RESEARCH ARTICLE

Electron-Capture and -Transfer Dissociation of Peptides Tagged with Tunable Fixed-Charge Groups: Structures and Dissociation Energetics

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Abstract

Pyridiniummethylcarbonyl moieties that were previously designed on the basis of electronic structure analysis are now utilized as fixed-charge tags with tunable electronic properties to be used for N-terminal peptide derivatization and sequencing by electron-transfer dissociation. Dipeptides AK and KA were derivatized at the peptide N-terminus with 4-dimethylaminopyridinium-N-acetyl (DMAP-ac) and pyridinium-N-acetyl (pyrid-ac) tags of increasing intrinsic recombination energies. Upon the capture of a free electron or electron transfer from fluoranthene anions, (DMAP-ac-**AK**+H)²⁺, (DMAP-ac-**KA**+H)²⁺, (pyrid-ac-**AK**+H)²⁺ and (pyrid-ac-**KA**+H)²⁺ ions, as well as underivatized (**AK**+2H)²⁺, completely dissociated. The fixed-charge tags steered the dissociation upon electron transfer to form abundant backbone N–C_α bond cleavages, whereas the underivatized peptide mainly underwent H-atom and side-chain losses. Precursor ion structures for the tagged peptides were analyzed by an exhaustive conformational search combined with B3LYP/6-31+G(d,p) geometry optimization and single-point energy calculations in order to select the global energy minima. Structures, relative energies, transition states, ion–molecule complexes, and dissociation products were identified for several charge-reduced species from the tagged peptides. The electronic properties of the charge tags and their interactions with the peptide moieties are discussed. Electrospray ionization and electron-transfer dissociation of larger peptides are illustrated with a DMAP-tagged pentapeptide.

Key words: Fixed-charge tags, Peptide derivatization, Electron-transfer dissociation, Ion structures, Ab initio calculations

Introduction

Electron-transfer dissociation (ETD) is one of the newer ion activation methods that is particularly useful for

peptide sequencing [1]. In typical ETD experiments, multiply protonated peptides are produced by electrospray ionization, stored in a radiofrequency ion trap, and partially discharged by electron transfer from a molecular anion donor. Another experimental technique, called electron-capture-induced dissociation (ECID [2]), makes use of fast ion beams that are partially discharged by collisional electron transfer from gaseous alkali metal atoms. A common feature of ETD and ECID is that charge-reduced peptide ions undergo dissociations through the loss of

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hydrogen atoms, ammonia, amino acid side chains, and backbone N–C α bond cleavages. The latter afford sequence fragments containing the N-terminal residues (even-electron c-type ions) and those containing C-terminal residues (odd-electron z-type ions).

The mechanism of N–C α bond cleavage has been of intense interest, especially regarding its dependence on the nature and sequence of amino acid residues [3–6], precursor ion charge state [7–9], and peptide secondary structure [10–14]. One of the questions that has been addressed by experiment [3] and theory [15] was the nature and position of charge sites in peptide ions and their effects on electron-based dissociations. The position of the charge site can be established by incorporating highly basic arginine residues into model peptides that provide unequivocal protonation sites [16]. However, a more general approach is to derivatize in situ the desired peptide with a charge-carrying or readily ionizable group [17–24], and thus affix the charge position in the sequence. Various charge tags have been implemented on an ad hoc basis of availability and convenience, such as 2,4,6-trimethylpyridinium [25], 2,2'-bipyridyl [26], tris (2,4,6-trimethoxyphenyl)phosphonium [24, 27–29], trimethylammoniumalkyl [30], etc. The charge tags function as potential electron attachment sites, depending on their intrinsic recombination energies (REs) relative to those of the charged peptide residue [31]. We have analyzed through computation fixed-charge pyridinium tags in which the intrinsic RE could be varied by selecting ring substituents (R) [31]. The REs ranged from 5.65 eV for electron-withdrawing groups (R = CN), through 5.03 eV for unsubstituted pyridinium (R = H), to 4.10 eV for electron-donating groups (R = N(CH $_3$) $_2$) (Scheme 1).

Molecular orbital analysis of small peptides that were tagged with pyridinium groups indicated that the 4-dimethylaminopyridinium tag had the most extensive interaction between the ring and peptide π^* -molecular orbitals due to the closest match of the corresponding recombination energies [32]. According to the current mechanisms of electron-based peptide dissociations, the so-called Utah–Washington (UW) model [33] postulates that amide π^* orbitals play a crucial role in N–C α bond cleavage [34, 35]. It was therefore of interest to investigate by experiment and

electron structure theory the behavior of model charge-tagged peptide ions. To make the models amenable to both experiment and high-level computational analysis, we selected Ala-Lys (AK) and Lys-Ala (KA) dipeptides that were tagged at the N-termini by N-pyridiniummethylcarbonyl and N-(4-dimethylaminopyridinium)methylcarbonyl groups (Scheme 1). We now report high-resolution ETD mass spectra of doubly charged ions carrying these tags. We wish to show that the charge tags substantially enhance backbone N–C α bond dissociations compared to underivatized peptides. We also report a detailed analysis of the energetics and mechanisms of peptide cation-radical dissociations that was performed at high levels of ab initio theory. The results of this pilot study will be used for a comprehensive investigation of electron-based dissociations of a large set of charge-tagged peptides. An example of the charge tagging and ETD of a pentapeptide of a tryptic type is reported here.

Experimental

Materials and Methods

The fixed-charge tagged dipeptides pyrid-ac-AK (93% pure), pyrid-ac-KA (99% pure), DMAP-ac-AK (97% pure), and DMAP-ac-KA (96% pure) were custom synthesized by PolyPeptide Laboratories (Strasbourg, France) and used as received. Alternatively, the following in-house developed preparative procedure can be used for DMAP derivatives. A peptide solution [100 μ l of 3 mM peptide solution in acetonitrile/water (1/1 v/v)] and 300 μ l of 30 mM triethylamine in water were mixed with 100 μ l of 12 mM *N*-hydroxysuccinimidyl iodoacetate (ProChem, Inc., Rockford, IL, USA, catalog no. CL214) on a vortex mixer for 10 min. Then 50 μ l of a 120 mM 4-(dimethylamino)pyridine (Sigma–Aldrich, St. Louis, MO, USA, catalog no. 522805-5G) solution in acetonitrile was added, and the resulting mixture was stirred at 75 °C for 2 h. The solution was then diluted with acetonitrile/water/acetic acid (48.5/48.5/1) to a concentration of 10 μ M and used without further purification

Scheme 1. Charge-tagged AK and KA peptide ions

for direct infusion using the electrospray ion source of a mass spectrometer to afford abundant doubly charged ions.

