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Electron capture-induced dissociation of AK dipeptide dications: Influence of ion velocity, crown-ether complexation and collision gas

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ABSTRACT

The fragmentation of doubly protonated AK dipeptide ions has been investigated after collisional electron transfer. Electron capture leads to three dominant channels, H loss, NH_3 loss, and N–C_α bond breakage to give either c^+ or z^+ fragment ions. The relative importance of these channels has been explored as a function of ion velocity, the degree of complexation with crown ether, and collision gas. Our results indicate that H loss and NH_3 loss are competing channels whereas the probability of N–C_α bond breakage is more or less constant.

1. Introduction

Electron capture dissociation (ECD) of peptides and proteins in combination with mass spectrometry is now a common method to obtain the sequence of amino acids [1–7]. It benefits from the selective cleavage of N–C_α bonds and generates an even backbone fragmentation pattern. ECD, which involves capture of free electrons, has been studied in great detail but the actual mechanism is still an open question. Instead of causing dissociation by the attachment of free low-energy electrons, dissociation can also be initiated by electron transfer from an anion in low-energy collisions (electron transfer dissociation (ETD)) [8–12] or by electron capture from a neutral gas target in high energy collisions, typically tens of kiloelectronvolt (electron capture-induced dissociation (ECID)) [13–16].

In Aarhus we carry out ECID to mimic ECD, and a typical spectrum for the doubly protonated dipeptide $[AK+2H]^{2+}$ (A = alanine, K = lysine) is shown in Fig. 1. There are three main dissociation channels after electron capture from sodium or cesium, loss of hydrogen, loss of ammonia, and cleavage of the N–C_α bond to give either c^+- or z^+-ions as indicated in Fig. 1. The ammonia is lost almost exclusively from the N-terminal end as recently shown from ^{15}N-labeling experiments [15]. There are minor peaks due to collision-induced dissociation (CID); these can be identified from experiments with neon as collision gas where no electron transfer occurs. For larger peptides such as bradykinin and substance P, the intact charge-reduced cation was detected and in a significantly higher yield than in ECD, by an order of magnitude or more [14,17]. However, with respect to the distributions and relative intensities of z^+ and c^+ fragments, ECID and ECD spectra are similar (Fig. 2). This result is intriguing for at least two reasons: (1) the time scales for the experiments are very different, typically a few microseconds for ECID (flight time after electron capture to detector) vs. tens of milliseconds for ECD (trap experiment) and (2) the energetics of the capture processes are different [18]. In ECD the recombination energy of 4–6 eV is fully gained by the peptide whereas in ECID the excitation energy depends on the electronic state populated in the electron transfer process.

In our experiments, ions are typically accelerated to 100-keV kinetic energy and collided with sodium, cesium, or with other

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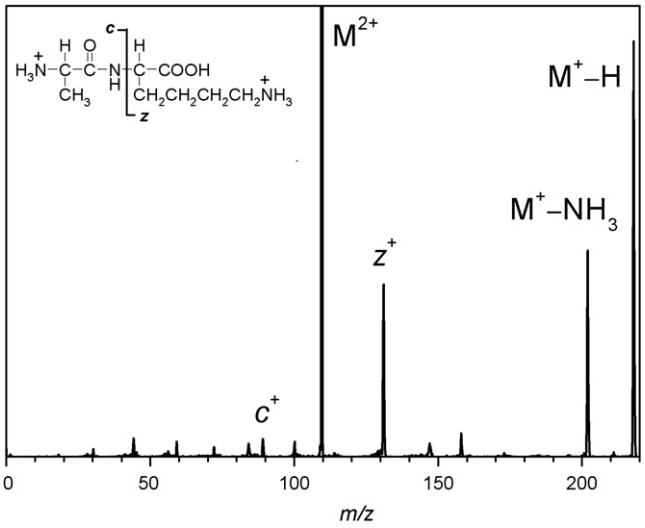

