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Serine effects on collision-induced dissociation and photodissociation of peptide cation radicals of the $z^{+\bullet}$-type

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ABSTRACT

The serine residue displays specific effects on the dissociations of peptide fragment cation-radicals of the $z^{+\bullet}$-type which are produced by electron transfer dissociation. Energy-resolved collision-induced dissociation (ER-CID), time-resolved infrared multiphoton dissociation (TR-IRMPD), and single-photon UV photodissociation at 355 nm revealed several competitive dissociation pathways consisting of loss of OH radical, water, and backbone cleavages occurring at *N*-terminal and *C*-terminal positions relative to the serine residue. The activation modes using slow-heating and UV photon absorption resulted in different relative intensities of fragment ions. This indicated that the dissociations proceeded through several channels with different energy-dependent kinetics. The experimental data were interpreted with the help of electron structure calculations that provided fully optimized structures and relative energies for *cis* and *trans* amide isomers of the $z_4^{+\bullet}$ ions as well as isomerization, dissociation, and transition state energies. UV photon absorption by the $z_4^{+\bullet}$ ions was due to C_α-radical amide groups created by ETD that provided a new chromophore absorbing at 355 nm.

1. Introduction

Effects of amino acid residues on gas-phase peptide ion dissociations have been extensively studied for various ion activation modes. Aspartic acid [1–3], proline [4] and histidine [5] are known to affect collision-induced dissociations (CID) of even-electron ions by enhancing backbone cleavage in their vicinity. Proline [6] and histidine [7–10] have major effects on hampering peptide ion backbone dissociations induced by electron attachment such as electron capture (ECD) [11] and electron transfer dissociation (ETD) [12]. In contrast, the serine residue has been found not to be prominent in affecting low-energy CID or ECD of peptide ions [6] although effects on the high-energy (kilo-electronvolt) CID spectra [13] and dissociations of negative peptide ions [14] have been noted. Radical-driven dissociations of ternary copper complexes showed a dominant loss of formaldehyde which was specific for the serine residue [15].

Fragment ions formed by ECD or ETD presumably retain intact partial amino acid sequences and thus can be used to infer additional sequence information from their CID [16–18]. In particular, fragment ions of the $z^{+\bullet}$-type have been addressed in detail as a matter of both survey [16–18] and mechanistic [19–21] studies. $z^{+\bullet}$-type ions are formed by backbone cleavage of bonds between the amide nitrogens and the alpha carbon atoms of the adjacent amino acid residues and represent truncated and deaminated peptide cation-radicals extending from the *C*-terminus. $z^{+\bullet}$-type ions represent a bridge between the chemistry of hydrogen-rich and hydrogen-deficient peptide cation-radicals; their structure and dissociations have been of much interest [22].

The amino acid residues that affect $z^{+\bullet}$-ion dissociations have been assigned to three categories [20]. The first type involves amino acids (Phe Tyr His Trp Val) that strongly promote backbone dissociations at positions two residues toward the *C*-terminus, forming $z_{n-2}^{+\bullet}$ fragment ions from $z_n^{+\bullet}$ precursors. The second type involves amino acids that undergo facile radical-induced side-chain dissociations such as loss of C_3H_7 from Leu or loss of SH from Cys. The third type involves z ions from amino acids in which backbone dissociations compete with side-chain losses, such as in Asp and Asn [20].

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We now report an energy-resolved CID, time-resolved infrared multiphoton photodissociation (IRMPD) and UV photodissociation (UVPD) study of serine-containing $z^{+\bullet}$ ions in which the serine residue promotes *N*-terminal backbone cleavages. This feature is related to radical reactions involving the serine side chain, as revealed by extensive electron structure calculations of isomer structures in addition to relative, transition-state, and dissociation energies that provide a comprehensive description of the cation-radical unimolecular chemistry.

2. Experimental

2.1. Materials and methods

All peptides used in this work were custom-synthesized by NEOPeptide Laboratories (Cambridge, MA). ETD mass spectra were measured on a Thermo Fisher (San Jose, CA, USA) LTQ XL quadrupole linear ion trap (QLT) instrument, outfitted with a chemical ionization source for the production of fluoranthene anion radicals as ETD reagent, as described previously [23]. High-resolution ETD spectra were measured under the same conditions on a modified Orbitrap mass spectrometer [24] at nominal resolution of 60,000. The typical data acquisition parameters were as follows: *q* value 0.25, ion–ion reaction time 100–200 ms, CID excitation time 30 ms. The LTQ XL mass spectrometer was modified to allow for the introduction of IR photons to the ion trapping region of the QLT [25]. For the energy-resolved experiments, CID normalized collision energies (NCE) of zero to 50 correspond roughly to supplemental AC excitation voltages of 0 to ~2.5 V. Infrared multiphoton dissociation was carried out by operating the laser in a mode whereby the laser was externally triggered via a TTL pulse from pin14 of the J1 connector. Laser power was set as indicated in the text.