Electron-transfer dissociation mass spectra were measured on a Thermo Fisher (San Jose, CA, USA) LTQ Orbitrap Velos hybrid mass spectrometer and an LTQ XL linear ion trap instrument, both outfitted with a chemical ionization source for the production of fluoranthene anion radicals as ETD reagent. Precursor dications were mass isolated with a window of 3–4 m/z units to accommodate the nearest ^{13}C isotopologs and allowed to react for 100 and 200 ms with fluoranthene anions. Electron-capture dissociation (ECD) mass spectra were obtained on a Thermo Fisher LTQ-FT-ICR instrument. Precursor ions were isolated in the LTQ with a mass-selection window of 1.8–2.5 m/z units, transferred to the ion cyclotron resonance (ICR) cell, and irradiated by electrons from an external source. The electron energy could not be measured directly, and the corresponding electron emitter bias was set at 3 V, which provided the best ECD efficiency. The irradiation pulse width was 300 ms.

Calculations

Conformational Search and Quantum Chemistry Calculations

Our strategy for finding the lowest energy conformations is to first generate a large number of candidate structures in the molecular dynamics regime, then refine the relative energies and re-optimize geometries with semi-empirical and ab initio methods. Molecular dynamics calculations were performed using NAMD [36]. Semi-empirical and ab initio calculations were performed using Gaussian 09 [37].

Molecular dynamics force-field parameters have not previously been developed for charge-tagged residues. We obtained charge distributions and force constants with ab initio calculations in order to develop a set of parameters for the charge tags. This parameter set is not intended to be sufficiently characterized to accurately reproduce all thermodynamic properties, but merely to be accurate enough to generate qualitatively reasonable candidate structures. Other residues are parameterized using established values from the CHARMM force field [38].

Once the force-field parameters have been determined, replica exchange molecular dynamics (REMD) [39] are performed on each sequence. Calculations are run for 10 ns with a step size of 1 fs with eight temperature replicas from 300 to 800 K. One thousand structures from each replica are selected at regular intervals for a total of 8000 initial candidate structures. Each candidate structure is then optimized at the PM6 level [40]. The PM6-optimized structures are then analyzed for potential hydrogen bonds. Structures with the same hydrogen bonds are grouped together and the lowest energy structure from each group is taken to form a new list of candidate structures. The single point energy for each candidate structure is calculated at the

B3LYP/6-31+G(d,p) level [41–43], and the structures from each sequence with the lowest energy are re-optimized at the same level and the local minimum confirmed with frequency calculation. B3LYP/6-311++G(2d,p) and MP2/6-311++G(2d,p) [44] single point energies are calculated for each re-optimized structure. The energies calculated from each of these methods were averaged to cancel known errors in each method [45]. Calculations of radical states began with the optimized dication structures. Time-dependent density functional theory [46] (TD-DFT) was used to calculate the first 15 excited states of the vertically attached radical configuration at B3LYP/6-311++G(2d,p). Selected structures were re-optimized with the charge-reduced electronic configuration at the B3LYP/6-31+G(d,p) level, and the structures were confirmed as local minima or first-order saddle points by harmonic frequency calculations. Single point energies were calculated with B3LYP/6-311++G(2d,p) and spin-projected MP2/6-311++G(2d,p) and averaged to provide improved relative energies for cation radicals.

Results

Electrospray of the fixed-charge tagged peptides gave abundant dications (DMP-ac-**AK**+H) $^{2+}$ (**1** $^{2+}$), (DMP-ac-**KA**+H) $^{2+}$ (**2** $^{2+}$), (pyrid-ac-**AK**+H) $^{2+}$ (**3** $^{2+}$), and (pyrid-ac-**KA**+H) $^{2+}$ (**4** $^{2+}$). All precursor and fragment ions were characterized by accurate mass measurements to ascertain their elemental compositions and those of the neutral fragments. The accurate m/z values are listed in Table 1. For backbone fragments, we use the standard nomenclature [47, 48] that was adapted for the presence of the N-terminal tag, which is considered a pseudo-residue. Fragment ions corresponding by their elemental composition to the tag plus amide are denoted as **c**₀.

Electron Transfer to DMAP-ac-AK/KA and pyrid-ac-AK/KA Ions

The ETD spectra measured on the Velos-Orbitrap and LTQ instruments showed essentially the same fragment ions. The Velos Orbitrap ETD mass spectra are shown in Figure 1. The LTQ ETD mass spectra are given in Figure S1 of the Electronic Supplementary Material (ESM). Both types of ETD spectra showed no charge-reduced ions for **1** $^{2+}$ –**4** $^{2+}$. This was unequivocally established by accurate mass measurements at the corresponding m/z 381 for the DMAP-tagged peptides and m/z 338 for the pyrid-tagged ones, which showed only baseline-resolved ^{15}N and ^{13}C isotope satellites of the respective abundant m/z 380 and m/z 337 ions (Table 1). Hence, the loss of an H atom was a major dissociation upon electron transfer to all our charge-tagged peptide ions.

The ETD mass spectrum of **1** $^{2+}$ (Figure 1a) showed major backbone fragments at m/z 251 (**c**₁) and m/z 180 (**c**₀), resulting from N–C $_{\alpha}$ bond cleavage and charge retention in

