examined whether ions corresponding to b_n-type ions formed from different precursors have the same structure and for the case of b_2 ions, we have compared their reactivity to that of the protonated diketopiperazine (B) [33g]. When the CID MS^3 spectra from the b_2 ions of protonated G_2 and G_3 were compared [Fig. 4(A) and (B)], both spectra were identical and characterized by the loss of CO to form the a_2 immonium ion. However, these spectra were different from that of the protonated diketopiperazine [Fig. 4(C)]. The gas phase H/D exchange profiles of the b_2 ions [Fig. 4(D) and (E)] also exhibited identical behavior where all three exchangeable hydrogen atoms were exchanged for deuterium. Again, in a similar result to that observed for the CID experiments, the bimolecular reactivity of these ions was noticeably different from that of the protonated diketopiperazine, which essentially underwent only one H/D exchange with D_2O [Fig. 4(F)]. A possible explanation of this poor reactivity is that the diketopiperazines rigid cyclic structure cannot form multiple sites of hydrogen bonding [47].

In contrast to the CID and gas phase H/D exchange reactivities of the b_2 and b_3 (data not shown) ions, whose results are all consistent with that of a protonated oxazolone, a different picture emerges when the putative b_4 product ions produced from the G_4 oligomer [*m/z* 229, see Fig. 2(C)] was probed via CID and MS^3 experiments. In comparison with the b_4 ion formed from protonated G_5 [Fig. 2(D)], the G_4 puta-

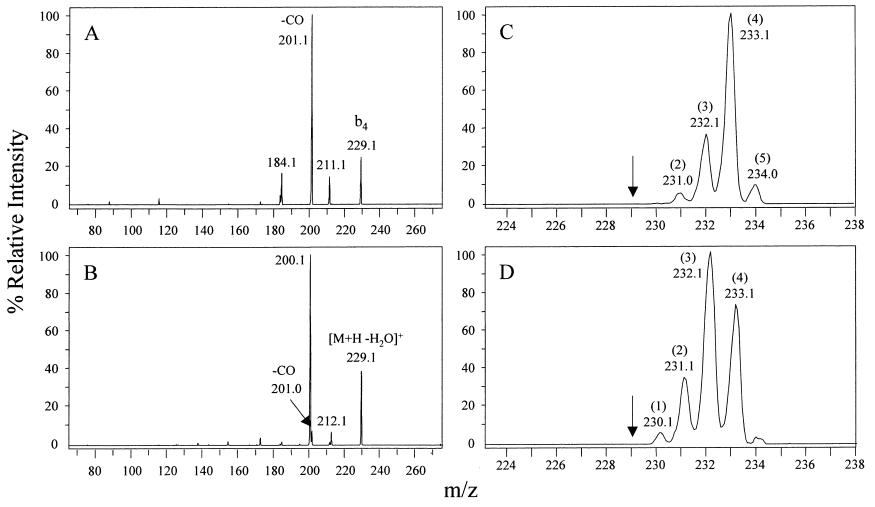


Fig. 5. Probing the structure of the putative b_4 product ions of G_4 and G_5 via MS^3 CID and gas phase H/D exchange: (A) MS^3 CID spectrum of the b_4 product ion at *m/z* 229 from G_5; (B) MS^3 CID spectrum of the $[M + H - H_2O]^+$ product ion at *m/z* 229 from G_4; (C) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the b_4 product ion at *m/z* 229 from G_5; (B) gas phase H/D exchange reaction [same reactions conditions as those in Fig. 1(B)] of the $[M + H - H_2O]^+$ ion at *m/z* 229 from G_4. The mass of the ion selected for H/D exchange in each spectra is indicated by an arrow. The number of exchanges for each product ion is given in parentheses.

tive b_4 ion MS^3 product ion spectra was found to be substantially different [compare Fig. 5(A) and (B)]. Whereas the b_4 ion produced from the protonated G_5 oligomer fragmented primarily by loss of CO to yield the a_4 immonium ion at *m/z* 201 [Fig. 5(A)], the product ion spectrum of the putative b_4 ion (i.e. the $[M + H - H_2O]^+$ ion) from protonated G_4 displayed an ion at *m/z* 200 (possibly via the neutral loss of $HN{=}CH_2$) as the primary product [Fig. 5(B)]. This data suggests that whereas the b_4 ion from the G_5 oligomer MS/MS experiment has a classical oxazolone structure, the putative b_4 ion from the protonated G_4 oligomer does not. Gas phase H/D exchange of the *m/z* 229 product ions from both the G_5 and G_4 oligomer MS/MS experiments also suggested different structures for the two ions [Fig. 5(C) and (D), respectively], based on their different reactivities. Thus, whereas the b_4 ion from the protonated G_5 oligomer showed a maximum of five exchanges (with the base peak at 4) [Fig. 5(C)], the putative b_4 ion from the protonated G_4 oligomer was seen to undergo a maximum of only four exchanges [Fig. 5(D)], with the base peak observed at 3, each from a predicted maximum of 5.

A similar result to that found for the putative b_4 ion (i.e. the $[M + H - H_2O]^+$ ion) from protonated G_4 was also observed upon MS^3 of the putative b_5 ion (*m/z* 286) produced by CID of the $[M + H]^+$ ion of the G_5 oligomer (data not shown). Here, the expected a-type ion and smaller b-type ions were only observed as minor products, with losses of NH_3, H_2O, and the loss of the neutral mass of 29 Da comprising the major fragment ions.

