The simplest b_2^+ ion: Determining its structure from its energetics by a direct comparison of the threshold collision-induced dissociation of protonated oxazolone and diketopiperazine

P.B. Armentrout*, Amy A. Clark

315 S. 1400 E. Rm 2020, Chemistry Department, University of Utah, Salt Lake City, UT 84112, United States

ABSTRACT

Energy-resolved collision-induced dissociation of b_2^+ ions derived from protonated GGG and GAG is compared with that of protonated diketopiperazine (H^+DKP). Absolute dissociation cross sections are obtained using a guided ion beam tandem mass spectrometer and analyzed using statistical methods. The fragmentation pattern of the b_2^+ ion from H^+GGG differs significantly from that of H^+DKP, although decarbonylation (forming the a_2^+ ion) is the lowest energy pathway found for both species. The threshold measured for b_2^+ decarbonylation differs appreciably from that of H^+DKP, but both processes have onsets that are consistent with those obtained from quantum chemical calculations, positively identifying the b_2^+ ion as the protonated 2-aminomethyl-5-oxazolone, H^+AMOx. The threshold for the b_2^+ ion derived from H^+GAG has a slightly lower threshold, again consistent with theory. Examination of the higher energy dissociation pathways observed shows that these generally involve subsequent dissociation of the primary a_2^+ product ion. As a consequence, statistical analysis of these pathways for threshold information is difficult and ultimately provides little agreement with theory. This disagreement is rationalized on the basis of considerable kinetic energy release that has been observed previously by Harrison for these subsequent reactions. The present results are also favorably compared with previous results obtained by Siu and coworkers, although a number of refinements in the analysis and mechanisms are suggested.

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1. Introduction

The gas phase fragmentation of protonated peptides often occurs at the peptide linkages, producing b ions when the charge is retained on the N-terminal fragment and y ions when the C-terminal fragment carries the charge. Structural identification of b ions along with their energetics and mechanisms of formation has been an active focus of much investigation recently because such information could aid in improving models for peptide fragmentation, thereby extending the utility of peptide sequencing by mass spectrometric approaches. For the smallest b_2^+ species, there are two principal structures: the six-membered cyclic diketopiperazine (DKP), formed by nucleophilic attack of the N-terminal amino group at the carbonyl carbon of the second residue; and the five-membered cyclic oxazolone (Ox), formed by nucleophilic attack of the carbonyl oxygen on the first reside at the carbonyl carbon of the second residue. A variety of mass spectrometric experiments and comparisons to theory, including seminal contributions from Harrison, have suggested that the oxazolone structure is generally

formed [1–8]. Recent studies using infrared multiple photon dissociation (IRMPD) spectroscopy combined with theory have provided more direct evidence for such identifications, again usually finding Ox structures [9–15]; however, the b_2^+ ion derived from protonated histidine–alanine (HA) shows a mixture of the DKP and Ox structures [16].

Although identification of the structures of the fragments of peptides is important information, improving models of peptide fragmentation would also benefit from experimental determinations of both mechanistic and energetic details, information that IRMPD studies are not capable of providing. Energy-resolved collision-induced dissociation (CID) studies can provide much of the needed energetic information, and in addition can provide an alternative means of determining structure as well as exploring mechanisms by comparisons to theory. However, such quantitative experimental characterizations of peptides are few [17–25], with most not capable of acquiring accurate energetic information. An early pioneering study of Klassen and Kebarle [18] examined kinetic energy-resolved CID fragmentation of protonated G_n ($n = 1$–4) and several related compounds, acquiring thresholds for the primary dissociation pathways. Siu and coworkers examined the energy-resolved CID of protonated GGG, AGG, and GAG and extracted threshold energy information that compares well with

* Corresponding author. Tel.: +1 801 581 7885; fax: +1 801 581 8433.
E-mail address: armentrout@chem.utah.edu (P.B. Armentrout).

theory [20,21]. Recently, we completed a similar study of protonated glycine and diglycine, again obtaining information that could be compared favorably with theory [23–25]. These latter studies illustrate the utility of having good quantitative thermodynamic information available to confirm theoretical studies of mechanisms and product structures.

In addition to the examination of the dissociation of intact amino acids and peptides, in-source fragmentations can be used to generate fragment ions, which can then be subject to interrogation using IRMPD (as in the studies referenced above) or energy-resolved CID. Indeed, Siu and coworkers have previously examined energy-resolved collision-induced dissociation of the b_2^+ and a_2^+ ions formed by decomposition of H^+GGG [20]. For the b_2^+ ion, they observed two products: loss of CO to form the a_2^+ product ion and formation of the a_1^+ ion, $CH_2NH_2^+$, although no cross sections for these processes are provided. For the a_2^+ ion, the primary product is a_1^+ along with an additional minor product ion at m/z 59, but no energetic information is given for this product. Their energy-dependent cross sections were analyzed using our data analysis program, CRUNCH, to obtain threshold energies (with and without kinetic shifts), although none of the additional parameters used to reproduce the data were reported (such that the shape and magnitudes of the cross sections remain unknown). Theoretical calculations at a B3LYP/6-31++G(d,p) level were used to outline the mechanisms for these fragmentations, with good agreement reported between experimental and theoretical threshold energies. Similar experiments and calculations were carried out for the b_2^+ and a_2^+ ions formed from H^+GAG and H^+AGG [21].