UV photodissociation (UVPD) measurements were performed on another modified LTQ XL ETD mass spectrometer that was furnished with a pulsed E/O Q-switched Nd:YAG diode laser (EKSPLA NL301, Altos Photonics, Bozeman, MT) and a third harmonics generator providing up to 110 mJ/pulse at 355 nm with a 20 Hz pulse frequency and 3–6 ns pulse width. For the UVPD experiments, the laser power was set to 15–18 mJ/pulse. This ensured one-photon absorption, as established by a calibration that showed a linear ion depletion curve as a function of the laser power. Laser pulses were triggered by LabView software that received a signal from a TTL pulse from pin14 of the J1 connector on the LTQ console that was controlled from within the mass spectrometer data acquisition software (Xcalibur Version 3.7). ETD fragment ions were generated at 100–300 ms ion–ion interaction time, selected by mass, and stored in the ion trap for 400 ms while the laser was off. The trapped z_4 ions were then irradiated by a variable number of laser pulses (0–7) during the 400 ms storage time, and the UVPD products were mass analyzed. A typical spectrum consisted of 50 accumulated UVPD scans. For comparison, low-level CID mass spectra at NCE = 13 were recorded over a 400-ms excitation time.

2.2. Calculations

All electron structure calculations were performed using the Gaussian 09 suite of programs as described previously [26]. Ion geometries were optimized with density functional theory calculations using the hybrid B3LYP [27,28] and M06-2X [29] functionals in the spin-unrestricted format and the 6-31+G(d,p) basis set. The geometries in the Cartesian coordinate format (standard orientation) are available from the corresponding author upon request. The optimized structures were confirmed as local energy minima or first-order saddle points by harmonic frequency calculations. In addition, Møller–Plesset theory [30], UMP2 (frozen core), with the 6-311++G (2d,p) basis set was used to obtain single-point energies, which were corrected for contribution of higher spin states by the standard spin annihilation procedure [31,32]. The UB3LYP and PMP2 single-point energies were averaged (B3-PMP2) to cancel small errors inherent to both approximations, as reported previously [33–35].

3. Results and discussion

3.1. z_4 ion formation and dissociations

Electron transfer dissociation of the doubly charged (AASAR + 2H)$^{2+}$ ion (*m*/*z* 238) forms a series of $z_1^{+\bullet}$–$z_4^{+\bullet}$ backbone fragment ions (Fig. S1, Supplementary data). The spectrum also shows a prominent fragment ion at *m*/*z* 459 which is formed by loss of ammonia from charge-reduced (AASAR + 2H)$^{\bullet}$. Such species are sometimes denoted as $z^{+\bullet}$-type fragment ions, provided the ammonia molecule was eliminated from the protonated *N*-terminus, which is typical for peptide ions containing lysine *C*-terminal residues [36]. However, there is evidence [37] that ammonia eliminated from arginine *C*-terminated peptide cation-radicals partly originates from the guanidine group, rendering the fragment ion structure uncertain.

The z_4 ion at *m*/*z* 388 was selected and subjected to either collisional activation or photoexcitation. The overall ETD-CID-MS^3 mass spectrum is shown in Fig. 1a. The spectrum contains a prominent fragment ion due to loss of water (*m*/*z* 370), which is accompanied by an *m*/*z* 371 ion by loss of OH radical. The other prominent fragment ions are at *m*/*z* 358 (loss of CH_2O), 316 (loss of C_3H_6NO), 315 (loss of C_3H_7NO) and *m*/*z* 230 (z_2). The identity of the neutral fragments was supported by accurate mass measurements in high-resolution ETD-CID-MS^3 mass spectra that showed agreement with theoretical masses within ±0.0007 Da (Fig. S2, Supplementary data), allowing unambiguous neutral fragment assignment. The MS^3 mass spectrum of the $z_4^{+\bullet}$ ion from AASAR is compared to those of $z_4^{+\bullet}$ ions from AACAR, AADAR, and AAAAR (Fig. 1b–d, respectively). These ions show residue-specific dissociations by loss of SH and CH_2S from AACAD, CO_2 from AADAR, and C_2H_3NO from AAAAR. The losses of OH, water, CH_2O and C_3H_xNO (x = 6, 7) are specific for the serine residue as they are nearly absent in the MS^3 spectra of the other $z_4^{+\bullet}$ ions. The $z_4^{+\bullet}$ ion from AACAR shows a minor peak at *m*/*z* 331, which can be assigned to loss of a 73 Da molecule, presumably C_3H_7NO.

3.2. Energy-resolved CID

The energy-resolved intensities of the *m*/*z* 388 precursor and major fragment ions are plotted in Fig. 2. The *m*/*z* 315 fragment ion shows the lowest energy onset at NCE ≈ 11 followed by a rapid increase of this ion intensity. The next threshold at NCE ≈ 12 belongs to the *m*/*z* 358 fragment ion (loss of CH_2O), followed by a shallow curve of its increasing intensity. The *m*/*z* 370 (loss of water) and *m*/*z* 230 ($z_2^{+\bullet}$) ions show indistinguishable onsets at NCE ≈ 13. Of these two, the loss of water fragment ion intensity shows a substantially steeper increase with the precursor ion excitation energy.

Note that the CID-MS^3 spectra were generated by resonant excitation of the *m*/*z* 388 ion, whereas the fragment ions formed from it were not accelerated for further collisional activation. Conversely, the fragment ions could lose internal energy by collisions with the He buffer gas in the linear ion trap. This is evidenced by CID-MS^4 mass spectra of mass-selected MS^3 fragment ions. Upon resonant excitation, the *m*/*z* 358 ion underwent major losses of water and CO_2 giving fragment ions at *m*/*z* 340 and 314, respectively (Fig. S3, Supplementary data). These secondary fragments were weak or nearly absent in the CID-MS^3 spectrum in Fig. 1a, indicating that the *m*/*z* 358 ion did not have sufficient