3.5. Probing the structure of the $[M + H - H_2O]^+$ product ion from G_4 and G_5 via MS/MS experiments on O-methyl ester derivatives

The C-terminal loss of H_2O, with concomitant formation of the b_n ion (where n = the number of residues in a peptide), versus loss of H_2O from other sites throughout the peptide can be probed experimentally by the formation of C-terminal methyl ester derivatives and subsequent CID. Previously, this approach has been employed to demonstrate that H_2O loss from protonated G_3 occurs exclusively at the C terminal [41c]. In an attempt to determine the site of H_2O loss from the protonated G_4 and G_5 peptides, and

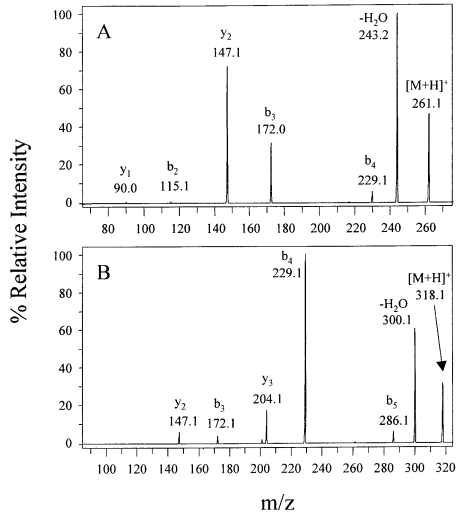


Fig. 6. MS/MS spectra of the $[M + H]^+$ ions of (A) G_4-OMe and (B) G_5-OMe.

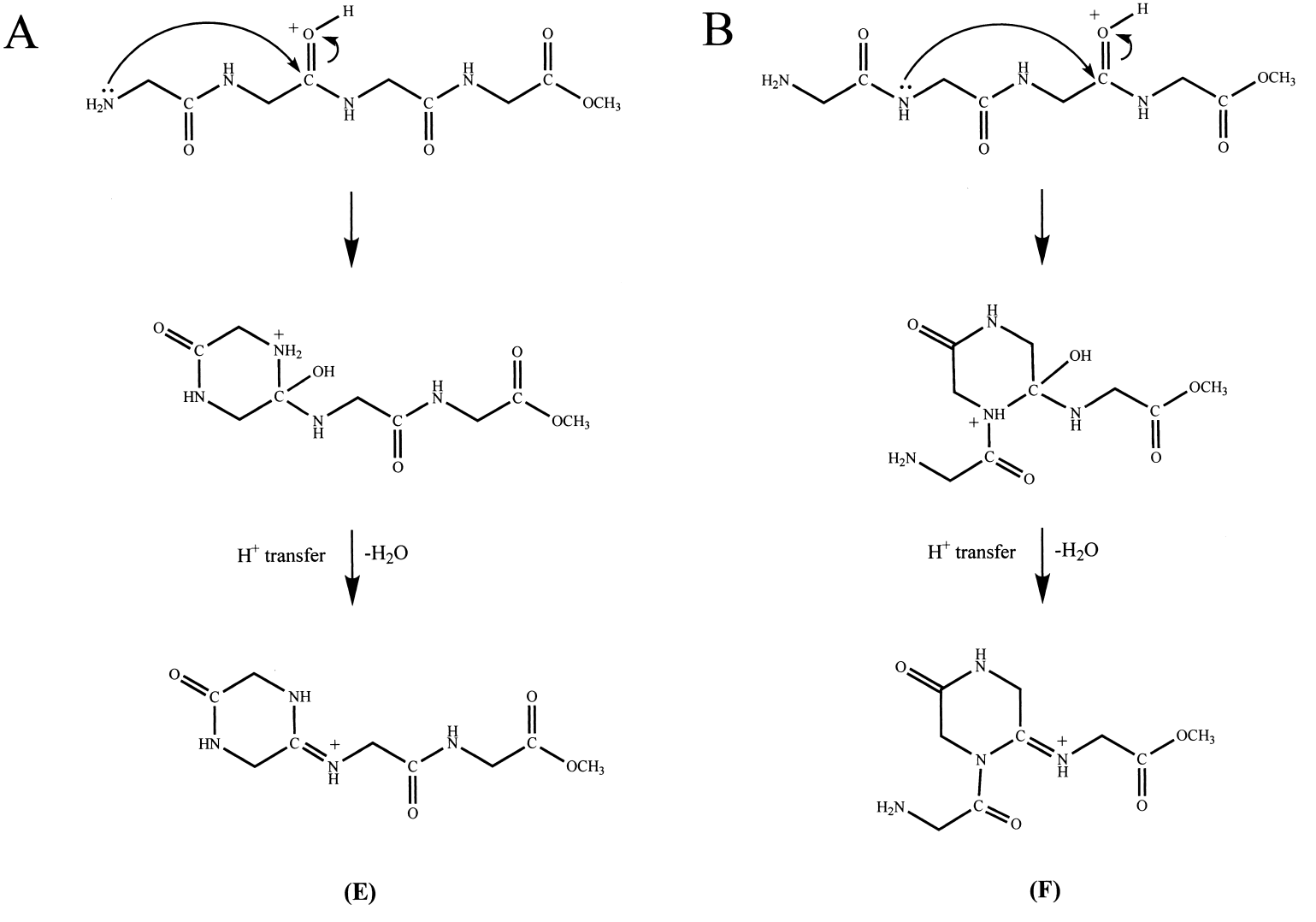
to gain insights into the mechanism for this loss, the O-methyl ester derivatives of G_4 and G_5 were prepared and their CID MS/MS spectra [Fig. 6(A) and (B), respectively] examined. The loss of CH_3OH [*m/z* 229 and 286 in Fig. 6(A) and (B), respectively] is indicative of the formation of the classical oxazolone b-type ion. It can be seen, however, that these ions are present as only minor products when compared to the larger loss of H_2O. In the absence of side chain influences, this loss of H_2O must occur via backbone–backbone reactions remote to the C terminal and must therefore involve the oxygen atoms of the other carbonyl groups.

Previously, we have demonstrated several other pathways for H_2O loss involving side chain–backbone [41] and backbone–side chain [48] neighboring group participation reactions. We have recently shown, using protonated glycyl-glycine-OMe, that the product formed by the backbone–backbone loss of water via a retro-Ritter type reaction is thermodynamically unfavored [41b]. Such a process, however, cannot be totally ruled out for more complex systems (such as the larger peptide systems studied here) because the "proton shuttle" events required for proton transfer and fragmentation leading to water loss may become more favored.