In the present work, we initiate our own exploration of peptide fragment ions by examining the simplest b_2^+ ion, the major product ion formed in the fragmentation of H^+GGG. This provides an ideal test system as both previous IRMPD [13] and energy-resolved CID [20] work is available for comparison. IRMPD studies identify the structure of the b_2^+ ion as protonated 2-aminomethyl-5-oxazolone (H^+AMOx, protonated on the ring nitrogen) by good agreement with theory and by direct comparison with an authentic sample of protonated diketopiperazine (H^+DKP, protonated on the oxygen). The CID work similarly finds consistency between theory and experiment when the b_2^+ ion is identified as H^+AMOx, although no direct comparisons to H^+DKP were made. As described in the Supplemental Information, there are some questions regarding these triple quadrupole experiments, such that a reinvestigation of these systems is also warranted. In addition, the present study extends this work: (a) by examining the b_2^+ fragmentation of H^+GGG and H^+GAG over a broader range of energies and with more sensitivity, (b) by directly comparing the fragmentation of the b_2^+ ion with that of protonated DKP, and (c) by revealing additional details regarding the relevant potential energy surfaces through an exploration at higher levels of theory.

2. Experimental and computational

2.1. General experimental procedures

Cross sections for CID of the ions investigated here are measured using a guided ion beam tandem mass spectrometer that has been described in detail previously [26,27]. Ions are generated using an electrospray ionization (ESI) source [28–31] and an in-source fragmentation technique [32] for generation of thermalized b_2^+ ions from H^+GGG and H^+GAG. Details of the experimental methods and means of data analysis [33–35] can be found in the Supplemental Information. Measured cross sections have absolute and relative uncertainties of ±20% and 5%, respectively. The energy scale has an absolute uncertainty of ±0.03 eV in the center-of-mass frame.

2.2. Computational approach

Model structures and vibrational frequencies for reactants, products, transition states, and intermediate species were performed at the B3LYP/6-311+G(d,p) level with energetics corrected for zero point energies determined by single point calculations at B3LYP/6-311+G(2d,2p) and MP2(full)/6-311+G(2d,2p) levels. Recent work has shown that these levels of theory provide accurate comparisons with experimental energetics for such protonated systems, with mean absolute deviations of about 10 kJ/mol [23,24,36]. Details of the procedures used can be found in the Supplemental Information. We also compare these results with lower level B3LYP/6-31++G(d,p) results of Siu and coworkers [20,21]. In the Supplemental Information, a comparison of these approaches with previous literature results [23] suggests that the double-ζ basis set yields energies that are high. Indeed, for the present systems, these calculations average $16 ± 10$ kJ/mol higher than the present triple-ζ B3LYP/6-311+G(d,p) results.

2.3. Nomenclature

To identify the various conformations of the molecules in this work, we use a nomenclature derived from the peptides that act as precursors to the b_2^+ ions and their fragments. This nomenclature specifies the site of protonation in brackets followed by a designation of any dihedral angles going from nitrogen that was the N terminus (N_1) along the backbone of the molecules, where c (cis) stands for angles < 45°, g (gauche) for angles between 45° and 135°, and t (trans) for angles > 135°. Thus the ground state (GS) of H^+AMOx is $[N_2]$-c where the N_2 designation indicates the proton is on the ring nitrogen and the aminomethyl group is cis relative to the ring ($\angle NCCN$ dihedral angle). The GS of H^+DKP is $[O_c]$ indicating that the carbonyl oxygen is protonated and the proton has a cis orientation along the $\angle CCOH$ dihedral. The position of the N-terminal amino group of H^+AMOx (when not protonated or involved in a hydrogen bond) can also be rotated but is often such that the lone-pair is cis with respect to the CC bond. When the amino group is rotated to the trans position, the $\angle NCCN$ dihedral is augmented by a subscript t. Transition states are indicated by TS followed by the protonation site and backbone conformation. TSs for proton transfer steps are named like $TS[N_1-N_2]$-c and those for dihedral angle rotations as $TS[N_2]$-(cg). TSs involving bond cleavage indicate the bond being broken by ∼ inside curly brackets, e.g., $\{OC\sim O\}$. Although more complicated than simply numbering species, we believe this nomenclature allows better visualization of the species.

3. Results and discussion

3.1. Cross sections for collision-induced dissociation

Kinetic energy dependent experimental cross sections were obtained for the interaction of Xe with the b_2^+ ion of H^+GGG, H^+DKP, and the b_2^+ ion of H^+GAG, Fig. 1. Data shown are a mean of results taken at xenon pressures of ∼0.05 and 0.1 mTorr, as little pressure dependence was detected within our experimental uncertainties at these lower pressures, but at the higher pressure of 0.2 mTorr, shifts in the apparent thresholds are obvious, especially for the higher energy channels. Three processes are observed in the b_2^+ (m/z 115) system. The primary product is loss of CO to form the a_2^+ product ion at m/z 87 in reaction (1). The cross section for this species reaches a maximum near the onset of the species observed at higher energies, m/z 59 and 30, indicating subsequent decomposition or competition. These peaks can be identified as $H^+(CH_2NH)_2$ and $CH_2NH_2^+$ (a_1^+), respectively. The small