Fig. 1. ECID spectrum of $M^{2+} = [AK + 2H]^{2+}$ obtained after electron capture from Na.

atomic or molecular targets. Alkali metal targets are often chosen because of the weakly bound valence s-electron that is readily transferred to the doubly charged peptide ion. Other gases can be used despite a lower total electron capture cross-section. The concern of the present work is to elucidate how the relative importance of the three fragmentation channels, H loss, NH_3 loss and N–C_α bond cleavage in the case of the AK dipeptide depends on the acceleration voltage, the ionization energy of the collision gas, and coordination of crown ether (CE, 18-crown-6 ether $(C_2H_4O)_6$)

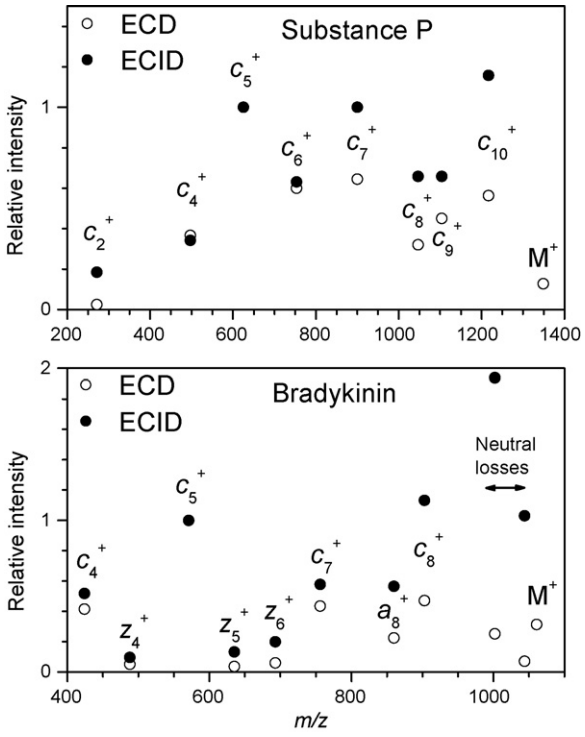

Fig. 2. Comparison of fragment ion distributions obtained from ECD and ECID of doubly protonated substance P (RPKPEEFFGLM) and bradykinin (RPPGFSPRR). The intensity of the c_5^+-ion is set to 1 both for ECD and ECID. The ECID values for the M^+-ion are 6.2 and 7.8 for substance P and bradykinin, respectively. ECD data were taken from Ref. [17] and the spectrum of substance P was kindly provided by Dr. K.F. Haselmann, and ECID data from Refs. [13,14].

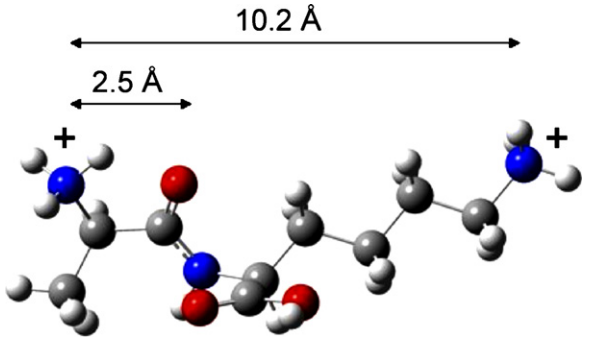

Fig. 3. One possible structure of $[AK + 2H]^{2+}$ obtained at the B3LYP/6-31+G(d) level of theory.

to the ammonium groups. The latter affects the chemistry in at least three different ways, it prohibits internal hydrogen or proton transfer after electron capture, it lowers the probability of internal ionic hydrogen bonding between the ammonium group and the amide or the carboxylic acid group to give a more extended structure, and it reduces the energy for electron recombination. The AK dipeptide was chosen since it is small and relatively easy to handle with quantum chemistry models. The Coulomb repulsion between the two charged ammonium groups limits the conformational flexibility and dictates somewhat extended structures even though internal ionic hydrogen bonding does play a role. A possible structure of $[AK + 2H]^{2+}$ at the B3LYP/6-31+G(d) level of theory is shown in Fig. 3.