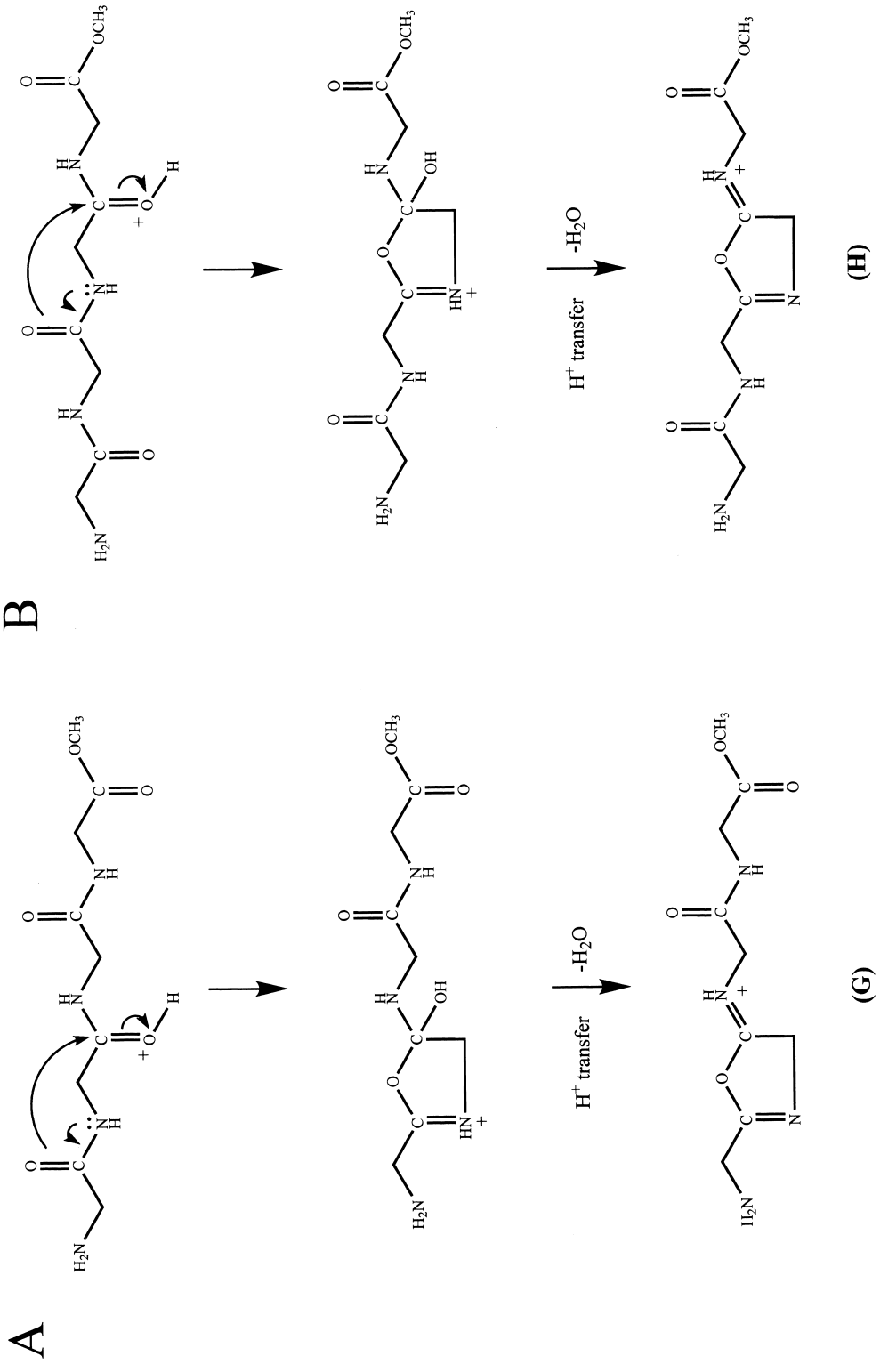
3.6. Probing the structure of the $[M + H - H_2O]^+$ ion of protonated G_4 via MS/MS and MS^3 experiments on various methylated derivatives of the protonated G_4-OMe oligomer

Alternate pathways for the loss of water from protonated G_4 via backbone–backbone neighboring group participation reactions involving either diketopiperazine- or oxazolone-like cyclic intermediates are shown in Schemes 5, 6, and 7, respectively [Structures (**E**)–(**J**)]. Note that the mechanism shown in Scheme 5(A) would lead to formation of the same intermediate shown in Scheme 3 for the alternate y_{n-2} product ion mechanism. Similarly, the oxazolone-like cyclic intermediates shown in Scheme 6(A) and (B) are common to the pathway shown in Scheme 1 for formation of b-type and y-type product ions. Note that in Schemes 5 and 6, the neighboring group is on the N-terminal side and attacks an O-protonated carbonyl group that is closer to the C terminus. The reverse situation operates in Scheme 7, where the neighboring group, which is closer to the C terminus attacks an O-protonated carbonyl group on the N-terminal side, resulting in the "reverse" oxazolone-like intermediates shown in Scheme 7(A) and (B). Interestingly, similar intermediates have been previously proposed to explain the rearrangement reactions involving the formation of $(b_{n-1} + H_2O)$ ions [49]. The loss of water via either of these mechanisms would lead to the structures (**I**) and (**J**). Note that these are simply different protonated forms of the structures (**G**) and (**H**) shown in Scheme 6. The MS^3 spectra of the product ions formed from either Schemes 6(A) and 7(A) or Schemes 6(B) and 7(B) would be expected to be indistinguishable from each other (provided that the "mobile proton" model of fragmentation is operating). We have shown previously that product ions formed via side chain–backbone interactions can compete with formation of the structurally relevant y- and b-type sequence ions [41c]. The mechanisms described above (Schemes 5–7), indicate that competing backbone–backbone interactions involving common intermediates may potentially influence "sequence" versus "nonsequence" ion formation.

