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On the Mechanism of Electron-Capture-Induced Dissociation of Peptide Dications from ^{15}N-Labeling and Crown-Ether Complexation

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^{15}N-labeling of di- and tripeptides reveals that electron capture to doubly protonated peptides results almost exclusively in ammonia loss from the N-terminal end, which clearly shows that a significant fraction of electron capture occurs at this end. In accordance with this finding, the competing channel of N−C_α bond breakage leads to $z^{+\bullet}$ ions and neutral *c* fragments after electron capture to small dications. In larger peptides that live long enough for internal proton exchanges to occur, c^+ ions are also formed and in some cases in dominant yield. Attachment of one or two crown ethers to ammonium groups is likely to reduce the probability of proton transfer, which enhances the formation of $z^{+\bullet}$ relative to c^+. The total yield of $z^{+\bullet}$ and c^+ is, however, more or less unchanged, which indicates that proton transfer or hydrogen transfer from a NH_3 group to the amide group is not required for the N−C_α bond breakage.

Introduction

Electron capture (EC) by peptide cations in the gas-phase results in extensive backbone fragmentation to give the sequence-specific c^+ and $z^{+\bullet}$ ions in competition with hydrogen and ammonia loss.[1] The mechanism of electron capture dissociation (ECD) is a hotly debated subject. One recent model assumes EC at the amide C═O bond followed by fast proton abstraction from a nearby ammonium driven by the Coulomb attraction.[2] Coulomb-assisted attachment to S−S and C−S bonds was also proposed by Simons and co-workers.[3] Here, experimental data that indicate electron capture by the N-terminal ammonium group in addition to some support for capture at the amide bond is presented. Electron capture by small ^{15}N-labeled peptide dications was investigated by colliding such ions with cesium atoms in an accelerator mass spectrometer to provide information about the subsequent ammonia loss channel. The peptides studied were $[AK + 2H]^{2+}$, $[KK + 2H]^{2+}$, $[AAK + 2H]^{2+}$, and $[GHK + 2H]^{2+}$ (A = alanine, K = lysine, G = glycine, H = histidine), all with lysine at the C-terminal end to allow for double protonation of the peptides. The N-terminal ends were ^{15}N-labeled to identify from which end ammonia loss occurred. In other experiments, internal proton exchanges were prohibited by the attachment of crown ether to one or to both ammonium groups. Our results indicate that the amide group plays a major role in the electron capture process, directing the electron to the nearest ammonium group and that the probability of N−C_α bond breakage does not strongly rely on crown ether attachment, whereas the relative ratio between $z^{+\bullet}$ and c^+ ions does.

In a series of papers, we have shown that capture of electrons by peptide cations from alkali-metal atoms produce fragmentation similar to that resulting from the capture of thermally generated free electrons.[1,4] In the former process, the electron is transferred from either sodium or cesium atoms to ions traveling at high velocity, the interaction time being a few femtoseconds. This technique is named electron-capture-induced dissociation (ECID). The similar fragmentation spectra obtained from ECD and ECID indicate similar dissociation mechanisms.

In this work, solid-phase synthesis was used to make ^{15}N-labeled peptides (see the Supporting Information), which were electrosprayed to produce doubly protonated peptides in the gas phase. After acceleration to a kinetic energy of 100 keV, ions of interest were mass selected by a magnet and collided with cesium vapor in a collision cell.[5] The cesium pressure was high enough that more than $^2/_3$ of the ion beam was converted to singly charged ions. Velocities of $[KK + 2H]^{2+}$ and of the dication with one crown ether attached are 2.6×10^5 m/s and 1.9×10^5 m/s, respectively, which results in flight times from the collision cell to the electrostatic analyzer of about 6 and 8 μs, respectively (flight distance of about 1.5 m). Other experiments were also performed at a lower pressure under single-collision conditions. The resulting product ions were scanned in an electrostatic analyzer to provide ECID spectra.