Table 1. Accurate ion m/z values in ETD and ECD mass spectra

Peptide ion	ETD	ECD	El. composition	Calc.
1²⁺	-	-	C ₁₈ H ₃₁ N ₅ O ₄	381.2376
	381.2323	-	(¹³ C)C ₁₇ H ₃₀ N ₅ O ₄	381.2331
	381.2259	-	C ₁₈ H ₃₀ (¹⁵ N)N ₄ O ₄	381.2268
	380.2289	380.2287	C ₁₈ H ₃₀ N ₅ O ₄	380.2298
	366.2133	-	C ₁₇ H ₂₈ N ₅ O ₄	366.2141
	259.1526	-	C ₁₁ H ₂₁ N ₃ O ₄	259.1532
	252.1342	252.1338	C ₁₂ H ₁₈ N ₃ O ₃	252.1348
	251.1502	251.1498	C ₁₂ H ₁₉ N ₄ O ₂	251.1508
	-	234.1233	C ₁₂ H ₁₆ N ₃ O ₂	234.1242
	-	206.1284	C ₁₁ H ₁₆ N ₃ O	206.1293
	190.6182	190.6179	C ₁₈ H ₃₁ N ₅ O ₄ ²⁺	190.6188
	180.1130	180.1128	C ₉ H ₁₄ N ₃ O	180.1137
	-	163.0863	C ₉ H ₁₁ N ₂ O	163.0871
	-	149.0706	C ₈ H ₉ N ₂ O	149.0715
	123.0916	123.0914	C ₇ H ₁₁ N ₂	123.0922
	-	84.0806	C ₅ H ₁₀ N	84.0813
2²⁺	381.2324	-	(¹³ C)C ₁₇ H ₃₀ N ₅ O ₄	381.2331
	381.2260	-	C ₁₈ H ₃₀ (¹⁵ N)N ₄ O ₄	381.2268
	380.2290	380.2287	C ₁₈ H ₃₀ N ₅ O ₄	380.2298
	366.2135	-	C ₁₇ H ₂₈ N ₅ O ₄	366.2141
	308.2080	-	C ₁₅ H ₂₆ N ₅ O ₂	308.2086
	-	291.1811	C ₁₅ H ₂₃ N ₄ O ₂	291.1821
	259.1527	-	C ₁₁ H ₂₁ O ₄ N ₃	259.1532
	190.6182	190.6179	C ₁₈ H ₃₁ N ₅ O ₄ ²⁺	190.6188
	180.1131	180.1128	C ₉ H ₁₄ N ₃ O	180.1129
	-	163.0863	C ₉ H ₁₁ N ₂ O	163.0871
	-	149.0707	C ₈ H ₉ N ₂ O	149.0715
	123.0916	123.0914	C ₇ H ₁₁ N ₂	123.0922
	-	84.0806	C ₅ H ₁₀ N	84.0813
	-	-	C ₁₆ H ₂₆ N ₄ O ₄	338.1954
3²⁺	338.1902	-	(¹³ C)C ₁₅ H ₂₅ N ₄ O ₄	338.1909
	338.1838	-	C ₁₆ H ₂₅ (¹⁵ N)N ₃ O ₄	338.1846
	337.1868	337.1874	C ₁₆ H ₂₅ N ₄ O ₄	337.1876
	320.1603	-	C ₁₆ H ₂₂ N ₃ O ₄	320.1610
	259.1526	-	C ₁₁ H ₂₁ N ₃ O ₄	259.1532
	241.1421	-	C ₁₁ H ₁₉ N ₃ O ₃	241.1426
	-	240.1344	C ₁₁ H ₁₈ N ₃ O ₃	240.1348
	216.1342	-	C ₉ H ₁₈ N ₃ O ₃	216.1348
	209.0920	209.0920	C ₁₀ H ₁₃ N ₂ O ₃	209.0926
	208.1080	-	C ₁₀ H ₁₄ N ₃ O ₂	208.1086
	-	191.0814	C ₁₀ H ₁₁ N ₂ O ₂	191.0821
	169.0971	169.0970	C ₁₆ H ₂₆ N ₄ O ₄ ²⁺	169.0977
	163.0866	163.0865	C ₉ H ₁₁ N ₂ O	163.0871
	147.1128	147.1127	C ₆ H ₁₅ N ₂ O ₂	147.1134
	137.0709	137.0708	C ₇ H ₉ N ₂ O	137.0715
	131.0940	131.0940	C ₆ H ₁₃ NO ₂	131.0946
4²⁺	-	120.0443	C ₇ H ₆ NO	120.0449
	84.0808	84.0807	C ₅ H ₁₀ N	84.0813
	-	80.0494	C ₅ H ₆ N	80.0500
	338.1902	-	(¹³ C)C ₁₅ H ₂₅ N ₄ O ₄	338.1909
	338.1837	-	C ₁₆ H ₂₅ (¹⁵ N)N ₃ O ₄	338.1846
	337.1868	337.1873	C ₁₆ H ₂₅ N ₄ O ₄	337.1876
	320.1603	-	C ₁₆ H ₂₂ N ₃ O ₄	320.1610
	266.1498	266.1500	C ₁₃ H ₂₀ N ₃ O ₃	266.1505
	265.1658	-	C ₁₃ H ₂₁ N ₄ O ₂	265.1665
	259.1526	-	C ₁₁ H ₂₁ N ₃ O ₄	259.1532
	248.1393	248.1394	C ₁₃ H ₁₈ N ₃ O ₂	248.1399
	241.1420	-	C ₁₁ H ₁₉ N ₃ O ₃	241.1426
	-	240.1343	C ₁₁ H ₁₈ N ₃ O ₃	240.1348
	-	209.0920	C ₁₀ H ₁₃ N ₂ O ₃	209.0926
	199.1077	199.1076	C ₉ H ₁₅ N ₂ O ₃	199.1083
	169.0971	169.0970	C ₁₆ H ₂₆ N ₄ O ₄ ²⁺	169.0977
	143.1178	-	C ₇ H ₁₅ N ₂ O	143.1184
	137.0709	137.0708	C ₇ H ₉ N ₂ O	137.0715
	120.0443	120.0442	C ₇ H ₆ NO	120.0449
	-	90.0548	C ₃ H ₈ NO ₂	90.0555
	-	84.0807	C ₅ H ₁₀ N	84.0813
	-	80.0494	C ₅ H ₆ N	80.0500

Figure 1. ETD mass spectra of doubly charged ions from (a) DMAP-ac-AK (1^{2+}), (b) DMAP-ac-KA (2^{2+}), (c) pyrid-ac-AK (3^{2+}), and (d) pyrid-ac-KA (4^{2+}) collected on a hybrid LTQ Orbitrap Velos instrument. Relative intensities are given as percentages of the sum of the charge-reduced ion intensities

the tag. In addition, a competing N- C_α bond cleavage at the DMAP moiety gave rise to fragments at m/z 259 and 123, the latter corresponding to protonated 4-dimethylaminopyridine. Note that these are complementary ions that differ in the position of the charging proton. The greater relative abundance of the $(\text{DMAP} + \text{H})^+$ ion indicates that DMAP is more basic than the $^-\text{CH}_2\text{CO-Ala-Lys}$ dipeptide residue.

The ETD mass spectrum of 2^{2+} (Figure 1b) was quite analogous to that of 1^{2+} in showing the c_1 (m/z 308) and c_0 (m/z 180) backbone fragments, as well as those due to the tag loss. Note that the tag loss peak (m/z 259) was substantially weaker

from 2^{2+} than from 1^{2+} . The general appearance of the ETD spectra of the DMAP-tagged peptides was that they were very clean and showed practically no fragments from side-chain losses or consecutive dissociations.

The ETD spectra of 3^{2+} and 4^{2+} showed the c_1 backbone fragments at m/z 208 and 265, respectively, whereas the c_0 ion at m/z 137 was very weak. Loss of the tag (m/z 259) was more pronounced for 3^{2+} . A few additional sequence fragments were found, such as the y_1 ion at m/z 147 from 3^{2+} and the m/z 266 ion from 4^{2+} . According to the accurate mass measurements (Table 1), the m/z 266 fragment arises

through the elimination of an alanine residue ($\text{C}_3\text{H}_5\text{NO}$) from the m/z 337 ion, and must involve a carboxyl OH group migration.