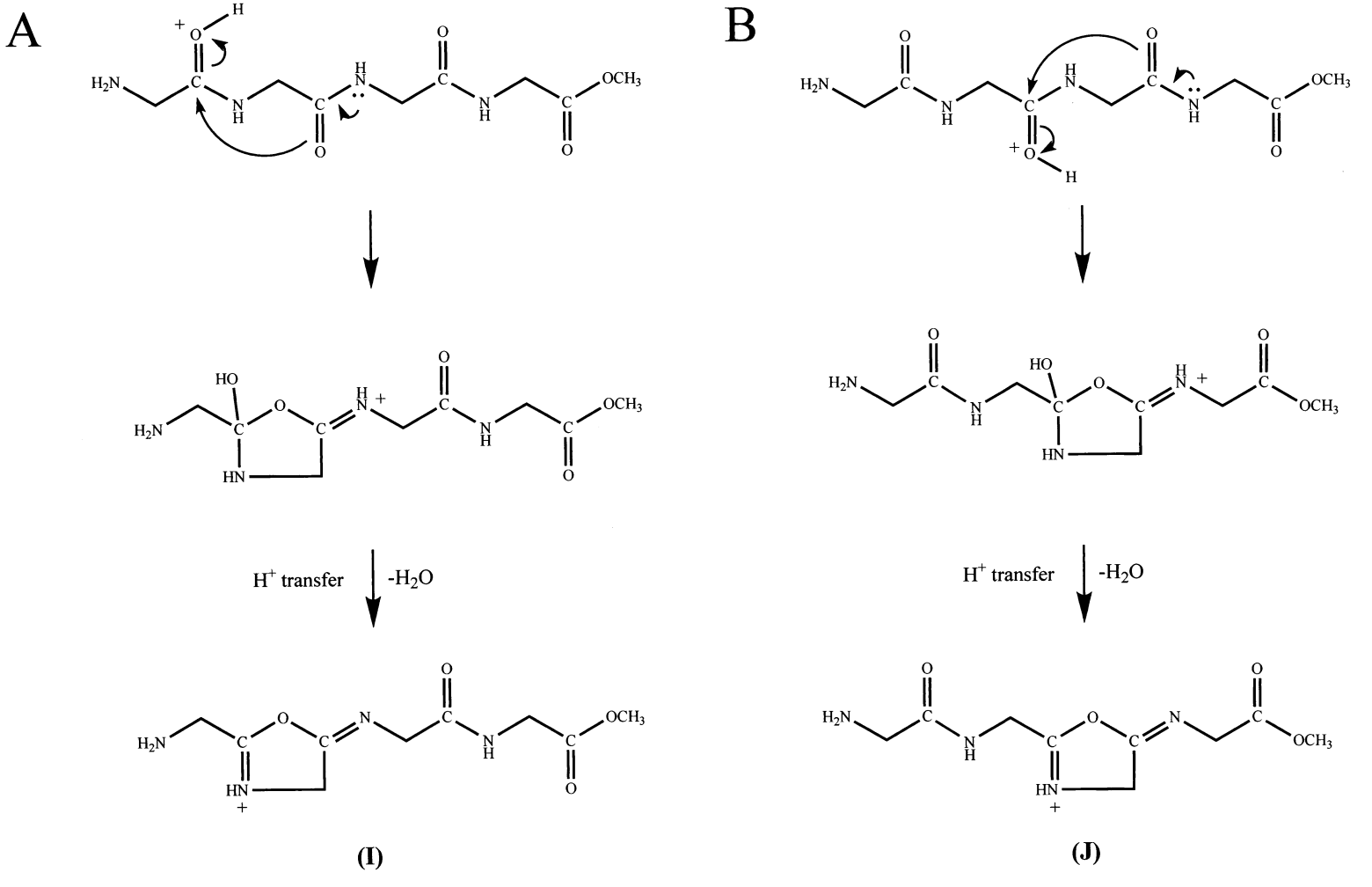
Given that a number of different pathways leading



Scheme 5.



Scheme 6.



Scheme 7.

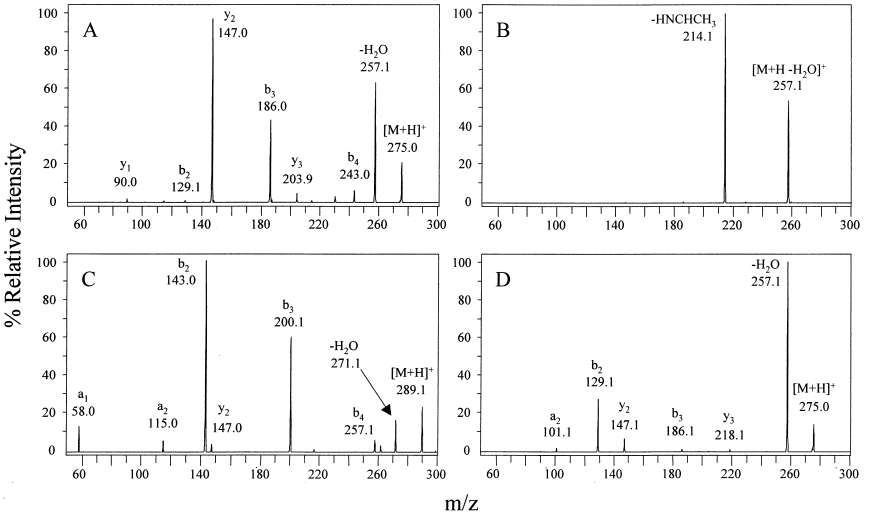


Fig. 7. MS/MS and MS^3 spectra of the $[M + H]^+$ ions of methylated derivatives of G_4-OMe: (A) MS/MS of the $[M + H]^+$ ion (*m/z* 275) of Alanyl-G_3-OMe; (B) MS^3 spectra of the $[M + H - H_2O]^+$ ion (*m/z* 257) of Alanyl-G_3-OMe; (C) MS/MS of the $[M + H]^+$ ion (*m/z* 289) of N,N-dimethyl-G_4-OMe; (D) MS/MS of the $[M + H]^+$ ion (*m/z* 275) of Glycyl-sarcosyl-G_2-OMe.

