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SHORT COMMUNICATION

Using Gas-Phase Guest–Host Chemistry to Probe the Structures of *b* Ions of Peptides

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Abstract

Middle-sized b_n ($n \geq 5$) fragments of protonated peptides undergo selective complex formation with ammonia under experimental conditions typically used to probe hydrogen–deuterium exchange in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Other usual peptide fragments like y, a, a^*, etc., and small b_n ($n \leq 4$) fragments do not form stable ammonia adducts. We propose that complex formation of b_n ions with ammonia is characteristic to macrocyclic isomers of these fragments. Experiments on a protonated cyclic peptide and N-terminal acetylated peptides fully support this hypothesis; the protonated cyclic peptide does form ammonia adducts while linear b_n ions of acetylated peptides do not undergo complexation. Density functional theory (DFT) calculations on the proton-bound dimers of all-Ala b_4, b_5, and b_7 ions and ammonia indicate that the ionizing proton initially located on the peptide fragment transfers to ammonia upon adduct formation. The ammonium ion is then solvated by N^+-H…O H-bonds; this stabilization is much stronger for macrocyclic b_n isomers due to the stable cage-like structure formed and entropy effects. The present study demonstrates that gas-phase guest–host chemistry can be used to selectively probe structural features (i.e., macrocyclic or linear) of fragments of protonated peptides. Stable ammonia adducts of b_9, b_9-A, and b_9-2A of A_8YA, and b_{13} of A_{20}YVFL are observed indicating that even these large b-type ions form macrocyclic structures.

Key words: Peptide, Fragment, Guest–host, Adduct, Modeling, HDX

Introduction

Understanding the dissociation of peptide ions and the structures and reactivity of their fragments is of great importance for developing improved sequencing software for proteomics. Protonated peptides fragment in a rather complex reaction pattern [1, 2] and many aspects of the underlying collision-induced dissociation (CID) chemistry are not fully understood yet. One of the consequences is that current sequencing programs that implement oversimplified fragmentation models often assign erroneous peptide sequences. It is expected that detailed understanding of peptide fragmentation will eventually lead to more robust protein sequencing software.

This promise fuelled a large number of recent studies on the structures and reactivity of CID product ions. These investigations utilized a great variety of experimental tools like MS/MS [3, 4], 'action' infrared (IR) spectroscopy [5, 6], ion mobility spectrometry (IMS) [7], gas-phase H/D exchange (HDX) [8, 9], ion-molecule reactions [10], etc., to provide information on stable structures of peptide fragments and the dissociation chemistries forming them. Here we demonstrate that the structures of peptide fragments can be probed utilizing isomer specific adduct formation with ammonia.

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Experimental and Computational Details

Experiments on a large set of peptides [G_6, A_5, YAGFL-NH_2, cyclo-(YAGFL), A_6MA, A_8YA, A_{20}YVLF, CGSVLVR, etc.] were performed in a Bruker (Bruker Daltonics, Billerica, MA) Apex Qh 9.4 T hybrid FT-ICR instrument. After selection of peptide ions in the Q quadrupole mass-filter, their fragments were formed in the hexapole (h) functioning as a collision cell (eV QCID), and the resulting fragment ion population was introduced into and trapped in the ICR cell where fragments could interact with ammonia (either ND_3 or NH_3) for time periods ranging between 0.0 and 10.0 s prior to high resolution/high mass accuracy ion detection. We used molecular dynamics simulations (using the AMBER [11] force field and Discover (Biosym Technologies, San Diego, CA, USA) and DFT calculations (B3LYP/6-31+G{d,p}, Gaussian [12]) to determine the energetically most favored linear and macrocyclic isomers of the all-Ala b_4 and b_5 ions, and the macrocyclic b_7 ions and their ammonia complexes. Detailed descriptions of our experimental and computational strategies are given in the Supporting Information.

Results and Discussion

Figure 1 displays the product ion spectra (7 eV QCID) of protonated G_6 acquired after exposure to ND_3 for 0.0, 2.0, and 5.0 s, respectively. Two major processes are observed: (1) HDX of the parent and all fragment ions, and (2) formation of a stable (detectable) complex of b_5 (*m/z* 286) and deuterated ammonia (*m/z* 306). The kinetics of HDX is usually monitored to derive structural information of the investigated ions; here we focus on complex formation of b_5 and ND_3 that is already advanced at 2.0 s ND_3 exposure and becomes nearly complete at 5.0 s. While the CID spectrum of protonated G_6 contains other *b* (b_4 and b_3), *a* (a_5 and a_4), *a** (a_5^* and a_4^*), *b-*H_2O (b_5-H_2O and b_4-H_2O), and *y* (y_4 and y_3) type fragments that all undergo facile HDX, only b_5 forms a stable complex with ND_3.