We also attempted to obtain ETD mass spectra of nonderivatized $(\text{AK}+2\text{H})^{2+}$ and $(\text{KA}+2\text{H})^{2+}$ ions. The AK peptide provided doubly charged precursor ions with intensities that were sufficient for ETD measurements. However, the ETD spectrum showed a very poor yield for charge reduction, even at prolonged ion-ion interaction times (Figure S2 of the ESM). The fragmentations observed upon electron transfer were the loss of H (m/z 218) followed by the elimination of ammonia (m/z 201) and water (m/z 200), and backbone cleavage giving the y_1 ion at m/z 147 and its further dissociation products at m/z 130, 129, 114, and 100. The KA peptide produced mainly singly charged ions, so ETD was not performed.

Electron Transfer to Larger Tagged Peptides

Tagging with DMAP was also investigated for larger peptides that may be more relevant to analytical applications of ETD in peptide sequencing. Although a critical evaluation of the procedure and its benefits and drawbacks when applied to a large number of tryptic peptides is beyond the scope of this report, encouraging results were obtained for pentapeptides of a tryptic type, as illustrated with AAAHR. Figure 2a shows the electrospray mass spectrum of the reaction mixture directly after tagging and without any purification. The mass spectrum is dominated by a triply charged ion of $(\text{DMAP-AAAHR}+2\text{H})^{3+}$, whereas the peak of the unreacted peptide (m/z 263.2) is weak, indicating good conversion. The ETD spectrum of the triply charged ion (Figure 2b) shows complete series of c_1 – c_4 as well as z_1 – z_4 ions. A notable feature is the very low relative intensities of sequence $c-1$ and $z+1$ fragment ions that underwent hydrogen atom transfer. This contrasts with the ETD spectrum of the doubly charged ion from untagged AAAHR (Figure 2c), which shows only z ions, some of which are accompanied by $z+1$ satellites (e.g., for the z_1 and z_3 fragments). Thus, tagging with DMAP increases the charge state of peptide ions to make them suitable for ETD, and improves sequence coverage by providing fragment ions from both the N and C termini.

Electron Capture by DMAP-ac-AK/KA and pyrid-ac-AK/KA Ions

To compare different modes of charge reduction, the electron-capture dissociation (ECD) [49] mass spectra were also recorded. Figure 3 shows that the ECD spectra are very different from the ETD spectra in Figure 1. In particular, the ECD spectrum of 1^{2+} (Figure 3a) showed a very weak m/z 380 fragment ion from the loss of H from the charge-reduced ion, which was absent. Major fragments were observed at m/z 252, 234, and 206, which corresponded to elimination of the lysine residue ($\text{C}_6\text{H}_{12}\text{N}_2\text{O}$) from the m/z

380 ion, followed by elimination of water and H_2CO_2 . These fragments indicate that this dissociation commences by a carboxyl OH group migration from the Lys to the Ala residue. The c_1 fragment at m/z 251 is very weak, whereas the c_0 fragment at m/z 180 and its dissociation product at m/z 163 by loss of ammonia are relatively abundant. Fragmentation at the N-terminus forms the $(\text{DMAP} + \text{H})^+$ ion at m/z 123, whereas the complementary m/z 259 ion is absent. The ECD spectrum of 2^{2+} (Figure 3b) is analogous to that of 1^{2+} . The spectrum in Figure 3b shows a weak m/z 380 ion that eliminates alanine ($\text{C}_3\text{H}_7\text{NO}_2$) to give the m/z 291 fragment. The other fragments are c_0 (m/z 180) and its dissociation products (m/z 163 and 149), as well as the $\text{C}_5\text{H}_{10}\text{N}^+$ ion at m/z 84 from the lysine side chain.

The ECD spectra of 3^{2+} and 4^{2+} showed extremely weak m/z 337 ions by charge reduction and loss of an H atom. This was followed by loss of the C-terminal residue, m/z 209 and m/z 266 for loss of lysine and $\text{C}_3\text{H}_5\text{O}$, respectively, and the subsequent elimination of the remaining amino acid to give the common pyridiniumketene fragment at m/z 120. Other common ECD fragments were $\text{C}_5\text{H}_{10}\text{N}^+$ (m/z 84) from the lysine side chain and protonated pyridine (m/z 80).

The differences between the ECD and ETD mass spectra of peptide ions are currently not well understood, but may be due to different electronic states accessed upon the capture of a free electron in ECD and the transfer of an electron from an anionic donor in ETD [50]. An obvious difference is the energetics of ECD and ETD, as discussed below.

Discussion

The ETD and ECD mass spectra showed that both the nature of the fixed-charge tag and the mode of charge reduction had substantial effects on the dissociations. From the point of view of ETD efficiency, the fixed-charge tags substantially improved double charging of dipeptides and triple charging of pentapeptides and provided abundant precursor ions. Thus, pyridinium-based charge tags are expected to have a favorable effect on the formation of multiply charged ions from larger peptides, especially those that do not contain basic amino acid residues. From the point of view of sequence information, ETD of the DMAP-tagged peptides appeared to be the most informative, and therefore we focused on interpreting the spectra of 1^{2+} and 2^{2+} . The interpretation starts with precursor ion structures, which are discussed next.

Precursor Ion Structures of DMAP-ac-AK/KA

The tautomers we considered for both charge-tagged peptides were protonated in the lysine ϵ -amino group, which corresponded to the most basic site in the dipeptides [15]. An extensive search of the dication conformational potential energy surface (PES) revealed several structures for 1^{2+} and

Figure 2. (a) Electrospray mass spectrum of a reaction mixture from tagging peptide AAAHR with DMAP. (b) ETD mass spectrum (500 ms reaction time) of the triply charged (DMAP-ac-AAAHR + 2H)³⁺ ion at m/z 229.8. (c) ETD mass spectrum (200 ms reaction time) of a doubly charged (AAAHR + 2H)²⁺ ion at m/z 263.2. Relative intensities are given as percentages of the sum of the charge-reduced ion intensities

2²⁺ as local energy minima (Scheme 2). The global energy minimum for **1²⁺** was structure **1a²⁺** by combined B3LYP and MP2/6-311++G(2d,p) enthalpy calculations and also according to free energies (Table 2). The ϵ -ammonium group in **1a²⁺** has an H-bond to the carboxyl carbonyl oxygen while maintaining effective spatial separation of the two charged groups to minimize Coulomb repulsion [15]. This can be gauged by the distance between the pyridine and Lys ϵ -ammonium N atoms, which is 12.6 Å in **1a²⁺**. The other low-energy conformers have less favorable combinations of lysine H-bonding and charge separation. For example, **1b²⁺** has the lysine ϵ -ammonium internally solvated by both the COOH and

Ala amide carbonyls, which leads to increased Coulomb repulsion and also decreases the ion entropy due to restricted rotations about backbone single bonds. Similar effects play a role in **1c²⁺**. Conformer **1d²⁺** with an extended lysine side chain is disfavored by enthalpy because of a loss of lysine H-bonding, but gains entropy by unimpeded backbone and side-chain rotations.