Results for $[GHK + 2H]^{2+}$ and $[AAK + 2H]^{2+}$ are shown in Figure 1 where the dominant channels after electron capture are hydrogen loss, ammonia loss and N−C_α bond breakage as

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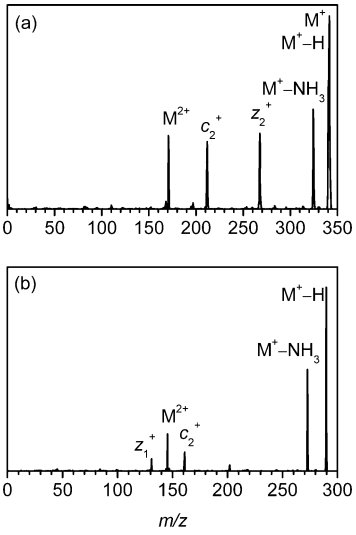

Figure 1. ECID spectra of (a) $[GHK + 2H]^{2+}$ and (b) $[AAK + 2H]^{2+}$. M^{2+} denotes the parent ion.

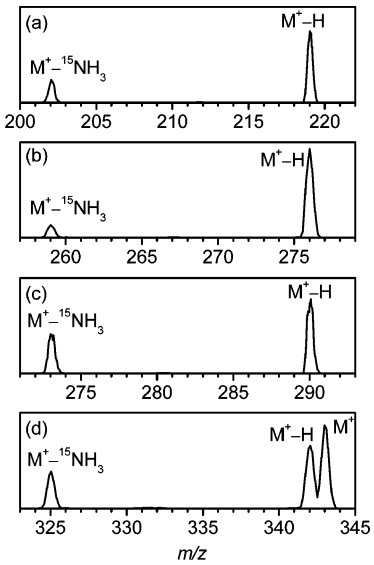

Figure 2. Partial ECID spectra of (a) $[AK + 2H]^{2+}$, (b) $[KK + 2H]^{2+}$, (c) $[AAK + 2H]^{2+}$, and (d) $[GHK + 2H]^{2+}$. M^+ denotes the intact reduced parent ion. All four peptides are ^{15}N-labeled at the N-terminal end.

described earlier.[4b] In the case of $[GHK + 2H]^{2+}$, the intact charge-reduced ion, $[GHK + 2H]^{+\bullet}$, was observed.

Higher resolution ECID spectra of $[AK + 2H]^{2+}$, $[KK + 2H]^{2+}$, $[AAK + 2H]^{2+}$, and $[GHK + 2H]^{2+}$ in the region of the ammonia loss peaks are shown in Figure 2. In all cases, there is a peak that corresponds to an ion with mass 18 Da less than that of the charge-reduced parent ion, which clearly demonstrates that $^{15}NH_3$ is lost. Loss of $^{14}NH_3$ (mass 17) from the lysine side chain occurs with a probability less than 3%, which indicates that electron capture followed by ammonia loss is governed by the nearby environment of the ammonium group. Elimination of NH_3 mainly from the N-terminus is consistent with the computational analysis by Tureček and Syrstad.[6] Yao et al.[7] predicted that H loss is likely to originate from the side-chain NH_3 group where it wins over the loss of ammonia, which is also consistent with our results. However, we cannot exclude the possibility that some or all H loss occurs from the N-terminal end.

Electron capture by the N-terminal end may result in a greater abundance of $z^{+\bullet}$ versus c^+ after $N-C_\alpha$ cleavage. Interestingly,

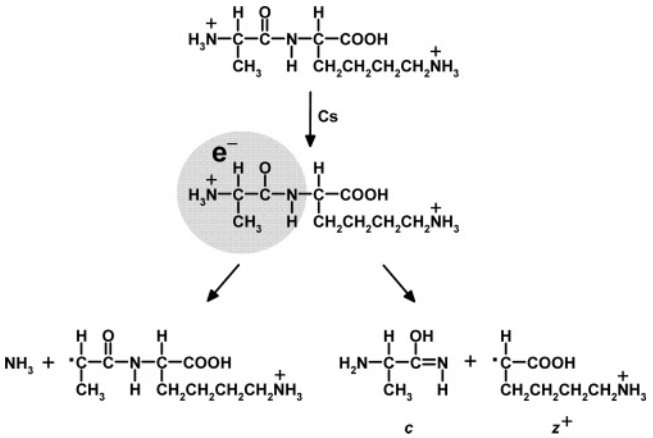

Figure 3. Electron capture to the N-terminal end leads to ammonia loss from this end and $z^{+\bullet}$ ions.