to the formation of isomeric product ions are possible for the loss of water from the examples discussed above, the challenge lies in evaluating whether a single product ion structure is particularly favored or whether a mixture of isomeric product ions are present. One possible method of evaluating different product ion structures is through the synthesis of derivatives in which hydrogen atoms are replaced by methyl groups at specific sites throughout the peptide, followed by an examination of their MS/MS and MS^3 spectra following CID. In our previous studies on elucidating the mechanisms for H_2O loss from cysteine containing peptides, we utilized various methylated derivatives to "switch off" the channels for H_2O loss [41b]. However, whereas this approach was successful, these derivatives were also observed to "switch on" new fragmentation channels. Therefore, in the larger systems studied here, the possible effects of these methyl groups on local proton affinities, internal hydrogen bonding, and peptide conformations, all of which may induce changes to the observed fragmentation channels, must be considered. Note that by the replacement of a hydrogen with a methyl group, many of the proposed intermediates in Schemes 1–7 now possess a "fixed" charge. If these intermediates are still to play a role in the loss of H_2O or other bond cleavage reactions, charge remote fragmentation mechanisms may become operable.

The CID MS/MS spectra of several methyl labeled derivatives (**K**)–(**M**), synthesized in order to further explore the potential pathways for H_2O loss from the protonated G_4 oligomer described above, have been examined. Evidence for the presence of a free N terminal in the dehydration product ions studied was supplied upon examination of the MS/MS [Fig. 7(A)] and MS^3 [Fig. 7(B)] spectra of the N-terminal alanine containing peptide (**K**) (Alanyl-G_3-OMe). Both the ion types and abundances observed in the MS/MS spectrum are comparable to those seen in the MS/MS spectrum of the G_4-OMe oligomer, indicating that substitution of a hydrogen atom for a methyl group at the N-terminal amino acid α-carbon position has no appreciable effect on the fragmentation channels in the tetrapeptide system. Furthermore, the loss of the neutral imine ($NHCHCH_3$) (*m/z* 214) upon CID of the $[M + H - H_2O]^+$ ion [Fig. 7(C)] in a MS^3 experiment confirms that the loss of 29 Da from the G_4 oligomer $[M + H - H_2O]^+$ ion corresponded to the loss of $HNCH_2$ as proposed above, and thereby supports the suggestion that the N-terminal amino

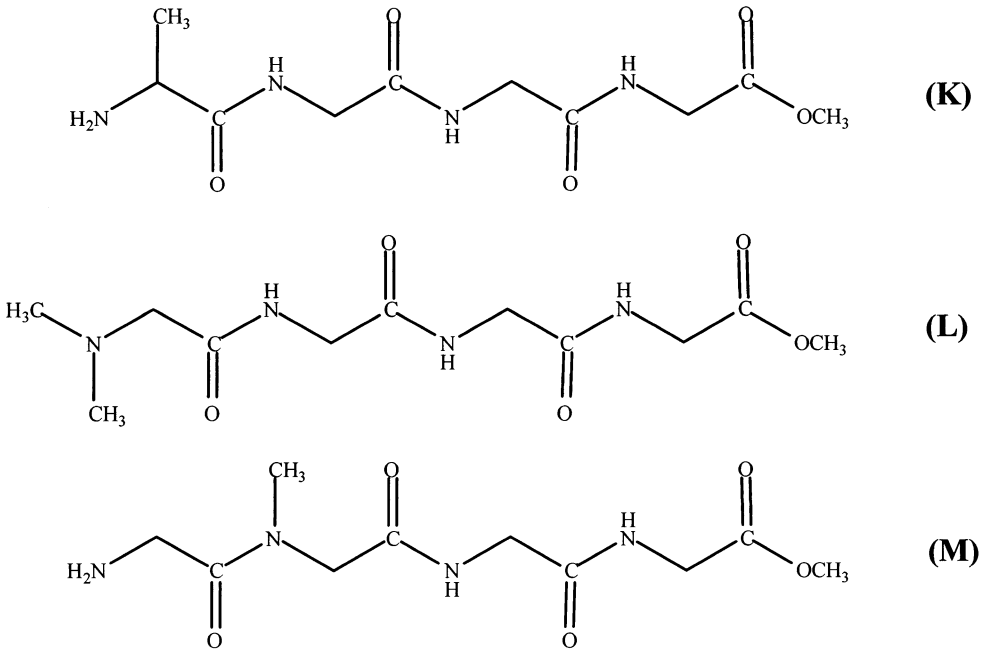


Diagram 2.

acid residue is not fixed within a cyclic product [thereby ruling out structure (**E**) in Scheme 5].

If the product ion formed by loss of H_2O from the protonated G_4-OMe oligomer is the diketopiperazine (**E**), then the MS/MS spectrum of the N,N-dimethyl substituted G_4-OMe peptide (**L**) should exhibit no H_2O loss because neither of the acidic protons on the amino nitrogen are available for transfer to effect bond cleavage, because of the formation of a fixed charge intermediate. However, H_2O loss (*m/z* 271) was observed from the $[M + H]^+$ precursor [see Fig. 7(C)], albeit at a reduced yield compared to the G_4-OMe peptide MS/MS [Fig. 6(A)], suggesting that the proposed diketopiperazine-like product ion is not the major structure associated with this process. In addition to water loss in the CID MS/MS, the yield of the b_2 and b_3 ions (*m/z* 143 and 200, respectively) and the a-type ions (a_1, *m/z* 58, and a_2, *m/z* 115) were observed at markedly increased levels compared to G_4-OMe, presumably because of the increased proton affinity of the N-terminal fixed charge fragment ions thus formed.

