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Influence of temperature and crown ether complex formation on the charge partitioning between *z* and *c* fragments formed after electron capture by small peptide dications

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ABSTRACT

Electron capture by peptide dications results in N–C_α bond cleavage to give c^+ and z or c and z^+ fragments. In this work we have investigated how crown ether (18-crown-6 = CE) complex formation and a change in the internal energy affect the charge division between the z and c fragments. Both complex formation and a high temperature have the effect of breaking internal ionic hydrogen bonds. The crown ether complex also lowers the probability of internal proton transfer between the two fragments, and reduces the recombination energy of the charged group it targets. The systems under study were doubly protonated di- and tripeptides, $[AK+2H]^{2+}$, $[AR+2H]^{2+}$, $[KK+2H]^{2+}$ and $[GHK+2H]^{2+}$ (A = alanine, K = lysine, R = arginine, G = glycine and H = histidine). For crown ether complexes the formation of z^+ ions was always preferred over c^+ ions. In the case of $[GHK+2H]^{2+}$, the bare ion dissociated into $z_2^+ + c_1$ and $z_1 + c_2^+$ from cleavage of the first and second N–C_α bond, respectively, whereas z_1^+ fragment ions had higher yield than c_2^+ for $[GHK+2H]^{2+}$(CE). The internal energy of the ions was changed by storing them in a 22-pole ion trap in which they were equilibrated to a temperature between −60 and 90 °C in collisions with helium gas. The average internal energy increased by about 0.4 eV from the lowest to the highest temperature for the dipeptides and 0.6 eV for the tripeptide. More fragmentation occurred at the higher temperature, as observed by an increase in the formation of b^+ and y^+ ions after breakage of the peptide bond of vibrationally hot even-electron cations and from secondary reactions of z^+ radical cations within the time window of the experiment. However, the z^+ to c^+ partitioning was not found to depend significantly on temperature in the measured range. In addition the decay of $[GHK+H]^+/[GHK+2H]^{\bullet+}$ and $[AK+H]^+$ formed after electron capture by $[GHK+2H]^{2+}$ and $[AK+2H]^{2+}$ was found to occur on a microsecond to millisecond timescale. The data are well described by a power-law decay, which implies that the internal energy distribution is broad after electron capture and/or hydrogen loss.

1. Introduction

Electron capture dissociation (ECD) [1] of peptide and protein cations in combination with mass spectrometry is increasingly used for sequencing. The N–C_α bonds are selectively cleaved to give either z or c fragment ions (see Fig. 1). In addition, an even backbone fragmentation pattern is generated, which makes it relatively easy to obtain the order of amino acids. ECD involves capture of free electrons and has been studied in great detail [1–7] along with electron transfer dissociation (ETD) where dissociation is initiated by electron transfer from an anion [8–12].

In our experiments we mimic ECD by the transfer of electrons from alkali metals to peptide cations in keV collisions [13–18] and the technique is denoted electron capture-induced dissociation (ECID). The interaction time of the collision partners is a few femtoseconds, and the electron transfer is therefore nearly vertical, which means that the nuclei do not move during the electron transfer process. The initial structure of the charge-reduced monocation can then be assumed to be identical to that of the dication, which is an advantage when performing quantum mechanical calculations. For small peptides, collision-induced dissociation can normally be neglected compared to ECID [14–16].

Although the electron capture process has been studied extensively, there are still open questions regarding the exact fragmentation mechanism. In this work, we have looked further into charge division between c and z fragments, and the possible link

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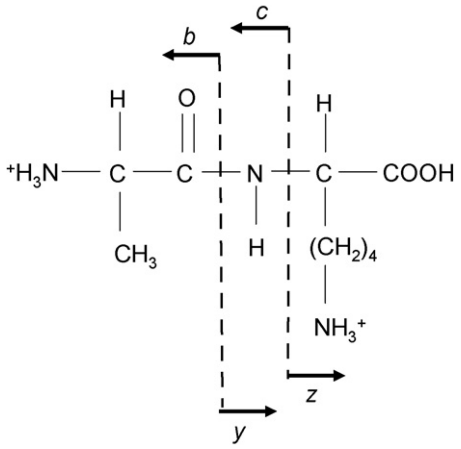

Fig. 1. An illustration of $[AK+2H]^{2+}$, showing the cleavages resulting in *b*, *c*, *y* and *z* fragments.

to conformation, protonation sites, and recombination energies. Conformational heterogeneity and multiple protonation sites have been invoked in several studies to explain why the sequence coverage is increased when the temperature is raised [19–27]. This is based on the idea that electron capture by different initial structures leads to radical cations that dissociate differently. Other studies performed on ubiquitin where multiple conformers were separated by FAIMS (high-field asymmetric waveform ion mobility spectrometry) prior to ECD [28] showed that in the dissociation of compact and extended conformers the same bonds were broken but the yields of fragment ions differed.