The most stable conformer of **2²⁺** (**2a²⁺**, Scheme 2) had the lysine ϵ -ammonium internally solvated by the carboxyl carbonyl. A virtually isoenergetic conformer (**2b²⁺**) showed the lysine ϵ -ammonium H-bonding to the COOH and amide carbonyls, but was entropically disfavored against **2a²⁺**,

Figure 3. ECD mass spectra of doubly charged ions from (a) DMAP-ac-AK (12^+), (b) DMAP-ac-KA (22^+), (c) pyrid-ac-AK (32^+), and (d) pyrid-ac-KA (42^+) collected on an LTQ FT-ICR instrument. Relative intensities are given as percentages of the sum of the charge-reduced ion intensities

which represented the global free energy minimum (Table 2). Another low-energy conformer ($2c^{2+}$) had the lysine ϵ -ammonium internally solvated by the carboxyl group only and was isoenergetic by free energy with $2b^{2+}$. Structures $1a^{2+}$ and $2a^{2+}$ were considered precursors for charge reduction and further radical-induced dissociations.

Electron Transfer Energetics

The energy balance ΔE for electron transfer between the anion-radical donor and the peptide ion acceptor is

expressed by Equation (1) [50]:

$$\Delta E = RE_a(\text{ion}) - [EA(\text{donor}) + \Delta E_{\text{exc}}(\text{donor})], \quad (1)$$

where $RE_a(\text{ion})$ is the ion adiabatic recombination energy, $EA(\text{donor})$ is the electron affinity of the neutral donor molecule, which is equal to the anion adiabatic electron binding energy, and $\Delta E_{\text{exc}}(\text{donor})$ is the excitation energy for the electronic state of the neutral donor molecule. Positive ΔE means that the electron transfer is exoergic. For fluoranthene, in particular, $EA=0.63$ eV [51] and $\Delta E_{\text{exc}}(\text{donor}) = 0, 3.06, 3.45, 3.83,$ and 4.32 eV for the S_0 -

Scheme 2. B3LYP/6-31+G(d,p) optimized structures of the lowest-energy conformers $1a^{2+}$ – $1e^{2+}$ and $2a^{2+}$ – $2e^{2+}$

S_4 singlet electronic states [52–54]. The calculated recombination energies of 1^{2+} and 2^{2+} conformers are collated in Table 3 and range from 5.83 to 6.5 eV. When used in Equation (1), the RE indicate that electron transfer from [fluoranthene] $^{\cdot-}$ to the charge-tagged peptides is always exoergic. However, the excitation energy deposited in the charge-reduced peptide ion depends on the electronic state of the departing fluoranthene molecule and can range from 0.88 for the S_4 state to 5.2 eV for the S_0 state. In contrast, the capture of a free electron results in the excitation of the charge-reduced ion, which is expressed by the adiabatic recombination energy of the precursor ion. The $RE_a(\text{ion})$ values in Table 3 indicate that a substantially greater excitation energy is imparted to the charge-reduced ions by ECD compared to ETD. These figures are important when discussing the dissociation energetics.

Cation-Radical Structures of DMAP-ac-AK/KA

Electron attachment to $1a^{2+}$ was 5.83–6.07 eV exoergic to produce the isomeric cation radicals $1a^{\cdot+}$ and $1b^{\cdot+}$ (Scheme 3). Of these two cation-radical conformers, the latter was 23 kJ mol $^{-1}$ more stable than the former (Table 4). $1b^{\cdot+}$ has the same bond connectivity as $1c^{2+}$ but shows a shorter H-bond between the tag acetyl oxygen and a Lys ammonium hydrogen (1.716 Å). $1a^{\cdot+}$ has the same bond connectivity as $1b^{2+}$ but shows a shorter H-bond between the tag acetyl oxygen and the Lys amide hydrogen (1.809 Å). Both $1a^{\cdot+}$ and $1b^{\cdot+}$ show >97% of the spin density spread about the entire 4-dimethylaminopyridine group. This moiety was also found via calculation to have a -0.18 negative charge in both of the mentioned species, whereas the positive charge is carried by the lysine ϵ -ammonium

Table 2. Relative enthalpies and free energies of doubly charged DMAP-acetyl-dipeptides

Ion	Relative energy ^a			
	B3LYP	B3LYP	MP2	B3-MP2
	6-31+G(d,p)	6-311++G(2d,p)		
(DMAP-ac-AK+H) ²⁺				
1a ²⁺	−14	−15	1	−7 (−16) ^b
1b ²⁺	0	0	0	0 (0) ^b
1c ²⁺	6	5	10	8 (6) ^b
1d ²⁺	16	13	29	21 (−0.1) ^b
1e ²⁺	30	27	33	30 (15) ^b
(DMAP-ac-KA+H) ²⁺				
2a ²⁺	5	−1	2	0.6 (−7) ^b
2b ²⁺	0	0	0	0 (0) ^b
2c ²⁺	11	3	10	7 (−0.8) ^b
2d ²⁺	32	26	30	28 (21) ^b
2e ²⁺	38	30	42	36 (19) ^b

^aIn units of kJ mol^{−1}, and including B3LYP/6-31+G(d,p) zero-point vibrational energies and 298 K enthalpies^bRelative free energies at 298 K

group. This electron distribution corresponds to the ground electronic state of **1⁺** and is consistent with the lower intrinsic recombination energy of the internally solvated lysine ϵ -ammonium group compared to that of 4-dimethylaminopyridinium [31, 32].

Charge reduction of **2²⁺** ions brings about the formation of cation radicals, which can exist as several conformers differing in the lysine ϵ -ammonium hydrogen bonding. The general structural feature of these cation radicals (**2⁺**) is that they maintain the lysine ϵ -ammonium as the charge-carrying group, whereas the odd electron is delocalized over the 4-dimethylaminopyridine moiety. Of the two most stable conformers, **2a⁺** has the same H-bonding pattern as **2a²⁺** but shows a shorter hydrogen bond between the tag acetyl oxygen and the lysine amide hydrogen at 1.974 Å. The removal of coulombic repulsion between the charge groups in **2a²⁺** upon electron attachment allows side-chain folding in the charge-reduced cation radical, leading to conformer **2b⁺**, which is the global energy minimum (Table 4). **2b⁺** displays a unique internal solvation of the charged group in that it develops a hydrogen bond between the dimethylamine

nitrogen and one of the ϵ -ammonium hydrogens at 1.903 Å (Scheme 3).