2. Experiment

The experimental setup has been described in detail elsewhere [19,20]. Doubly protonated AK dipeptides were generated by electrospray (water/methanol (1:1) with 5% acetic acid), introduced into an accelerator mass spectrometer, accelerated to kiloelectronvolt energies, m/z selected and passed through a heated metal vapor cell. Product ions were analyzed by a hemispherical electrostatic analyzer. Collisions with noble gases, NO and O_2 were also performed. In additional experiments, crown ether (18-crown-6 ether) was added to the solution to produce noncovalent peptide–crown-ether complexes.

3. Results and discussion

First we measured the cross-section for electron capture in collisions between $[AK + 2H]^{2+}$ and sodium as a function of the acceleration voltage between 10 kV and 70 kV, which is the lower and upper limit for our experiment. The cross-section increases strongly from 10 kV to 70 kV, by about a factor of five (Fig. 4). It is expected to reach a broad maximum, according to Massey's adiabatic criterion [21], before it falls off at considerably higher energies than the ones used here. Our data clearly show that there is an energy defect for electron transfer from sodium to $[AK + 2H]^{2+}$, and that quite high acceleration voltages are advantageous for ECID. In other words, there is no quasi-continuum of states resonant with the energy of the active electron from sodium for which very weak velocity dependence would be expected. The energy defect is the numerical difference between the ionization energy of sodium and the energy gained by the ion after capture of an electron from sodium.

Next we consider how the three dissociation channels after electron capture depend on the acceleration voltage. The relative probability of H loss in ECID, $P(-H)$, is obtained from the abundance of H-loss ions divided by the total abundance of ions that

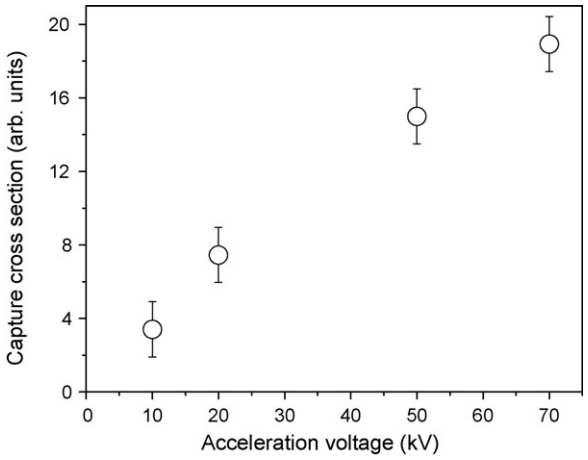

Fig. 4. Electron capture cross-section of $[AK+2H]^{2+}$ as a function of acceleration voltage. Na was used as target gas. The cross-section is in arbitrary units.

arise from electron capture. This dissociation process is the most likely, and it occurred for about half of the ECID events. The probability of NH_3 loss, $P(-NH_3)$, and those for the formation of either z^+ or c^+, $P(z^+)$ and $P(c^+)$, were deduced in similar ways. It appears from Fig. 5 that the probabilities for the formations of z^+- and c^+-ions are more or less independent of the acceleration voltage taking values of 0.3 and 0.03, respectively. On the other hand, the H-loss channel decreases in importance from a $P(-H)$ value of about 0.5 at the two lowest acceleration voltages to about 0.4 at the two highest voltages. This decrease is accompanied by an increase of the ammonia loss channel from a $P(-NH_3)$ value of 0.2 to a higher value of 0.3. These data thus suggest that the electronic state being populated in the electron transfer process depends somewhat on the acceleration voltage, and that the H-loss and NH_3-loss channels compete. If these two losses occur on the electronic ground state potential energy surface, the barriers are about 0.1 eV for H loss and 0.1–0.5 eV for NH_3 loss (lowest for loss of N-terminal ammonia) [18,22]. Small changes in excitation energy may significantly alter the branching ratio between these two channels. The excitation energy probably increases with the collision velocity, which according to our data seem to be in favor of NH_3 loss. Another possibility is that different conformers are present in the beam, and that they have different electron capture cross-sections depending on the energy defect and therefore the collision velocity.