An ion–molecule complex between the *c* and *z* fragments is often formed after breakage of the N–C_α bond, and the lifetime of this complex with respect to dissociation is determined by the internal energy and the size of the complex (i.e., degrees of freedom). The initial fragments from the N–C_α cleavage are a $z^\bullet$-radical and a *c* fragment with even number of electrons, and the $c^\bullet$ and *z* are then formed after hydrogen transfer from *c* to $z^\bullet$ within the ion–molecule complex [26,29–32]. Note that in our nomenclature, *z* and *c* have one more hydrogen atom than $z^\bullet$ and $c^\bullet$. The hydrogen transfer process, however, competes with dissociation of the complex, and hence the $z^\bullet/z$ ratio increases with internal energy along with a decrease of the $c^\bullet/c$ ratio. The division of charge between the *c* and *z* fragments may also depend on the lifetime of the complex compared to the time scale for internal proton transfer processes and on differences in recombination energies between the two initial charge sites [5,26,33–35]. Thus, for doubly charged peptides, there is a preferred neutralization of the cation with highest recombination energy, and this largely determines the fragmentation pattern [33,34].

Most of the previous work focused on larger peptides or proteins where several folding motifs are in play. We have instead chosen small doubly protonated peptides as subjects for study [14–16]. For dipeptides and tripeptides the Coulomb repulsion between the two excess charges determines the lowest-energy structures to a large extent and limits the number of important conformers to a few, two or three in the case of dipeptides [36]. Such systems are amenable to quantum mechanical calculations, and detailed reaction paths including transition states have been calculated by Tureček et al. [36].

We have earlier shown that complex formation by one or two crown ethers (18-crown-6 = CE) to doubly protonated di- and tripeptides significantly alters the c^+ to z^+ ratio, in favor of z^+ formation [15]. The crown ether affects the chemistry in at least three ways; it slows down or prevents internal proton transfer in the ion molecule complex formed after N–C_α bond breakage, it prevents internal ionic hydrogen bonding resulting in somewhat more extended structures, and it lowers the recombination energy of the charged group that it binds to [37–39]. The previous work was focused on lysine-containing peptides, and we therefore decided to carry out a similar experiment for crown ether complexes of $[AR+2H]^{2+}$ where the second charge is provided by protonated arginine instead of protonated lysine (A = alanine and R = arginine). ECID spectra for $[AR+2H]^{2+}(CE)_n$, $n = 0$, 1, and 2, are reported here together with those for $[GHK+2H]^{2+}(CE)_n$, $n = 0$, 1.

An increase of the internal energy of the peptide increases the probability of an extended structure and lowers the lifetime of the ion–molecule complex. In the present work, we therefore decided to investigate how the internal energy of the parent ion affects the charge division between *c* and *z* fragments by varying the temperature. Previous temperature dependent experiments were on relatively large peptides and hydrogen transfer processes [26,29–32] whereas the focus of this work is on smaller peptides, $[AK+2H]^{2+}$, $[AR+2H]^{2+}$, $[KK+2H]^{2+}$ and $[GHK+2H]^{2+}$. Our experiment is based on the accumulation of ions in a 22-pole ion trap filled with helium buffer gas at different temperatures, followed by acceleration to keV energies and ECID. Mass spectrometric analysis is done using an electrostatic ion storage ring. This instrument was chosen for practical reasons but it has the disadvantage that it does not provide high enough mass resolution to separate the even-electron cations from the radicals. Hence the focus is on the relative abundance between z^+ and c^+ ions, that is, how the charge is divided between the two fragments.

Finally, lifetimes of charge-reduced ions were measured for two of the peptides by monitoring the decay in the storage ring on the microsecond to millisecond time scale. This experiment provides important information on the internal energy distribution after electron capture and possibly hydrogen loss and is not easily done with other instruments.

2. Experimental setup

Compounds were purchased from Sigma–Aldrich and used without further purification. The crown ether measurements in the case of $[AR+2H]^{2+}$ and $[GHK+2H]^{2+}$ were made using an accelerator mass spectrometer [40,41]. Ions were produced by electrospray of peptides dissolved in water/methanol (1:1) with acetic acid added (5% in volume). Crown ether (18-crown-6), CE, was added in different amounts to produce complexes with either one or two CEs attached. $[AR+2H]^{2+}(CE)_n$, $n = 0$, 1, and 2 were accelerated to 22, 46 and 70 keV, respectively, and $[GHK+2H]^{2+}(CE)$ ions were accelerated to 100 keV. After mass selection by a magnet the ions passed through a cesium vapor cell where ECID took place as electrons were transferred from cesium atoms to the doubly charged peptide ions. After a flight time of 2–3 μs, the fragment ions were energy/charge analyzed by a hemispherical electrostatic analyzer and counted by a channeltron detector.