Dissociation Pathways of DMAP-ac-AK/KA Cation Radicals

Electron transfer to DMAP-tagged dipeptide ions induced the loss of a hydrogen atom, N–C α bond dissociations of both amide bonds, and another N–C α bond dissociation affecting the pyridine-linker bond. The energetics of the latter have been previously analyzed for a DMAP-tagged GK dipeptide and shown to have a low threshold energy (9 kJ mol^{−1}), and to require 67–70 kJ mol^{−1} in the pertinent transition state [31, 32]. We will use these values as benchmarks to be compared with the new data on N–C α bond dissociations of amide bonds in cation radicals **1⁺** and **2⁺**.

Dissociation of the Ala-Lys N–C α bond to produce the **c₁** ion was considered to start from **1a⁺**, which had a favorable conformation for H atom transfer from the Lys ϵ -ammonium to the pertinent amide carbonyl (Scheme 4). The H-atom transfer required 65 kJ mol^{−1} in **TS1** and formed aminoketyl

Table 3. Ion recombination energies

Ion	Recombination energy ^{a,b}			
	B3LYP	B3LYP	MP2	B3-MP2
	6-31+G(d,p)	6-311++G(2d,p)		6-311++G(2d,p)
1a²⁺ → 1a⁺	5.87	5.88 (5.17) ^c	5.78 (4.32) ^c	5.83 (4.74) ^c
1a²⁺ → 1b⁺	6.12	6.14	6.00	6.07
1b²⁺ → 1a⁺	6.02	6.04 (5.45) ^c	5.79 (4.94) ^c	5.92 (5.20) ^c
1c²⁺ → 1b⁺	6.34	6.36 (5.70) ^c	6.12 (5.24) ^c	6.24 (5.47) ^c
2a²⁺ → 2a⁺	6.07	6.08 (5.40) ^c	5.81 (4.91) ^c	5.95 (5.15) ^c
2a²⁺ → 2b⁺	6.18	6.20	6.12	6.16
2b²⁺ → 2a⁺	6.03	6.11 (5.49) ^c	5.80 (4.96) ^c	5.95 (5.23) ^c
2b²⁺ → 2b⁺	6.14	6.22	6.11	6.17
2d²⁺ → 2b⁺	6.47	6.48 (5.79) ^c	6.42 (5.37) ^c	6.45 (5.58) ^c

^aIn units of eV^bAdiabatic recombination energies include zero-point corrections^cVertical recombination energies without zero-point corrections

Scheme 3. B3LYP/6-31+G(d,p) optimized structures of the lowest-energy conformers **1a^{••}**, **1b^{••}**, **2a^{••}**, and **2b^{••}**

radical **1c^{••}**, which was 21 kJ mol⁻¹ above **1a^{••}** (Table 4). **TS1** is a late transition state in which the O–H bond has practically been formed at 1.072 Å (Scheme 4). It should be noted that aminoketyl radical **1c^{••}** can presumably exist in a variety of conformations, some of which may be more stable than structure **1c^{••}**. However, since an exhaustive conformational search of peptide cation radicals cannot be carried out, we proceeded with **1c^{••}** as a plausible intermediate for further dissociations. N–C_α bond cleavage in **1c^{••}** was found to proceed through **TS2**, which was 93 kJ mol⁻¹ above the reactant and 114 kJ mol⁻¹ above **1a^{••}**. The N–C_α bond

dissociation formed a complex of the incipient **c₁** ion in its enolimine tautomeric form and the neutral **z₁** radical (**1d^{••}**). Complex **1d^{••}** was 33 kJ mol⁻¹ below the starting charge-reduced ion **1a^{••}**, indicating that the N–C_α bond dissociation was *exothermic*. However, **c₁** and **z₁** fragment separation in **1d^{••}** was 69 kJ mol⁻¹ endothermic, making the overall fragmentation 36 kJ mol⁻¹ *endothermic* (Table 4). This example illustrates that the energies for N–C_α bond dissociations and the overall fragmentation upon ETD are very different and must be distinguished when discussing the ETD mechanisms. Furthermore, **1d^{••}** can follow an energetically more favorable

Table 4. Relative energies of (DAP-ac-AK+H) cation radicals

Reaction	Relative energy ^{a,b}			
	B3LYP	B3LYP	MP2	B3-MP2
	6-31+G(d,p)	6-311++G(2d,p)		6-311++G(2d,p)
1a^{••}→1b^{••}	-24	-25	-21	-23
1a^{••}→TS1	42	44	86	65
1a^{••}→1c^{••}	26	28	15	21
1c^{••}→TS2	87	84	101	93
1c^{••}→1d^{••}	-70	-75	-34	-55
1c^{••}→1e^{••}	-132	-136	-94	-115
1d^{••}→1e^{••}	-62	-61	-60	-61
1a^{••}→1d^{••}	-45	-47	-19	-33
1a^{••}→1e^{••}	-107	-108	-79	-94
1a^{••}→enolimine-AK_{c1}⁺ + AK_{z1}[•]	16	6	66	36
1a^{••}→amide-AK_{c1}⁺ + AK_{z1}[•]	-56	-64	1	-31
1b^{••}→TS3	-4	0	3	2
1b^{••}→1f^{••}	0	3	1	2
1f^{••}→TS4	61	60	74	67
1f^{••}→1g^{••}	-32	-36	-18	-27
1f^{••}→1h^{••}	-110	-113	-89	-101
1b^{••}→1g^{••}	-32	-33	-17	-25
1b^{••}→1h^{••}	-110	-109	-88	-99
1b^{••}→enolimine-AK_{c0}⁺ + AK_{z2}[•]	47	43	83	63
1b^{••}→amide-AK_{c0}⁺ + AK_{z2}[•]	-23	-27	17	-5

^aIn units of kJ mol⁻¹

^bIncluding B3LYP/6-31+G(d,p) zero-point energies and referring to 0 K

Scheme 4. Backbone dissociations of 1a⁺• Scheme 5. Backbone dissociations of 1b⁺•

pathway by isomerizing to another complex $1e^{++}$ (-61 kJ mol^{-1} relative to $1d^{++}$, Table 4), in which the c_1 ion moiety is in the more stable amide tautomeric form (Scheme S1 of the ESM). Fragment separation in $1e^{++}$ is also endothermic, but occurs at -31 kJ mol^{-1} relative to $1a^{++}$, making this dissociation pathway exothermic.