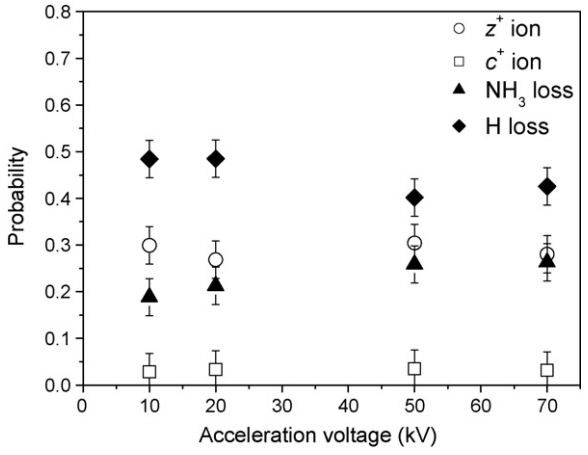

Fig. 5. Probability of formation of the different ECID ions of $[AK+2H]^{2+}$ after electron capture from Na as a function of the acceleration voltage.

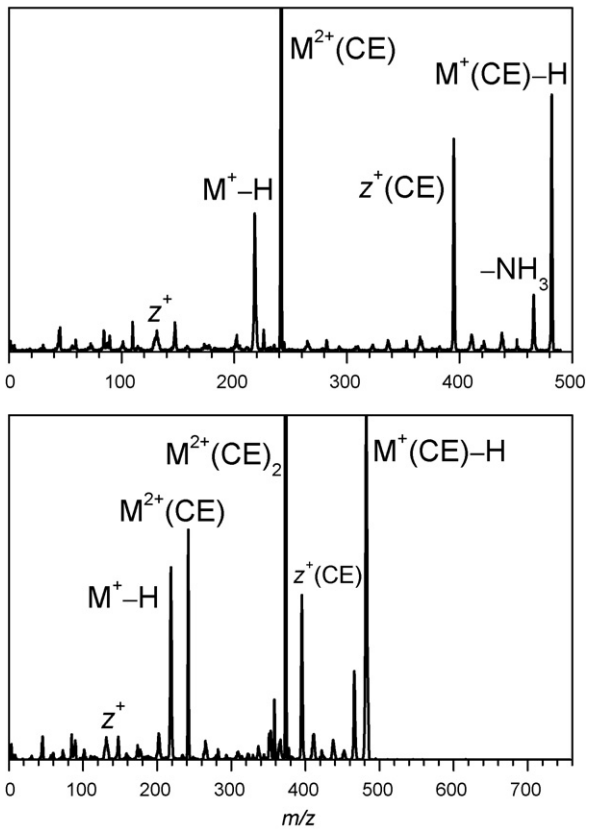

Fig. 6. ECID spectra of $M^{2+}(CE)$ and $M^{2+}(CE)_2$ ($M^{2+}=[AK+2H]^{2+}$, CE = crown ether) obtained after electron capture from Na.