Temperature dependence measurements were done using the electrostatic ion storage ring ELISA (see Fig. 2), which has been previously described in detail [42–44]. The ions were produced in an electrospray ion source and transported through a heated capillary and a lens system. A 22-pole ion trap which, under normal operation, is used to accumulate ions, served a second purpose in this experiment as it was used to control the temperature of the ions. The temperature of the ion trap was varied in the range −60 to 90 °C, and through collisions with helium (at an estimated pressure of a few mTorr) the ions were thermalized and acquired the surrounding temperature. Radiative cooling or heating of the ions is not considered to be important on the time scale of the experiment (0.1 s). After the ion trap, the ions were accelerated to 22 keV and mass selected before passing through a Na vapor cell, heated to

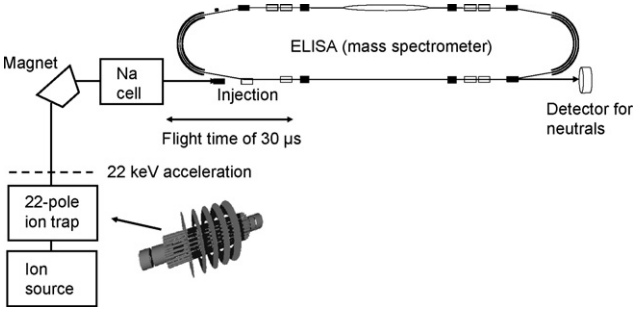

Fig. 2. A schematic of the experimental setup. See text for details.

250 °C, where they captured an electron. After about 30 μs (flight path indicated in Fig. 2), the fragment ions formed in the ECID process were analyzed using the ion storage ring ELISA [42–44] as a mass spectrometer. A recent development makes it possible to switch the ring element voltages in ELISA to match the mass of a fragment ion [44]. In this way the correct fragment ion beam could be selected, and this beam was stored in the ion storage ring for about 60 ms. The fragment ion beam was then dumped onto a microchannel plate (MCP) detector, and the signal gave one point in the mass spectrum. This scheme was repeated over the range of all fragment masses to give a complete mass spectrum. The pressure in the ring was in the order of 10^{-11} mbar to reduce the importance of collision-induced dissociation.

In a separate experiment, the lifetimes with respect to dissociation of the charge-reduced ions ($M^{\bullet+}$ or $M^+ - H$) of $M^{2+} = [GHK+2H]^{2+}$ and $[AK+2H]^{2+}$ were also measured by storing them in ELISA and monitoring the decay based on the yield of neutral fragments formed as a function of time.

Cs was used for the accelerator mass spectrometry experiments and Na for the storage ring experiments but previous experiments have shown that ECID is almost independent on the collision gas being Na or Cs [16].

3. Results and discussion

ECID mass spectra of $[AR+2H]^{2+}(CE)_n$ ($n = 0, 1, 2$) are shown in Fig. 3. The statistical uncertainties in the peaks are for all presented data sets around 5–20% depending on the peak height. For $[AR+2H]^{2+}(CE)_n$ the two charges are initially located on the N-terminal amino group and the highly basic side chain of arginine. The crown ethers bind to these charged sites, and the size of the chosen crown ether (18-crown-6) is optimum for the formation of three hydrogen bonds with ammonium [37–39]. The observed dissociation channels are loss of hydrogen, ammonia or $HN{=}C(NH_2)$, $C(NH_2)_3$, and breakage of the N–C_α bond to give z^+ and c^+ ions. The losses may be accompanied by loss of crown ether. The bare ion, $[AR+2H]^{2+}$, preferably fragments into c and z^+, and this remains the important channel upon crown ether attachment. For $n = 1$, both bare z^+ and c^+ fragment ions and z^+(CE) and c^+(CE) complexes were formed, the latter in higher abundance. This indicates that the electron is attached to the bare charged group, which is the one with the highest recombination energy, *RE*. The crown ether lowers the *RE* of an alkyl ammonium group by about 1.4 eV [38]. In agreement with this interpretation, Chamot-Rooke et al. [33] have shown that ECD of charge-tagged GK, AK and GR dications results in c^+ ions. The charge tag was introduced at the N-terminal amino group and has a lower *RE* than the other charged group. Hence the electron attaches to the protonated ε-amino lysine or to the guanidinium to give c^+ fragments. However, their work also suggested the involvement of excited electronic states to account for some of the dissociation occurring. For $n = 2$, we cannot tell from our results whether CE is lost before or after N–C_α bond breakage; it likely depends on the site of electron capture, –C(O)NH– or $–NH_3^+$(CE).