The sarcosine containing peptide (**M**) (Glycyl-sarcosyl-G_2-OMe) is a potentially useful probe of the dehydration product ion structures (**F**) and (**G**) and (**I**) [Scheme 5(B), Scheme 6(A), and Scheme 7(A), respectively] because substitution of the acidic amide nitrogen hydrogen atom for a methyl group should impede dehydration via these processes. Examination of the MS/MS spectrum of the sarcosine containing $[M + H]^+$ ion [Fig. 7(D)] reveals that the loss of H_2O (*m/z* 257) was observed as the major fragmentation channel, suggesting that the structures (**F**) (i.e. the diketopiperazine), (**G**) and (**I**) (i.e. the oxazolones) are not likely intermediates for the dehydration described.

By the process of attrition described above, it would seem that the products formed are those of the oxazolones (**H**) or (**J**) [Schemes 6(B) and 7(B), respectively]. Whereas mechanisms involving formation of either of these would also explain the absence of water loss from protonated G_3-OMe ion (note that the intermediate leading to the "reverse" oxazolone structure (**J**) would lead only to water loss and not amide bond cleavage) the caveats discussed above

regarding the potential alternate fragmentation pathways for methylated derivatives means that it is possible that charge remote fragmentation pathways are operable, or that isomeric populations of dehydration product ions are present. From the work presented here, the unequivocal determination of fragmentation mechanisms and product ion structures in larger systems, even with the use of structurally labeled derivatives, is clearly fraught with difficulties. Even for the simple glycine containing peptides discussed above, assigning product ions structures and elucidating fragmentation mechanisms continues to be a challenge. Future progress in alternative techniques such as gas phase ion–molecule chemistry or ion-mobility MS may lead to the development of more elegant probes of gas phase ion structure, thereby allowing further insights into the reactivity and structures of some of the intermediates proposed above.

4. Conclusions

The results presented here, as well as those described previously, clearly indicate that a detailed understanding of the fragmentation reactions of protonated peptides requires studies involving not only MS/MS based techniques, but also MS^n methods. The use of gas phase ion–molecule reactions (for example H/D exchange) and derivatives (such as methyl esters or isotopically labeled systems) can be used to provide further information. The results presented above indicate that caution must be taken in assuming that the loss of water from the protonated precursor results in formation of a b_n ion (where n = the number of amino acid residues in the peptide). Also, the b- and y-type "sequence ions" formed following CID may be influenced not only by the initial site of protonation but also by the proton affinities of the fragments formed, peptide conformation, and competing neighboring group participation reactions leading to the formation of "nonsequence" ion structures.

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References

[1] (a) A.L. Burlingame, R.K. Boyd, S.J. Gaskell, Anal. Chem. 68 (1996) 599R; (b) Mass Spectrometry, Meth. Enzym., J.A. McCloskey (Ed.), Academic, New York, 1990, Vol. 193; (c) Mass Spectrometry in the Biological Sciences, M.L. Gross (Ed.), Nato ASI Series C, Kluwer, Dordrecht, 1992, Vol. 353; (d) G. Siuzdak, Mass Spectrometry for Biotechnology, Academic, San Diego, 1996; (e) Protein and Peptide Analysis by MS, Meth. Mol. Biol., J.R. Chapman (Ed.), Humana, Totowa, 1996, Vol. 61; (f) E. Nordhoff, F. Kirpekar, P. Roepstorff, Mass Spectrom. Rev. 15 (1996) 67; (g) K.M. Murray, J. Mass Spectrom. 31 (1996) 1203.
[2] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wang, Mass Spectrom. Rev. 9 (1990) 37.
[3] F. Hillenkamp, M. Karas, R.C. Beavis, B.T. Chait, Anal. Chem. 63 (1991) 1193.
[4] (a) D.F. Hunt, J.R. Yates, J. Shabanowitz, S. Winston, C.H. Hauer, Proc. Natl. Acad. Sci. USA 83 (1986) 6233; (b) K.L. Busch, G.L. Glish, S.A. McLuckey, Mass Spectrometry/Mass Spectrometry. Techniques & Applications of Tandem Mass Spectrometry, VCH, New York, 1988.
[5] J.K. Eng, A.L. McCormack, J.R. Yates, J. Am. Soc. Mass Spectrom 5 (1994) 976.
[6] M.K. Green, C.B. Lebrilla, Mass Spectrom. Rev. 16 (1997) 53.
[7] A.R. Dongre, A. Somogyi, V.H. Wysocki, J. Mass Spectrom. 31 (1996) 339.
[8] (a) D.F. Hunt, J. Shabanowitz, J.R. Yates, J. Chem. Soc. Chem. Commun. (1987) 548; (b) S.A. Martin, J.A. Hill, C. Kittrell, K. Biemann, J. Am. Soc. Mass Spectrom. 1 (1990) 107; (c) E.R. Williams, J.J.P. Furlong, F.W. McLafferty, J. Am. Soc. Mass Spectrom. 1 (1990) 288.
[9] B.H. Wang, F.W. McLafferty, Org. Mass Spectrom. 25 (1990) 554.
[10] W.D. Price, E.R. Williams, J. Phys. Chem. A 100 (1997) 8844.
[11] (a) M.J. Polce, S. Beranova, M.J. Nold, C. Wesdemiotis, J. Mass Spectrom. 31 (1996) 1073; (b) M.M. Cordero, C.