Overall, the reactions leading to the formation of c_1 had to cross **TS2** as the highest point on the PES at 114 kJ mol^{-1} . This is higher than the presumed energy barrier for the loss of DMAP, which was calculated to be at $67\text{--}70 \text{ kJ mol}^{-1}$ in related peptide radicals [31, 32]. The fact that these two dissociations are kinetically competitive indicates that the calculated PES would have to be modified to accurately describe the actual dissociations. Modifications to this end may involve finding lower energy conformers for **TS2** or $1e^{++}$ or a higher energy TS for the DMAP loss, which both would kinetically favor Ala-Lys N- C_α bond dissociation.

Dissociation of the tag-Ala N- C_α bond to produce the c_0 ion was considered to start from conformer $1b^{++}$, which had an appropriate arrangement of the Lys ϵ -ammonium and the tag amide carbonyl (Scheme 5). H-atom migration in $1b^{++}$ required an extremely low activation energy in **TS3** and produced aminoketyl radical $1f^{++}$, which was practically isoenergetic with $1b^{++}$. N- C_α bond cleavage in $1f^{++}$ proceeded through **TS4** (67 kJ mol^{-1} relative to $1f^{++}$,

Table 4), forming a complex of the enolimine tautomeric form of the c_0 ion and the complementary z_2 radical ($1g^{++}$), which was 25 kJ mol^{-1} more stable than $1b^{++}$. A further dissociation of $1g^{++}$ can follow an endothermic pathway to separate the incipient fragments at 63 kJ mol^{-1} relative to $1b^{++}$. An energetically more favorable pathway involves enolimine–amide isomerization in $1g^{++}$, forming more stable complexes (e.g., $1h^{++}$, Scheme S2 of the ESM), which can dissociate to produce an amide tautomer of the c_0 ion at -5 kJ mol^{-1} relative to $1b^{++}$.

A salient feature of the dissociation pathways discussed above is the energetics of the first step, which is the Lys ϵ -ammonium H-atom migration. Analogous migrations have been studied for peptide radicals and cation radicals and shown to be substantially exothermic, requiring very low activation energies [55, 56]. In contrast, the isomerizations in the charge-tagged peptide cation radicals shown in Schemes 4 and 5 were uniformly endothermic and required substantial TS energies. The unusual properties of $1a^{++}$ and $1b^{++}$ can be understood by analyzing the electron distribution and excited electronic states that develop during the course of these reactions.

The ground electronic state of $1a^{++}$ (**X**) has the odd-electron density localized in the DMAP moiety, as represented by the corresponding π orbital (SOMO, Figure 4). This electronic configuration does not allow for a migration of the Lys ϵ -ammonium hydrogen atom, which requires odd-

Figure 4. Electronic states for $1a^{++}$ and **TS1** from TD-DFT calculations with B3LYP/6-311++G(2d,p). The orbital phases are distinguished by *gold* and *purple* colors. Energies are in kJ mol^{-1} for vertical excitations

Figure 5. Electronic states for $1b^{+\bullet}$ and $TS3$ from TD-DFT calculations with B3LYP/6-311++G(2d,p). The orbital phases are distinguished by gold and purple colors. Energies are in kJ mol^{-1} for vertical excitations

Scheme 6. Backbone dissociations of $2a^{+\bullet}$

Table 5. Relative energies of (DMAP-ac-KA + H) cation radicals

Reaction	Relative energy ^{a,b}			
	B3LYP	B3LYP	MP2	B3-MP2
	6-31+G(d,p)	6-311++G(2d,p)		6-311++G(2d,p)
$2a^{+\bullet} \rightarrow 2b^{+\bullet}$	-10	-11	-30	-21
$2a^{+\bullet} \rightarrow TS5$	41	42	57	49
$2a^{+\bullet} \rightarrow 2c^{+\bullet}$	22	22	29	26
$2c^{+\bullet} \rightarrow TS6$	67	65	78	71
$2c^{+\bullet} \rightarrow 2d^{+\bullet}$	30	26	56	41
$2a^{+\bullet} \rightarrow 2d^{+\bullet}$	8	4	26	15
$2a^{+\bullet} \rightarrow 2e^{+\bullet}$	-6	-11	20	5
$2e^{+\bullet} \rightarrow TS7$	115	114	131	123
$2e^{+\bullet} \rightarrow 2f^{+\bullet}$	-74	-73	-66	-70
$2a^{+\bullet} \rightarrow 2f^{+\bullet}$	-80	-84	-46	-65
$2a^{+\bullet} \rightarrow 2g^{+\bullet}$	-96	-97	-8	-88
$2a^{+\bullet} \rightarrow \text{enolimine-KA}_{C_1}^{+\bullet} + \text{KA}_{Z_2}^{\bullet}$	7	2	39	21
$2a^{+\bullet} \rightarrow \text{amide-KA}_{C_1}^{+\bullet} + \text{KA}_{Z_1}^{\bullet}$	-46	-50	-16	-33
$2b^{+\bullet} \rightarrow TS8$	42	43	108	76
$2b^{+\bullet} \rightarrow 2h^{+\bullet}$	3	5	31	18
$2g^{+\bullet} \rightarrow TS9$	42	41	49	45
$2g^{+\bullet} \rightarrow 2i^{+\bullet}$	-35	-38	-26	-32
$2g^{+\bullet} \rightarrow 2j^{+\bullet}$	-71	-77	-54	-65
$2b^{+\bullet} \rightarrow 2i^{+\bullet}$	-31	-33	5	-14
$2b^{+\bullet} \rightarrow 2j^{+\bullet}$	-68	-72	-23	-47
$2a^{+\bullet} \rightarrow \text{enolimine-KA}_{C_1}^{+\bullet} + \text{KA}_{Z_2}^{\bullet}$	66	56	135	95
$2b^{+\bullet} \rightarrow \text{amide-KA}_{C_0}^{+\bullet} + \text{KA}_{Z_2}^{\bullet}$	-5	-13	69	28