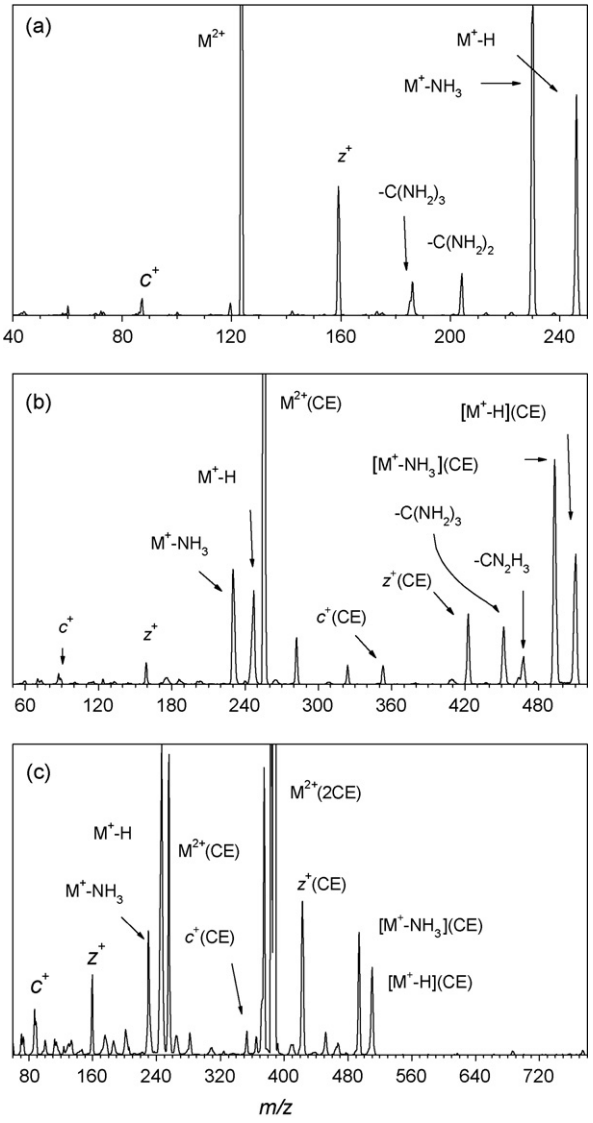

Fig. 3. Cs ECID mass spectra of $[AR+2H]^{2+}(CE)_n$: (a) $n = 0$ ($m/z = 123.5$), (b) $n = 1$ ($m/z = 255.5$), and (c) $n = 2$ ($m/z = 387.5$). The spectra were recorded at an accelerator mass spectrometer.

Previous measurements have shown that electron capture by $[GHK+2H]^{2+}$ ions leads to c_2^+ and z_2^+ ions from breakage of the second and first N–C_α bond, respectively, and in both cases retains the charge on the heaviest fragment [15]. The two protons are most likely located at the lysine-ε amino group and the imidazole ring of histidine, Gly-His(H^+)-Lys-NH_3^+. The proton affinity of the N-terminal amino group is about 0.6 eV less than that of the imidazole ring according to DFT calculations (B3LYP/6-31 + G(d,p)) [45]. For a simpler model, GH, the proton affinity of imidazole is 0.9 eV higher than that of the N-terminal amino group, which indicates that the proton affinity of imidazole in the tripeptide is reduced by 0.3 eV due to the Coulomb repulsion from the positive charge of the protonated lysine side chain. When the same experiment was carried out for the complex between $[GHK+2H]^{2+}$ and one crown ether, $[GHK+2H]^{2+}$(CE), the ECID spectrum revealed that z_1^+(CE), z_2^+(CE), c_2^+, and c_2^+(CE) ions are formed (Fig. 4). These results were briefly discussed in a recent letter [15] but the spectrum was not shown. There is no indication of the formation of c_1^+ ions but clearly the $c_2 + z_1^+$ channel is now open. There are at least two different

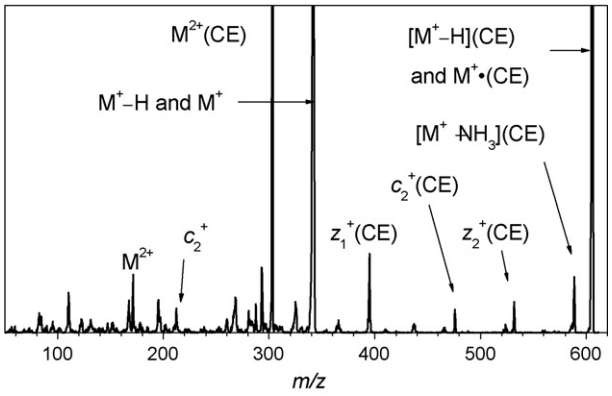

Fig. 4. Cs ECID mass spectrum of $[GHK+2H]^{2+}(CE)$ ($m/z = 171$). The spectrum was recorded at an accelerator mass spectrometer.

complexes present in the ion beam since we can assign peaks to correspond to $z_1^+(CE)$ and $c_2^+(CE)$ ions. Thus, CE is located either at the N-terminal ammonium group or at the lysine ammonium group. The similar abundances of c_2^+ and $c_2^+(CE)$ and the presence of $z_1^+(CE)$ but the absence of z_1^+ indicates that the crown ether preferentially binds to the lysine ammonium group, in agreement with work by Julian and Beauchamp [39]. Still, the fact that $c_2^+(CE)$ is formed implies that in some cases the CE is either located at the N-terminal ammonium or at a protonated imidazole ring. According to B3LYP/6-31 + G(d,p) calculations on the simpler model system, $[GH+H]^+(CE)$, the difference in energy between the structure where the crown ether binds to protonated imidazole, Gly-HisH$^+$(CE), and the structure where it binds to ammonium, (CE)$^+$H$_3$N-Gly-His, is 0.1 eV more favorable for the imidazole ring being the charge site. However, the Coulomb repulsion from the protonated lysine chain in the doubly charged complex is likely to favor (CE)$^+$H$_3$N-Gly-His-Lys-NH$_3^+$ over Gly-HisH$^+$(CE)-Lys-NH$_3^+$.