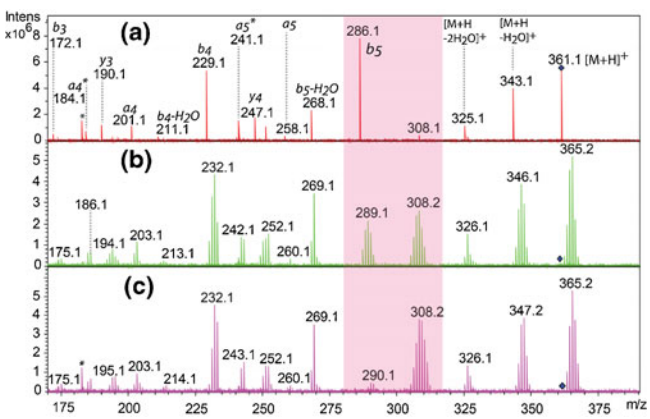

Figure 1. QCID (7 eV) product ion spectra of protonated G_6 acquired after (**a**) 0.0, (**b**) 2.0, (**c**) 5.0 s exposure to ND_3 in the ICR cell. The colored area highlights formation of the adduct of b_5 and ND_3. Filled diamonds indicate the parent ion at *m/z* 361.1

It is to be noted here that adducts formed by b_5 and ND_3 must be stable for at least 30 s since our product ion spectra are acquired after removing the residual ND_3 from the ICR cell on this time-scale. Furthermore, formation of the stable adducts of b_5 and ND_3 is not due only to a 'size-effect' since ions larger than b_5 ($[M + H]^+$, $[M + H - H_2O]^+$, and $[M + H - 2H_2O]^+$) are present and these undergo only HDX but no formation of ND_3 adducts. The b_5 and ND_3 complex (*m/z* 306.1) undergoes H/D back-exchange in our experiments (*m/z* 305.1, Figure S1); this most likely happens via collisions with water traces in the ICR. This back-exchange indicates that even the ND_3 adduct undergoes reactive collisions suggesting substantial stability of this complex.

Figure 2 displays the QCID (13 eV) product ion spectra of protonated A_8YA acquired after exposure to ND_3 for 0.0, 2.0, and 5.0 s, respectively. Similarly to G_6, all fragment ions undergo HDX with ND_3. Additionally, *b*-type fragments, namely b_9, b_9-A, b_9-2A, b_8, and b_7 form stable complexes with ND_3. Under the very same experimental conditions, a_n and b_n-H_2O ions do not form stable complexes with ND_3. Figure S2 demonstrates that even large *b* ions like b_{13} of triply protonated A_{20}YVLF undergo facile complexation with ammonia. Figure S3 displays the product ion spectra of doubly protonated CGSVLVR acquired after exposure to ND_3 for 0.0 and 2.0 s, respectively. These spectra are dominated by *y* and *y-*H_2O type fragments which all undergo facile HDX but none of these form stable complexes with ND_3.

Figures 1, 2, and S2, S3 and similar data on additional peptide ions (not shown here) indicate the following trends in terms of adduct formation of CID fragments of peptides with ammonia; *y*, *a*, *a**, y^0, and b^0 type fragments and small b_n ($n \leq 4$) ions do not form stable ammonia complexes under the experimental conditions applied. On the other hand, middle-sized b_n ($n \geq 5$) type ions undergo facile complex formation with ammonia in our experiments.