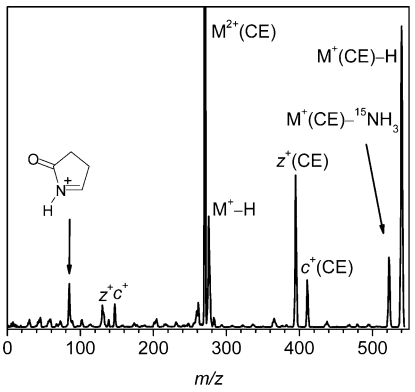

Figure 4. ECID spectrum of $[\alpha\text{-}^{15}N\text{-}KK + 2H]^{2+}$(CE), CE = 18-crown-6.

however, EC by $[AK + 2H]^{2+}$ produced mainly $z^{+\bullet}$ ions, EC by $[KK + 2H]^{2+}$ produced mainly c^+ ions, whereas c^+ and $z^{+\bullet}$ ions were formed in similar numbers after EC by $[AAK + 2H]^{2+}$ and $[GHK + 2H]^{2+}$.[3] The two *z* and *c* fragments may stay together as an ion−molecule complex allowing for proton exchanges.[8] In the case of $[KK + 2H]^{2+}$ and tripeptides, there may also be internal ionic hydrogen bonding between different amino acid residues. As a result, c^+ will be formed in competition with $z^{+\bullet}$ ions dependent on the lifetime of the complex and the proton affinity of the *c* versus *z* fragment.

Complexes of crown ether (CE = 18-crown-6) and peptides were investigated to lower the probability of proton transfer. The preferred site of binding is the side chain of lysine.[9] Adduct formation between $[AK + 2H]^{2+}$ and crown ether does not decrease the probability, *P*, of $N-C_\alpha$ cleavage after electron capture from sodium ($P = 0.32$, 0.41, and 0.33 for $[AK + 2H]^{2+}$, $[AK + 2H]^{2+}$(CE), and $[AK + 2H]^{2+}(CE)_2$, respectively, measured under single-collision conditions),[10] which indicates that proton or hydrogen atom transfer from $-NH_3$ is unimportant for this fragmentation. This is in accordance with previous work on peptides metalated with divalent metal ions where z^+ and c^+ ions were also formed after electron capture.[11] For the supramolecular complex between $[KK + 2H]^{2+}$ and one crown ether, ammonia loss occurs exclusively from the N-terminal end but the abundance of $z^{+\bullet}$ ions is 4 times larger than that of c^+ ions (Figure 4). A similar enhancement in $z^{+\bullet}$ formation was obtained for $[KK + 2H]^{2+}(CE)_2$. In agreement with these results, we also found that EC by $[GHK + 2H]^{2+}$(CE) and $[AAK + 2H]^{2+}$(CE) led to dominant formation of $z_1^{+\bullet}$ and $z_2^{+\bullet}$ ions and much diminished yield of c^+ ions because the ammonium group of the lysine side chain is less likely to donate its proton to the

neutral *c* fragment. It should be emphasized that association of crown ether with the ion also affects the stabilities of the charge sites and the recombination energies upon electron capture, which may also affect the eventual product partitioning.

In our previous work, we have found that the probability of $N-C_\alpha$ bond breakage is about 20−30% for a series of peptides ranging in size between 2 and 11 residues.[4b] Direct electron transfer to the amide site has been predicted to occur at a rate approximately 100 times less than that for transfer to the protonated amine site in the case of a singly charged model peptide,[12] but this number is likely different for doubly charged peptides where there is additional Coulomb stabilization of NCO^-. The authors also found that once the electron is captured at either the protonated amine site or the Coulomb-stabilized amide site it will remain on that site rather than undergo a subsequent intramolecular transfer to the other site. In contrast, an electron can undergo a through-bond transfer from a Rydberg state of $-NH_3$ to a S−S σ^* state.[13] These previous findings and those presented in this work are therefore consistent with the superbase mechanism,[2] but there may be other plausible explanations of these data.

To summarize, we have found that electron capture by dipeptide and tripeptide dications leads to ammonia loss from the N-terminal end, which implies that significant electron capture close to this group, if not directly, occurs. A competing reaction is $N-C_\alpha$ bond breakage to give $z^{+\bullet}$ or c^+ ions. Attachment of crown ether to ammonium groups should significantly reduce the probability of H or H^+ transfer from these sites, but the propensity for $N-C_\alpha$ bond breakage is more or less unchanged. The branching ratio between $z^{+\bullet}$ and c^+, however, increases in favor of $z^{+\bullet}$, again consistent with chemistry associated with the electron at the N-terminal end.

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Supporting Information Available: Synthetic procedures for ^{15}N-labeled peptides as well as the ECID spectrum of $[KK + 2H]^{2+}(CE)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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