For $[GHK+2H]^{2+}(CE)$ the total yield of z^+ ions (bare ions and CE complexes) was found to be higher than that of c^+. As discussed above, the crown ether preferentially binds to the lysine ammonium group, which results in a lower *RE* of this charge site compared to that of the bare protonated imidazole ring. The electron therefore neutralizes the imidazole to give a neutral c fragment and a charged z fragment after N–C$_\alpha$ bond breakage. For comparison, the main dissociation channel of $[KK+2H]^{2+}$ changes from $c^+ + z^\bullet$ to $c + z^{\bullet+}$ with the attachment of a single crown ether [15]. The recombination energies of $[KK+2H]^{2+}$ are similar for the two ionic sites, and the dominant fragment is by far the c^+ ion. With two crown ethers attached, $[KK+2H]^{2+}(CE)_2$, the recombination energies of the two ionic sites are again similar but the z^+ fragment ions are now more abundant than c^+ ions. Only for $[KK+2H]^{2+}(CE)$, the incoming electron experiences a difference between the two charged sites if energetics alone is considered. These results indicate that an interpretation of the charge division based solely on differences in recombination energies between the two charged groups is not sufficient to explain the data since the fragmentation of $[KK+2H]^{2+}$ and $[KK+2H]^{2+}(CE)_2$ is completely different. It appears that the charge division between c^+ and z^+ is also determined by the proton mobility in the ion–molecule complex. When crown ether targets the ammonium protons, dissociation of the ion–molecule complex is likely to be faster than internal proton transfer processes that involve concomitant movement of the crown ether and suggests that charge division depends on the lifetime of the ion–molecule complex, as is the case for hydrogen atom transfer processes.

Next we present the data from the temperature variation experiments. The purpose of this study is to investigate the influence of the initial structure of the parent ion on its dissociation after electron capture and a possible dependence on the lifetime of the ion–molecule complex formed after N–C$_\alpha$ bond cleavage. After electron capture we expect the internal energy to be similar in all cases. The ECID mass spectra of $M^{2+} = [AR+2H]^{2+}$, $[AK+2H]^{2+}$, $[KK+2H]^{2+}$ and $[GHK+2H]^{2+}$ at different temperatures are shown in Figs. 5–8. The spectra are similar to those obtained at the accelerator mass spectrometer [14–16], except for a much larger yield of b^+ and y^+ ions in the present experiment. This difference is ascribed to the higher internal excitation of the ions at the higher temperatures and the longer flight time before mass analysis (30 μs vs. 2–3 μs). The b^+ and y^+ ions may also originate from the dissociation of hot $M^+ - H$ ions [46–48] since ions that have lost hydrogen after electron capture dissociate on a microsecond to millisecond timescale (see below). The longer flight time before mass analysis makes it possible for us to see the fragment ions from consecutive reactions.

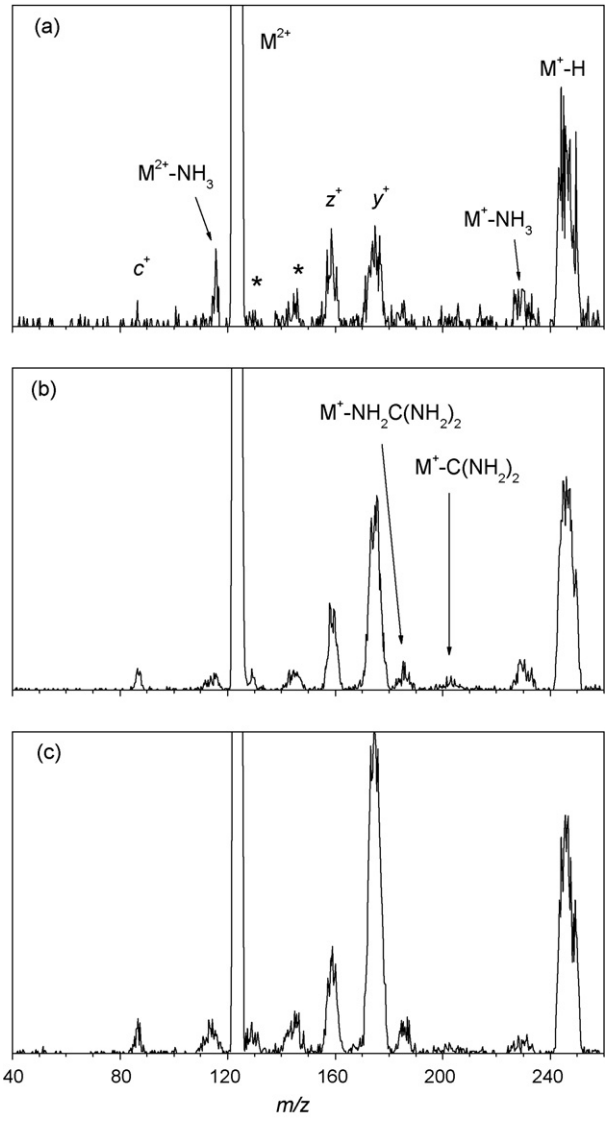

Fig. 5. Na ECID mass spectra of $[AR+2H]^{2+}$ (m/z 123.5) at temperatures of (a) −40 °C, (b) 25 °C and (c) 90 °C prior to electron capture. The peaks labeled with (*) are ascribed to secondary fragmentation of the z^+ ions. The spectra were recorded at ELISA.