The structures of middle-sized b_n ($n \geq 5$) ions were recently investigated in numerous studies [2, 13, 14], which considered

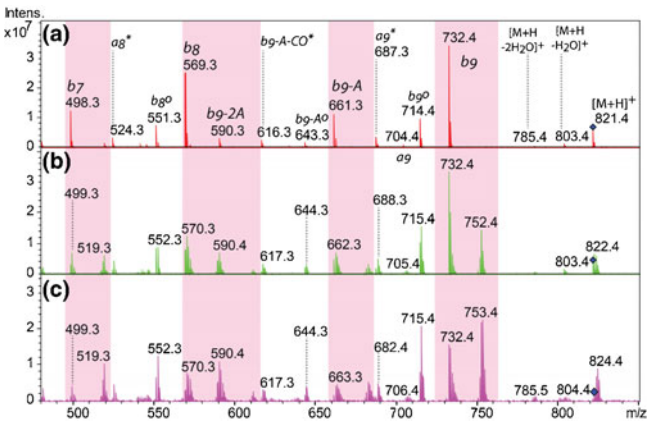

Figure 2. QCID (13 eV) product ion spectra of protonated A_8YA acquired after (**a**) 0.0, (**b**) 2.0, and (**c**) 5.0 s exposure to ND_3 in the ICR cell. The colored areas highlight formation of complexes of b_9, b_9-A, b_9-2A, b_8, and b_7 with ND_3. Filled diamonds indicate the parent ion at *m/z* 821

two main isomers, the linear form that is C-terminated by the five-membered oxazolone ring and the macrocyclic form that is a protonated cyclo-peptide (Scheme S1). It has been shown [13, 14] that one can form the macrocyclic isomer from the linear isomer originally created from intact peptides by head-to-tail cyclization; this reaction becomes pronounced for middle-sized b_n ($n \geq 5$) ions. Opening up the macro-ring at amide bonds other than the one created by the initial cyclization can lead to sequence scrambling [13, 14] and fragments that could hamper sequencing. We hypothesize here that only the macrocyclic isomers of middle-sized b_n ($n \geq 5$) ions undergo stable complex formation with ND_3.

To test this hypothesis protonated cyclo-(YAGFL) was isolated in the ICR cell and subsequently exposed to ND_3 for 0.0, 0.5, 2.0, and 5.0 s, respectively (Figure 3). Formation of the ND_3 adduct is already facile at 2.0 s and becomes nearly complete at 5.0 s. The adduct can be isolated and subsequently fragmented by IRMPD (Figure S4) forming protonated cyclo-(YAGFL) as dominant product. This indicates that the macrocyclic isomers of middle-sized b_n ($n \geq 5$) ions are indeed capable of forming stable complexes with ammonia under our experimental conditions. To test whether smaller protonated cyclic peptides undergo adduct formation with ammonia protonated cyclo-(AA) was probed in the ICR cell under the same conditions; no adduct formation (data not shown) was observed indicating that 'macrocyclic' b_2 ions do not form stable ammonia adducts under the conditions applied.

To further test our hypothesis we performed similar experiments on protonated A_6MA and Ac-A_6MA. Acetylation of the N-terminus eliminates the N-terminal amine group effectively freezing head-to-tail cyclization (Scheme S1) of linear *b* ions. [3] The *b*-type fragments of protonated A_6MA behave similarly (Figure S5) to those of protonated A_8YA; b_7, b_7-A, and b_6 form stable complexes with ND_3. On the other hand, the b_7 and b_6 ions of protonated Ac-A_6MA, which have linear structures, do not form stable ammonia adducts (Figure S5).

To gain further insight into the interaction of b_n ions and ammonia we performed detailed scans of the potential energy surfaces of the ammonia complexes of the b_4, b_5, and b_7 ions composed exclusively of alanine residues. Both linear and macrocyclic isomers were considered for b_4 and b_5, while only the latter was computed for b_7. Our calculations indicate that the C-terminal oxazolone ring nitrogen and amide oxygen are the most basic protonation sites for the linear and macro-cyclic isomers of the investigated bare b_n ions (Figure S6), respectively. Upon adduct formation the ionizing proton transfers to ammonia and the ammonium ion becomes solvated by the neutral (either linear or macro-cyclic) peptide fragment via strong N^+-H…O H-bonds. Both the macrocyclic and the linear isomers form three such H-bonds for the investigated b_4, b_5, and b_7 adducts (Figure S6). However, the spatial arrangement of amide oxygens for the macrocyclic isomers seems to be more favored allowing an extremely strong host–guest like interaction between the two monomers. This leads to more strongly bonded (Figure 4a) macrocyclic adducts (binding energy at 32.4, 25.6, and 26.2 kcal mol^{-1} for b_4, b_5, and b_7, respectively) than those featuring isomeric linear structures (binding energy at 20.1 and 15.6 kcal mol^{-1} for b_4 and b_5, respectively). Strong complexation necessarily lowers the flexibility and entropy of the peptide fragment; this unfavorable effect (accurately not predictable from our present calculations) is less pronounced for the already more constrained macrocyclic isomers than for linear forms. This entropy contribution can be as important as energetic effects for the formation of stable ammonia adducts.