In addition, we investigated the influence of crown ether coordination to the ammonium groups on the fragmentation patterns. The ECID spectra at 50-kV acceleration voltage of $[AK+2H]^{2+}(CE)$ and $[AK+2H]^{2+}(CE)_2$ are shown in Fig. 6. In the one crown ether case, the yield of $z^+(CE)$ is significantly larger than that of the bare z^+-ion, which indicates that the crown ether is mainly attached to the side chain of lysine. Also a CID spectrum of $[AK+2H]^{2+}(CE)$, obtained through collisions with neon, reveals a six times larger abundance of fragment ions with CE on a C-terminal fragment than on a N-terminal fragment (spectrum not shown). These findings are in agreement with the work by Julian and Beauchamp [23] who showed that CE binds preferentially to lysine. A significant yield of ions that have lost hydrogen but not the crown ether (Fig. 6) indicates that hydrogen loss from the N-terminus end occurs, and this channel is therefore in competition with ammonia loss from this end (ammonia loss occurs almost exclusively from this end [15]). It is uncertain whether hydrogen loss is always from the N-terminal since some ions have lost both hydrogen and crown ether. We can conclude that electron capture by the N-terminal end clearly occurs to give both hydrogen loss and ammonia loss but cannot exclude the possibility of electron capture by the lysine ammonium groups also.

The ECID spectrum of $[AK+2H]^{2+}(CE)_2$ reveals that the same channels are open as for the bare ion and one crown-ether complex (Fig. 6). As discussed in a recent paper [15], crown ether coordination does not prevent N–C_α bond breakage, and hydrogen or proton transfer to the amide is therefore not required for this dissociation to occur. Similar conclusions were drawn from experiments on metalated ions instead of protonated ions [24,25]. It is also seen that electron capture by $[AK+2H]^{2+}(CE)_2$ always results in the loss of at least one crown ether. We have calculated the probabilities for the

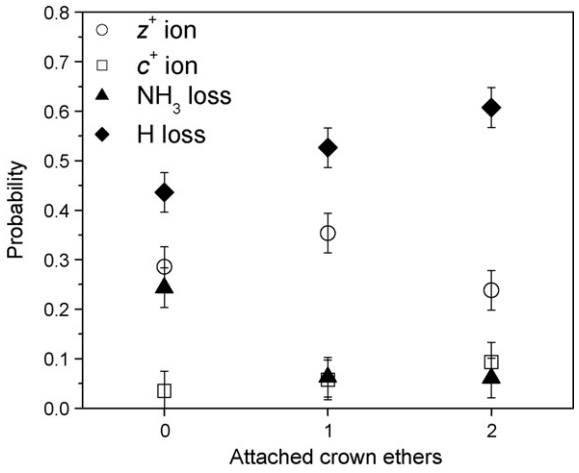

Fig. 7. Probability of formation of the different ECID ions after electron capture from Na as a function of the number of attached crown ethers to $[AK+2H]^{2+}$.

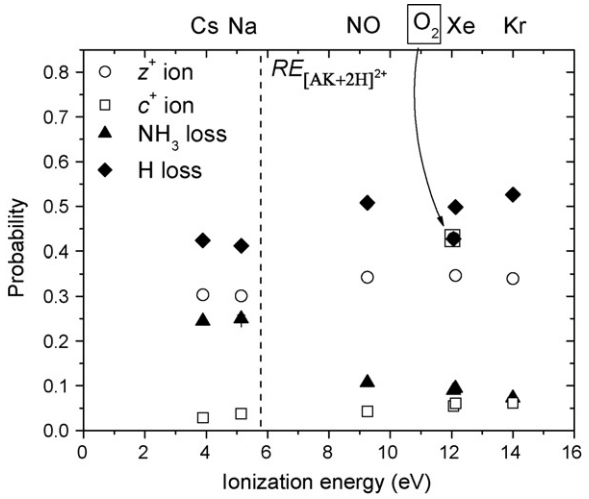

Fig. 8. Probability of formation of the different ECID ions after electron capture to $[AK+2H]^{2+}$ as a function of the ionization energies of the target gases. In the case of dioxygen, the probabilities for H loss and z^+-ion formation are the same, and the two data points (solid diamond and circle) are enclosed by a square to distinguish them from those due to xenon. The probabilities for c^+-ion formation and ammonia loss are similar for dioxygen and xenon. The dotted vertical line marks the calculated vertical recombination energy (RE) of $[AK+2H]^{2+}$.