At high temperatures we observed water loss from the b_2^+ ion for the tripeptide, which we believe is induced by the histidine side chain as seen in other studies [49,50]. The NH_3-loss channel is reduced in the present measurements, which is ascribed to further dissociation of the $M^+ - NH_3$ ions during the 60 ms storage of the

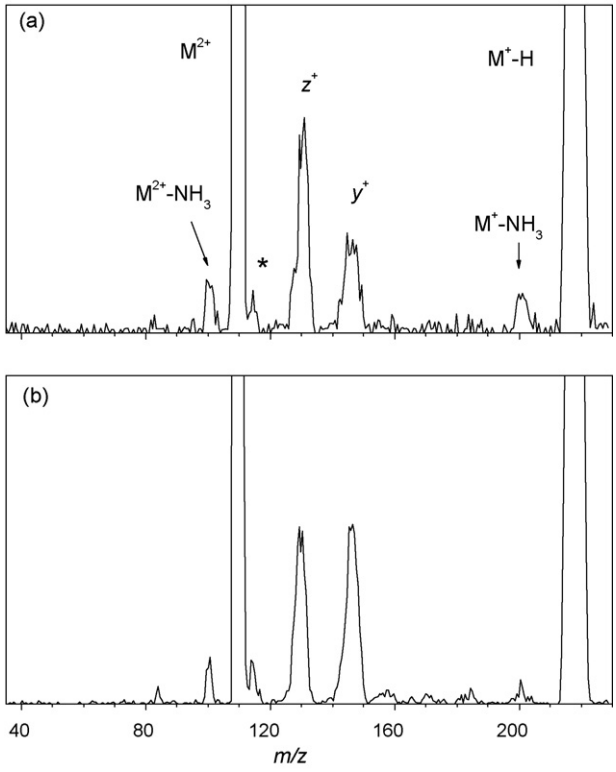

Fig. 6. Na ECID mass spectra of $[AK+2H]^{2+}$ (m/z 109.5) at temperatures of (a) −55 °C and (b) 90 °C prior to electron capture. The peak at m/z 84 is assigned to cleavage of the lysine side-chain in accordance with previous observations [52]. The peaks labeled with (*) are ascribed to secondary fragmentation of the z^+ ions. The spectra were recorded at ELISA.

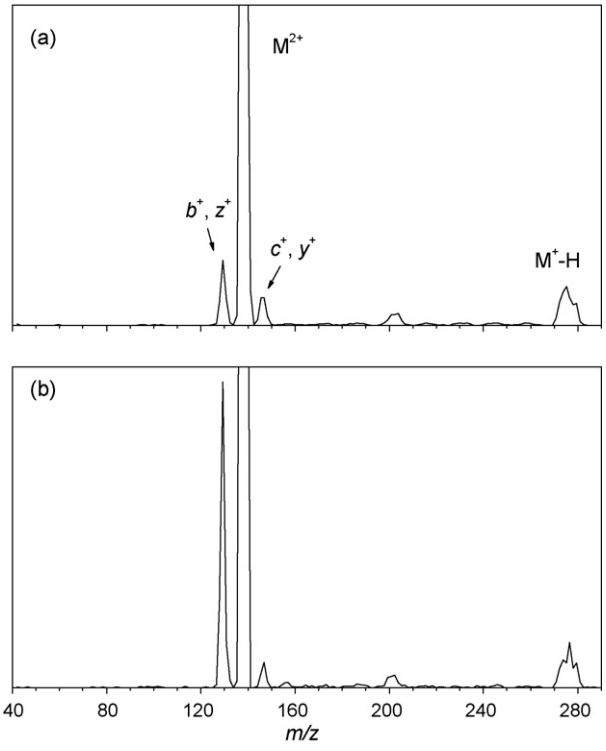

Fig. 7. Na ECID mass spectra of $[KK+2H]^{2+}$ (m/z 138) at temperatures of (a) −60 °C and (b) 95 °C prior to electron capture. The b^+ and z^+ peak is assigned to originate mainly from b^+ ions, see text for details. The peak at about m/z 201 has not been assigned. The spectra were recorded at ELISA.