Wesdemiotis, in T. Matsuo (Ed.), Biological Mass Spectrometry: Present and Future, Wiley, New York, 1994, p. 119.
[12] R.A.J. O'Hair, Chem. Aust. 65(8) (1998) 50.
[13] (a) J.L. Stephenson, S.A. McLuckey, J. Am. Chem. Soc. 119 (1997) 1688; (b) S.A. McLuckey, J.L. Stephenson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 8 (1997) 148.
[14] D.E. Clemmer, J.M. Jarrold, J. Mass Spectrom. 32 (1997) 577.
[15] P.A. Sullivan, J. Axelsson, S. Altmann, A.P. Quist, B.U.R. Sunqvist, C.T. Reimann, J. Am. Soc. Mass Spectrom. 7 (1996) 329.
[16] W.J. Hehre, J.A. Pople, L. Radom, Ab Initio Molecular Orbital Theory, Wiley, New York, 1986.
[17] (a) D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler, F.W. McLafferty, Proc. Natl. Acad. Sci. USA 90 (1993) 790; (b) F.W. McLafferty, Z. Guan, U. Haupts, T.D. Wood, N.L. Kelleher, J. Am. Chem. Soc. 120 (1998) 4732.
[18] Time of Flight Mass Spectrometry, R.E. Cotter (Ed.), American Chemical Society, Washington, DC, 1994.
[19] (a) Fourier Transform Mass Spectrometry: Evolution, Innovation and Applications, M.V. Buchanan (Ed.), ACS Symposium Series, American Chemical Society, Washington, DC, 1987 Vol. 359; (b) A.G. Marshall, F.R. Verdun, Fourier Transforms in NMR, Optical and Mass Spectrometry: A User's Handbook, Elsevier, New York, 1990; (c) FT-ICR/MS: Analytical Applications of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, B. Asamoto (Ed.), VCH, New York, 1991.
[20] (a) Practical Aspects of Ion Trap Mass Spectrometry, R.E. March, J.F.J. Todd (Eds.), Fundamentals of Ion Trap Mass Spectrometry, CRC Press, Boca Raton, 1995, Vol. 1; (b) Practical Aspects of Ion Trap Mass Spectrometry, R.E. March, J.F.J. Todd (Eds.), Ion Trap Instrumentation, CRC, Boca Raton, 1995, Vol. 2; (c) R.E. March, J. Mass Spectrom 32 (1997) 351.
[21] G.J. Van Berkel, G.L. Glish, S.A. McLuckey, Anal. Chem. 62 (1990) 1284.
[22] (a) J.S. Brodbelt-Lustig, R.G. Cooks, Talanta 36 (1989) 255; (b) B.D. Nourse, R.G. Cooks, Int. J. Mass Spectrom. Ion Processes 106 (1991) 249.
[23] S.A. McLuckey, D.E. Goeringer, J. Mass Spectrom. 32 (1997) 461.
[24] (a) S.A. McLuckey, D.E. Goeringer, Anal. Chem. 67 (1995) 2493; (b) S.A. McLuckey, G.L. Glish, G.J. Van Berkel, Anal. Chem. 63 (1992) 1971; (c) S.A. McLuckey, G.J. Van Berkel, G.L. Glish, J.C. Schwartz, in R.E. March, J.F.J. Todd (Eds.), Practical Aspects of Ion Trap Mass Spectrometry, Ion Trap Instrumentation, Vol. 2, CRC, Boca Raton, 1995, Chap. 3; (d) R.A.J. O'Hair, S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 162 (1997) 183.
[25] C.S. Hoaglund, S.J. Valentine, D.E. Clemmer, Anal. Chem. 69 (1997) 4156.
[26] (a) G.E. Reid, R.A.J. O'Hair, M.L. Styles, W.D. McFadyen, R.J. Simpson, Rapid Commun. Mass Spectrom. 12 (1998) 1701; (b) R.W. Vachet, J.A.R. Hartman, J.H. Callahan, J. Mass Spectrom. 33 (1998) 1209.
[27] J.L. Stephenson, S.A. McLuckey, Anal. Chem. 70 (1998) 3533.
[28] Z. Wu, C. Fenselau, J. Am. Soc. Mass Spectrom. 3 (1992) 863.
[29] (a) J. Wu, C.B. Lebrilla, J. Am. Chem. Soc. 115 (1993) 3270; (b) K. Zhang, D.M. Zimmerman, A. Chung-Philips, C.J. Cassady, J. Am. Chem. Soc. 115 (1993) 10 812; (c) K. Zhang, C.J. Cassady, A. Chung-Philips, J. Am. Chem. Soc. 116 (1994) 11 512; (d) J. Wu, C.B. Lebrilla, J. Am. Soc. Mass Spectrom. 6 (1995) 91; (e) C.J. Cassady, S.R. Carr, K. Zhang, A. Chung-Philips, J. Org. Chem. 60 (1995) 1704; (f) S.G. Lias, J.E. Bartmess, J.F. Liebman, J.L. Holmes, R.D. Levin, W.G. Mallard, J. Phys. Chem. Ref. Data 17 (1988) (suppl. 1); (g) E.P.L. Hunter, S.G. Lias, J. Phys. Chem. Ref. Data 27 (1998) 413.
[30] (a) S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 117 (1995) 12 840; (b) E.H. Gur, L.J. de Koning, N.M.M. Nibbering, J. Am. Soc. Mass Spectrom. 6 (1995) 466; (c) M.K. Green, E. Gard, J. Bregar, C.B. Lebrilla, J. Mass Spectrom. 39 (1995) 1103.
[31] E.H. Gur, L.J. de Koning, N.M.M. Nibbering, Int. J. Mass Spectrom. Ion. Processes 167/168 (1997) 135.
[32] T. Wyttenbach, J.E. Bushnell, M.T. Bowers, J. Am. Chem. Soc. 120 (1998) 5098.
[33] (a) R.W. Yeh, J.M. Grimley, M.M. Bursey, Biol. Mass Spectrom. 20 (1991) 443; (b) D.G. Morgan, M.M. Bursey, Biol. Mass Spectrom. 22 (1993) 502; (c) D.G. Morgan, M.M. Bursey, Org. Mass Spectrom. 29 (1994) 354; (d) S. Beranova, J. Cai, C. Wesdemiotis, J. Am. Chem. Soc. 117 (1995) 9492; (e) D.G. Morgan, M.M. Bursey, J. Mass Spectrom. 30 (1995) 290; (f) J.S. Klassen, P. Kerbarle, J. Am. Chem. Soc. 119 (1997) 6552; (g) J.A. Carroll, J. Wu, T.A. Do, C.B. Lebrilla, Proceedings of the 42nd ASMS Conference on Mass Spectrometry, Chicago, IL, 29 May–3 June 1994, p. 475; (h) K. Eckart, M.C. Holthausen, W. Koch, J. Spiess, J. Am. Soc. Mass Spectrom. 9 (1998) 1002; (i) W.D. van Dongen, W. Heerma, J. Haverkamp, C.G. de Koster, Rapid. Commun. Mass Spectrom. 10 (1996) 1237.
[34] (a) C.H. DePuy, V.M. Biebaum, in Structure/Reactivity and Thermochemistry of Ions, P. Ausloos, S.G. Lias (Eds.), Reidel, Dordrecht, 1987, p. 293; (b) S.G. Lias, J. Phys. Chem. 88 (1984) 4401.
[35] (a) D.R. Mueller, M. Eckersley, J. Richter, Org. Mass Spectrom. 23 (1988) 217; (b) P.T.M. Kenny, K. Nomoto, R. Orlando, Rapid Commun. Mass Spectrom. 6 (1992) 95; (c) T. Yalcin, C. Khouw, I.G. Csizmadia, M.R. Peterson, A.G. Harrison, J. Am. Soc. Mass Spectrom. 6 (1995) 1165; (d) T. Yalcin, I.G. Csizmadia, M.R. Peterson, A.G. Harrison, J. Am. Soc. Mass Spectrom. 7 (1996) 233; (e) D. Arnott, D. Kottmeier, N. Yates, J. Shabanowitz, D.F. Hunt, Proceedings of the 42nd ASMS Conference on Mass Spectrometry, Chicago, IL, 29 May–3 June 1994, p. 470; (f) K. Ambihapathy, T. Yalcin, H-W. Leung, A.G. Harrison, J. Mass Spectrom. 32 (1997) 209; (g) S.G. Summerfield, M.S. Bolgar, S.J. Gaskell, J. Mass Spectrom. 32 (1997) 225; (h) R.W. Vachet, K.L. Ray, G.L. Glish, J. Am. Soc. Mass Spectrom. 9 (1998) 341.
[36] (a) P. Roepstorff, J. Fohlman, Biol. Mass Spectrom. 11 (1994) 601; (b) I.A. Papayannopoulos, K. Biemann, Acc. Chem. Res. 27 (1994) 370.
[37] R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 8 (1994) 169.
[38] (a) A.R. Dongre, J.L. Jones, A. Somogyi, V.H. Wysocki, J. Am. Chem. Soc. 118 (1996) 8365; (b) A.G. Harrison, T. Yalcin, Int. J. Mass Spectrom. Ion Processes 165/166 (1997) 339.