formation of the four characteristic fragment ions as a function of the number, n, of crown ether attached (Fig. 7). The probability of formation of z^+-ions increases from $n=0$ to 1 but decreases from $n=1$ to 2. The probability of formation of c^+-ions increases by about a factor of two from $n=0$ to 2 but still this channel only gives a small contribution to the total dissociation of about 10%. We currently have no explanation for these changes in the probabilities of z^+ and c^+ formation. Interestingly, the addition of one or two crown ethers increases the probability for H loss and decreases the probability for ammonia loss. The recombination energy decreases with n, and it is therefore likely that the excitation energy after electron capture decreases with n as well. This finding is consistent with the dependence on the acceleration voltage discussed earlier where it was found that the ammonia loss increased with the acceleration voltage (i.e., excitation energy). It should, however, be mentioned that the ion velocity is lower for a heavier complex ion, which should favor hydrogen loss over ammonia loss (cf., Fig. 4).

Finally, we carried out experiments with different collision gases and measured again the probabilities for the different fragmentation channels, cf. Fig. 8. The gases were Cs, Na, NO, O_2, Xe and Kr. Despite a difference of as much as 10 eV in ionization energy between the gases, the results are surprisingly similar. The data seem, however, to fall into two groups: Cs and Na vs. NO, O_2, Xe, and Kr. We estimate the recombination energy of the dication to be about 5.8 eV based on calculations at the B3LYP/6-311++G(2d,p)//B3LYP/6-31+G(d) level of theory. This value is in between the ionization energies of the two groups, which implies that the electron capture is exothermic for Cs and Na but endothermic for the other gases. The major difference observed between the two groups is that H loss is more favored over NH_3 loss for gases with high ionization energy, which is again in accordance with the expectation of lower excitation energy after electron transfer or that capture occurs dominantly to conformers with high recombination energy for gases with high ionization energy. The probability of N–C_α bond cleavage is more or less independent of the collision gas.

A main concern of our work is the initially populated state after electron capture. In the case of free electrons, it seems highly likely that there is a competition between electron capture by the ammonium group and by the amide CO bond due to charge-stabilization by nearby positively charged ammonium groups, as suggested by the Tureček and coworkers [26,27] and the Simons and coworkers [28,29]. According to this model, the electron is either in a Rydberg orbital on the $-NH_3^+$ group or in a Coulomb-stabilized OCN π^* orbital. We have previously demonstrated that dissociation obtained by ECID can also be explained by such a model [14,15]; other models may explain the data as well but at least this model provides a simple and appealing picture. In any case, the similarity between the results obtained from ECD and ECID (Fig. 2) suggests that similar electronic states are in play and give similar relative yields of c^+ and z^+-ions. If so, during the collisional electron transfer, a significant part of captures (about 30%) is by the peptide backbone leading to N–C_α bond cleavage. Capture by the ammonium groups occurs with a probability of about 70%, which is significantly higher than that for ECD (Fig. 2). We suggest that the state populated on the ammonium group varies with the acceleration voltage and collision gas to explain the changes in the ratio of H loss to NH_3 loss. Thus, for gases with high ionization energy, a state close to the ground state is populated, if not the ground state itself. Likewise, for low acceleration voltages, a lower-lying state is populated. Conformational heterogeneity is another interpretation that implies that only conformers for which the energy defect of the electron transfer process is small are expected to play a role. Still, it is uncertain why the competition between capture to the backbone and the ammonium group seems to depend so little on the collision gas.

4. Conclusions

In collisional electron transfer between dipeptide dications and a gas, there is surprisingly small dependence of the ionization energy of the gas target, the ion velocity, and the attachment of crown ether to the ammonium groups on the fragmentation channels. This finding suggests that similar electronic states are populated in all cases. We have also found that for larger peptides the backbone cleavages happen with equal probabilities in ECD and ECID, which indicates similar mechanisms for the fragmentation.

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