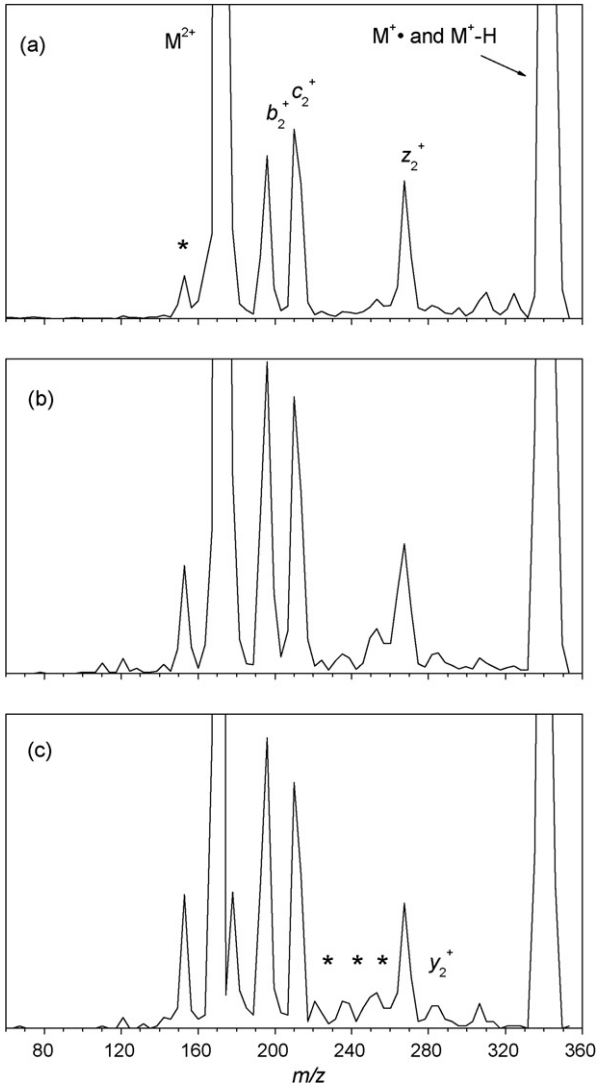

Fig. 8. Na ECID mass spectra of $[GHK+2H]^+$ (m/z 171) at temperatures of (a) −40 °C, (b) 25 °C and (c) 90 °C prior to electron capture. The peaks labeled with (*) are ascribed to secondary fragmentation of the z^+ ions. The peak at m/z 178 is due to loss of H_2O from the $b_2{}^+$ ion. The spectra were recorded at ELISA.

fragment ions. At higher temperatures we also observe secondary dissociation of the z^+ ions, in which they lose up to three heavy atoms with an appropriate number of hydrogen atoms. Unfortunately we could not determine the fragment masses exactly due to the mass resolution but the secondary fragmentation is in agreement with previous observations by O'Connor and co-workers [32] who have shown that the odd-electron $z^{\bullet+}$ ions are highly reactive, in contrast to the even-electron c fragments. We have earlier shown that for peptides of similar size, even-electron z^- anions are formed in a second capture collision process whereas no c^- anions were measured [51]. This indicates that internal hydrogen atom transfer in the initially formed c/z ion pair cannot compete with the dissociation of the ion–molecule complex. If hydrogen atom transfer had occurred, we would expect the un-even-electron c fragments to have positive electron affinities and that they would pick up electrons in secondary collisions to become anions.

The fragmentation of $[AK+2H]^{2+}$ after electron captures greatly favors production of z^+ ions over c^+ ions (no c^+ ions were identified) (Fig. 6), which is in accordance with earlier experiments [15]. For $[AR+2H]^{2+}$, z^+ ions are preferentially formed but the ratio between c^+ and z^+ ions approximately doubles as the temperature rises from

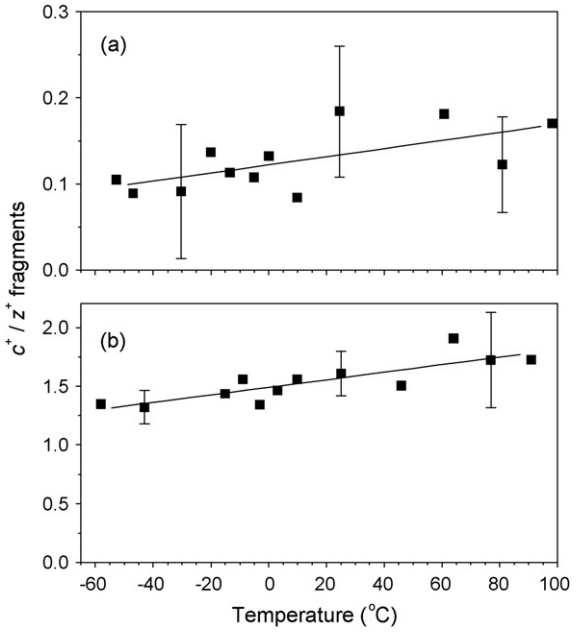

Fig. 9. The ratio of c^+/z^+ ion fragments after ECID for (a) $[AR+2H]^{2+}$, (b) $[GHK+2H]^{2+}$, without taking the secondary fragments from z^+ into account. Error bars represent statistical uncertainties.