Literature data from IR [6] and MS/MS [3, 4] studies on b_5 and larger b_n ions indicate these are present as macrocyclic structures under standard MS conditions. Our results here confirm these observations, the investigated b_n ($n \geq 5$) ions as large as b_{13} of $A_{20}YFLV$ and also *b*-type fragments formed by sequence scrambling [for example b_9-A and b_9-2A for A_8YA (Figure 2)] undergo facile adduct formation with ammonia providing compelling experimental evidence for having a macrocyclic structure. It is worth noting here that our experiments do not indicate adduct formation (data not shown) for the b_4 ion of A_5 even though the computed binding energy for the macrocyclic isomer is high at 32.4 kcal mol^{-1}. This observation can be explained by dominance of linear structures for bare b_4; this is supported by recent theoretical [15] and IR studies [Paizs, B.; Maître, P., unpublished results].

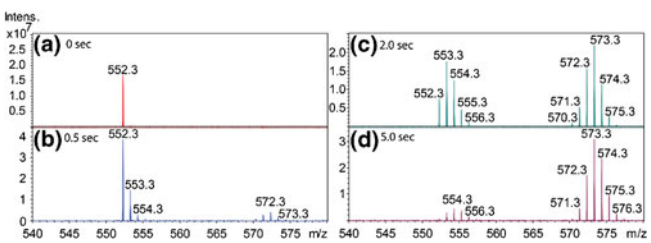

Figure 3. Adduct formation of protonated cyclo-(YAGFL) with ND_3 for (**a**) 0.0, (**b**) 0.5, (**c**) 2.0, (**d**) 5.0 s in the ICR cell

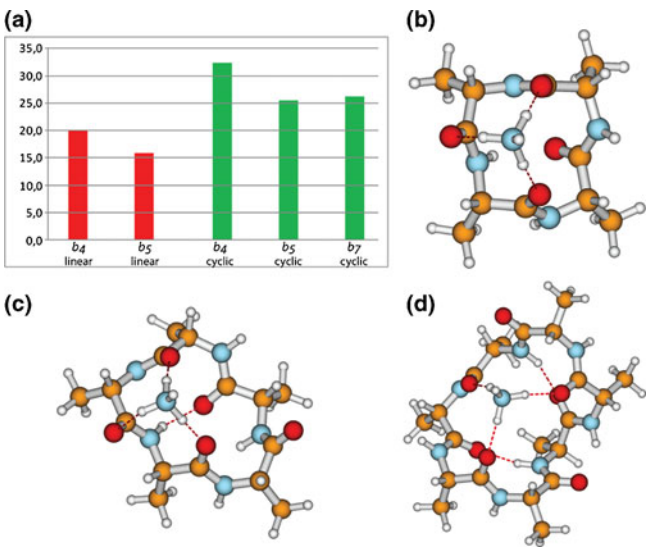

Figure 4. (**a**) Binding energies (kcal mol^{-1}) for selected linear and macro-cyclic isomers of all-Ala b_n ions. (**b**), (**c**), (**d**) Structures of ammonia adducts of macrocyclic b_4, b_5, and b_7 isomers

In conclusion, we present here strong experimental and theoretical evidence supporting isomer specific complex formation of a number of middle-sized b_n ($n \geq 5$) ions with ammonia under experimental conditions typically used to probe gas-phase HDX in FT-ICR MS. This observation enables us to use gas-phase guest–host chemistry to probe the structures of CID fragments significantly extending the outreach of our current physico-chemical tool-box available to study gas-phase peptide fragmentation chemistry. Our laboratories are currently investigating fine details of the underlying complexation chemistry using theory and 'action' IR spectroscopy. Further studies are underway on the kinetics of complex formation and related entropy effects, and on peptides with systematically varied amino acid composition using both ammonia and other amines for complexation. Since these experiments can be carried out in a semi high-throughput manner, we will investigate CID fragments of a large number of tryptic and Lys-C peptides.

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