^aIn units of kJ mol^{-1} ^bIncluding B3LYP/6-31+G(d,p) zero-point energies and referring to 0 K **Scheme 7.** Backbone dissociations of $2b^{+\bullet}$

electron density for a transfer to the recipient amide group [15, 55]. Note that such migrations typically display the characteristics of proton-coupled electron transfer (PCET) [57–59], and therefore should be referred to as such. PCET is possible in the **A**, **B**, and presumably also higher excited states of **1a⁺⁺** that have high odd-electron density in the Ala amide carbonyl, as represented by the pertinent orbitals for the **A–D** states (Figure 4). The activation energy associated with the PCET in **1a⁺⁺** ($E_{\text{TS1}}=65 \text{ kJ mol}^{-1}$ by B3-PMP2, 44 kJ mol^{-1} by B3LYP) results from the mixing of the ground and excited states in **TS1**, with upper bounds given by the excitation energies in **1a⁺⁺**, for example $\Delta E_{\text{exc}}=60$ and 103 kJ mol^{-1} for the **A** and **B** states, respectively (Figure 4). The loss of an H atom from **1a⁺⁺** can occur from the **D** state ($\Delta E_{\text{exc}}=122 \text{ kJ mol}^{-1}$), which has a character of a symmetry-adjusted ammonium $3s$ Rydberg (Figure 4). Rydberg $3s$ states in ammonium radicals are known to undergo facile H-atom loss [60–62]. However, one cannot a priori exclude an alternative pathway for the H loss to occur from aminoketyl radical **1c⁺⁺** in competition with the N–C $_{\alpha}$ bond cleavage through **TS2**. Although we did not study this reaction in detail here, previous studies of aminoketyl radicals indicated TS energies for H-atom loss ranging from 68 [55] to 91 kJ mol^{-1} [63], which could be competitive with N–C $_{\alpha}$ bond cleavage through **TS2** ($E_{\text{TS2}}=93 \text{ kJ mol}^{-1}$, Table 4).

In contrast to **1a⁺⁺**, PCET involving the Lys ϵ -ammonium hydrogen migration to the tag-Ala amide group in **1b⁺⁺** requires a low activation energy in **TS3**. This can be explained by the electron distribution in the ground electronic states, which show correlation between the odd-electron density in the corresponding SOMOs (Figure 5). Thus, the SOMO in **1b⁺⁺**, albeit mostly confined to the DMAP moiety, shows delocalization of electron density to the tag-Ala amide. Therefore, reaching the SOMO in **TS3** requires only minor mixing of the reactant **X** and **A** states, resulting in a low activation energy.

Backbone dissociations in **2a⁺⁺** can be discussed along the same lines as done for **1a⁺⁺** and **1b⁺⁺**. The dissociation leading to the **c₁** ion can be visualized as starting with a migration of a Lys ϵ -ammonium hydrogen to the Lys-Ala amide group, which requires 49 kJ mol^{-1} in **TS5** (Scheme 6, Table 5), and forms an aminoketyl intermediate (**2c⁺⁺**). Note that **2c⁺⁺** is 26 kJ mol^{-1} less stable than **2a⁺⁺**. N–C $_{\alpha}$ bond cleavage may proceed through **TS6**, which is 71 kJ mol^{-1} above **2c⁺⁺**, to form an ion–radical complex (**2d⁺⁺**). The latter is only weakly bound toward dissociation to the **c₁** ion and **z₁** radical (Scheme 6). However, complex **2d⁺⁺** can undergo exothermic isomerizations by enolimine rotations and ion–radical reorientation (**2e⁺⁺**), or prototropic isomerization of the enol imine moiety to a more stable amide [64, 65] (Scheme S3 of the ESM). Interestingly, the latter isomerization is only weakly catalyzed by the incipient **z₁** radical that helps to chaperon the enol OH proton to the imine nitrogen through **TS7** at 123 kJ mol^{-1} above **2e⁺⁺** (Table 5). As expected, the enolimine–amide isomerization is substantially exothermic, forming complex **2f⁺⁺** or its reorientation isomers.

Dissociation of the tag-Lys N–C $_{\alpha}$ bond to produce the **c₀** ion was considered to start from conformer **2b⁺⁺** (Scheme 7). PCET migration of a Lys ϵ -ammonium hydrogen to the tag-Lys amide group requires a high activation energy (**TS8**) to form aminoketyl radical **2h⁺⁺**, which is 18 kJ mol^{-1} above **2b⁺⁺**. N–C $_{\alpha}$ bond cleavage in **2g⁺⁺** requires 45 kJ mol^{-1} in **TS9** to give complex **2i⁺⁺** at 32 kJ mol^{-1} below **2g⁺⁺**. Fragment separation in **2i⁺⁺** is 109 kJ mol^{-1} endothermic, but can be avoided by exothermic ion–radical pair reorientation and prototropic enolimine–amide isomerization (**2j⁺⁺**). Dissociation of the latter complex is 75 kJ mol^{-1} endothermic, which brings the energy threshold for the separated **c₁** and **z₁** fragment to 28 kJ mol^{-1} relative to **2b⁺⁺**. This analysis indicates that reorientation in the **c**-ion–**z**-radical complexes followed by **c**-ion isomerization is the energetically favorable pathway for fragment formation.

We note that the activation energies for N–C $_{\alpha}$ bond dissociations between the Ala-Lys and Lys-Ala residues in **TS2** and **TS6**, respectively, are at the high end of the typical energy range for analogous dissociations in peptide radicals and cation radicals ($2\text{--}71 \text{ kJ mol}^{-1}$)[66]. Such relatively high TS energies are presumably due to the conformational changes from the aminoketyl reactants to the pertinent TS rather than to some unusual electronic properties. The reactants (**1d⁺⁺** and **2c⁺⁺**) are proper aminoketyl radicals [66], judging by their pyramidized geometry and odd-electron density. The conformational effects are illustrated with the N–C $_{\alpha}$ bond cleavage in **1d⁺⁺**, for which we found three transition states that differed in their conformations with energies ranging from 69 to 91 kJ mol^{-1} .

PCET involving the Lys ϵ -ammonium hydrogen migration to the Lys-Ala amide group in **2a⁺⁺** can also be explained using the electronic state energies and orbital analysis (Figure S3 of the ESM). The SOMO in **2a⁺⁺** shows odd-electron delocalization over the DMAP moiety, as confirmed by the calculated spin densities. To reach the TS for PCET (**TS5**), the electron density must flow into the Lys-Ala amide group. This can be realized by mixing the **X** and **A** states in **2a⁺⁺** to form the SOMO in **TS5** (Figure S3 of the ESM).

Conclusions

Electron-transfer dissociation of short peptide ions tagged with fixed-charge groups that were previously designed on the basis of electronic structure analysis and attached to the N-terminus showed increased abundance of backbone fragments and decreased side-chain losses when compared to the ETD of the parent, underivatized, peptide ions. The 4-dimethylaminopyridinium tag, attached to the peptide by a methylcarboxamide linker (DMAP-ac), appears to be particularly effective at providing informative ETD spectra with a balanced abundance of **c**-type fragment ions carrying the fixed-charge tag. Further exploration of the potential of the DMAP-ac tag for larger peptides is in progress in this laboratory.

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