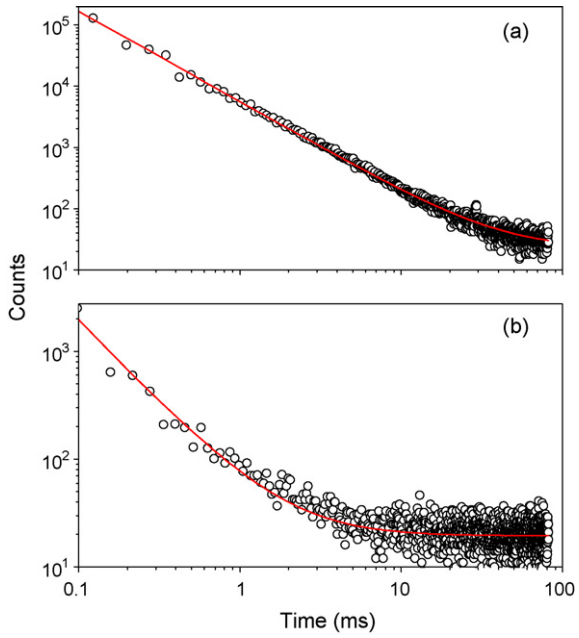

Fig. 10. Decay spectra of charge-reduced ions from (a) $[GHK+2H]^{2+}$ and (b) $[AK+2H]^{2+}$. The solid lines are fits to the function $Nt^{-1.5}+K$.

−60 to 90 °C (Fig. 9). However, from the fragment spectra in Fig. 5 we see that the z^+ products fragment further by losing heavy groups such as NH_3, as described above. Assuming these fragments originate from z^+ ions, the formation of c^+ and z^+ after ECID is almost independent of temperature. The same observation is made for the tripeptide $[GHK+2H]^{2+}$, where the measured increase of 40% in the $c_2{}^+$ to $z_2{}^+$ ratio vanishes when the fragments assigned as originating from the z^+ ions are taken into account.

In the case of $[KK+2H]^{2+}$, the b^+ and y^+ product ions are separated by only one mass unit from the c^+ and z^+ fragment ions, and could not be resolved in the present experiment. However, the lack of further fragmentation of the z^+, as seen in the case of $[AR+2H]^{2+}$ and $[AK+2H]^{2+}$ indicates that the peak at m/z around 130 is mainly due to b^+ ions. Hence the increase of this peak with temperature is attributed to an increase in the formation of b^+ ions, and the main ECID dissociation channel at high temperatures is still $c^+ + z$.

Calculations of the vibrational frequencies at the B3LYP/6-31 + G(d,p) level of theory [45] reveal that the average internal energy of $[AK+2H]^{2+}$, $[AR+2H]^{2+}$, $[KK+2H]^{2+}$, and $[GHK+2H]^{2+}$ is raised by about 0.4 eV when the temperature is increased from −60 to 90 °C for the dipeptides and 0.6 eV for the tripeptide. This change is apparently not enough to change the lifetime of the c/z ion pair or to change the distribution between different conformers to affect the partitioning between c^+ and z^+ significantly.

Finally, the decay of the charge-reduced ions of $[GHK+2H]^{2+}$ and $[AK+2H]^{2+}$ was measured by storing them and measuring their lifetimes with respect to dissociation into fragment ions and neutral fragments. In the case of GHK both $[GHK+2H]^{\bullet+}$ and $[GHK+H]^+$ were likely injected into the ring whereas in the case of AK only $[AK+H]^+$ was present after a few microseconds [14]. The decay spectra of the ions are shown in Fig. 10, and the dissociation was found to occur on a microsecond to millisecond time scale in both cases. The number of counts is proportional to the decay rate. The data are well described by power-law decay, $Nt^{-1.5}+K$ (N is a normalization constant, and the additive constant K accounts for fragmentation by collisions in the ring), implying that the internal energy distribution is broad [53–55]. For comparison, a power-law decay of photoexcited tryptophan was measured earlier, which was ascribed to the decay of the tryptophan radical cation formed after prompt hydrogen loss [56]. In the present case, the energy distribution may be broadened also as the result of capture to different electronic states in the electron transfer process from sodium. The negative power of −1.5 indicates that we are sampling at the low energy side of the energy distribution [55], and small changes in the time scale for the experiment will give large differences in the yields of daughter ions from vibrationally hot ions. This is in agreement with the much lower yield of b^+ and y^+ ions for sampling times of only 2–3 μs [14,15].

4. Conclusion

We have found that the partitioning between z^+ and c^+ after electron capture by doubly protonated di- and tripeptides is not significantly dependent on the temperature in the range from −60 to 90 °C. A higher internal energy does, however, result in more fragmentation of vibrationally hot ions. Our results indicate that a temperature of 90 °C is not high enough to lower the lifetime of the c/z ion pair to disfavor the formation of c^+ ions. In contrast, attachment of crown ether in general results in z^+ ions formed in higher yields than c^+ ions in the case of lysine-containing peptides. Electron capture by $[AR+2H]^{2+}$ favors z^+ over c^+ ions, independent of crown ether complex formation. To fully understand how the presence of crown ether affects fragmentation requires more studies. Finally, we have found that charge-reduced peptides have a broad distribution of lifetimes, resulting in a power-law decay which means that the internal energy distribution of the monocations is broad, and that small changes in the time scale for the experiment will give large differences in the yield of daughter ions from vibrationally hot ions.

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