[39] (a) M.M. Cordero, J.J. Houser, C. Wesdemiotis, Anal. Chem. 65 (1994) 1594; (b) M.J. Polce, S. Beranova, M.J. Nold, C. Wesdemiotis, J. Mass Spectrom. 31 (1996) 1073; (c) M.J. Nold, C. Wesdemiotis, T. Yalcin, A.G. Harrison, Int. J. Mass Spectrom. Ion Processes 164 (1997) 137.
[40] Y-P. Tu, A.G. Harrison, J. Am. Soc. Mass Spectrom. 9 (1998) 454.
[41] (a) W. Yu, J.E. Vath, M.C. Huberty, S.A. Martin, Anal. Chem. 65 (1993) 3015; (b) G.E. Reid, R.J. Simpson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 9 (1998) 945; (c) R.A.J. O'Hair, M.L. Styles, G.E. Reid, J. Am. Soc. Mass Spectrom. 9 (1998) 1275; (d) Y.-P. Tu, A.G. Harrison, Rapid Commun. Mass Spectrom. 12 (1998) 849.
[42] (a) O. Burlet, C-Y. Yang, S.J. Gaskell, J. Am. Soc. Mass Spectrom. 3 (1992) 337; (b) S.G. Summerfield, A. Whiting, S.J. Gaskell, Int. J. Mass Spectrom. Ion Processes 162 (1997) 149.
[43] J.A. Loo, J.X. He, W.L. Cody, J. Am. Chem. Soc. 120 (1998) 4542.
[44] G.E. Reid, R.J. Simpson, Anal. Biochem. 200 (1992) 301.
[45] S. Gronert, J. Am. Soc. Mass Spectrom. 9 (1998) 845.
[46] R.D. Kinser, D.P. Ridge, G. Hvistendahl, B. Rasmussen, E. Uggerud, Chem. Eur. J. 2 (1996) 1143.
[47] M.J. Nold, C. Wesdemiotis, Proceedings of the 45th ASMS Conference on Mass Spectrometry, Palm Springs, FL, 1–5 June 1997, p. 84.
[48] R.A.J. O'Hair, G.E. Reid, Rapid Commun. Mass Spectrom. 12 (1998) 999.
[49] (a) K.D. Ballard, S.J. Gaskell, J. Am. Chem. Soc. 114 (1992) 64; (b) J. Gonzalez, V. Besada, H. Garay, O. Reyes, G. Padron, Y. Tambara, T. Takao, Y. Shimonishi, J. Mass Spectrom. 31 (1996) 150; (c) R.W. Vachet, M.R. Asam, G.L. Glish, J. Am. Chem. Soc. 118 (1996) 6252.