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Article

Salt-Bridged Peptide Anion Gaseous Structures Enable Efficient Negative Ion Electron Capture Dissociation

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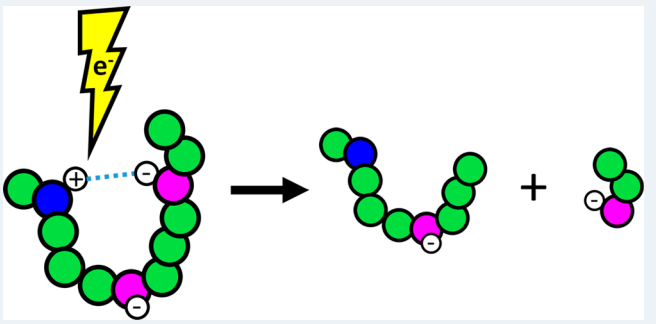
ABSTRACT: We previously discovered that electron attachment to gaseous peptide anions can occur within a relatively narrow electron energy range. The resulting charge-increased radical ions undergo dissociation analogous to conventional cation electron capture/transfer dissociation (ECD/ETD), thus enabling a novel tandem mass spectrometry (MS/MS) technique that we termed negative ion electron capture dissociation (niECD). We proposed that gaseous zwitterionic structures are required for niECD with electron capture either occurring at or being directed by a positively charged site. Here, we further evaluate this zwitterion mechanism by performing niECD of peptides derivatized to alter their ability to form zwitterionic gaseous structures. Introduction of a fixed positive charge tag, a highly basic guanidino group, or a highly acidic sulfonate group to promote zwitterionic structures in singly charged anions, rescued the niECD ability of a peptide refractory to niECD in its unmodified form. We also performed a systematic study of five sets of synthetic peptides with decreasing zwitterion propensity and found that niECD efficiency decreased accordingly, further supporting the zwitterion mechanism. However, traveling-wave ion mobility-mass spectrometry experiments, performed to gain further insight into the gas-phase structures of peptides showing high niECD efficiency, exhibited an inverse correlation between the orientationally averaged collision cross sections and niECD efficiency. These results indicate that compact salt-bridged structures are also a requirement for effective niECD.



INTRODUCTION

Electron irradiation as an activation method in tandem mass spectrometry (MS/MS)[1−4] is a valuable complement to conventional collision-based MS/MS due to, for example, its ability to improve sequence coverage in top-down proteomics and to determine post-translational modifications (PTMs). Originally most compatible with Fourier transform ion cyclotron resonance (FT-ICR) mass analyzers, free electron irradiation is subject to renewed interest due to the development of compact reaction cells compatible with virtually any MS/MS-enabled instrument.[5,6] The interaction between gaseous even-electron molecular ions (typically generated by electrospray ionization) and free electrons can trigger a range of dissociative reactions, depending upon the electron energy as well as the polarity of the precursor ion.[7−12] Among these electron-based MS/MS techniques, electron capture dissociation (ECD),[12] which involves attachment of low-energy (<1 eV) electrons to multiply charged cations, has found the broadest application for structural analysis of biomolecules, primarily proteins.[13−18] In ECD of peptides and proteins, radical-driven fragmentation of charge-reduced cations yields N-C_α backbone bond cleavage, resulting in predictable $c'/z^\bullet$-type (Zubarev nomenclature[10]) product ions without loss of labile PTMs.

In parallel with research on the analytical applications of ECD, there has been much interest in studying the associated dissociation chemistry. Two primary mechanisms have been proposed to explain the bond cleavage observed in ECD of peptides/proteins. In the Cornell model,[19] the electron is captured at/transferred to a protonated site and a hydrogen radical, $H^\bullet$, resulting from neutralization is released and recaptured by a nearby carbonyl oxygen atom, yielding an aminoketyl intermediate that dissociates via N-Cα backbone bond cleavage. In the Utah-Washington model,[20,21] it is argued that the electron is first captured in/transferred to the remote-charge but Coulomb-stabilized π^* orbital of a backbone amide, generating an aminoketyl radical anion (a super base), which abstracts a nearby proton. The resulting aminoketyl radical

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undergoes facile cleavage of the adjacent N-Cα bond with a low energy barrier.

Attachment of electrons to anions is counterintuitive due to Coulomb repulsion. However, we showed that such a process can indeed occur for both singly- and multiply deprotonated peptide anions within a rather narrow electron energy range, an intriguing reaction we termed negative ion electron capture dissociation (niECD).[11] The resulting charge-increased radical intermediates undergo extensive dissociation into the same c' and $z^{\bullet}$-type product ions as in conventional ECD of cations. Analogous to ECD, niECD is valuable for peptide sequencing and for PTM site determination due to retention of labile PTMs in peptide fragments.[11,22] niECD appears particularly promising for analysis of acidic peptides, such as biologically important phospho- and sulfopeptides, which show improved ionization in negative ion mode and are still challenging to analyze with currently available MS/MS techniques. In addition, the charge increase upon electron capture in niECD (in contrast to the charge decrease in ECD) improves fragment ion signal-to-noise ratios in the image current-based detection employed in FT-ICR instruments because the generated image current is proportional to the charge state.[9] The latter advantage affords higher fragmentation efficiencies than in conventional ECD, in theory >100%, similar to electron ionization dissociation in which positive ions are further ionized to form more highly charged cationic intermediates.[9] Moreover, niECD is compatible with (but not limited to) singly charged ions, thus potentially allowing coupling with matrix-assisted laser desorption/ionization (MALDI). In summary, niECD has potential to be analytically valuable; however, in order to fully harness its promise, the niECD mechanism requires additional investigation. For example, understanding of dissociation pathways offers the opportunity to design derivatives that control gas-phase chemistry for optimized fragmentation and/or wider application. In addition, gaseous anions exhibit different physical and chemical properties compared with their counterparts in solution. Mechanistic studies of niECD allow for the generation of novel insights into the gas-phase structures of biomolecular anions, which are less understood compared with biomolecular cations.

Our earlier work showed that niECD is not a universal reaction, i.e., several unmodified peptides were found to not undergo niECD.[11] We proposed that zwitterionic, overall negatively charged, gas-phase structures are necessary for successful niECD with electron capture either occurring at, or being directed by, a positively charged site, similar to the ECD mechanisms described above. The striking similarity in product ion types (c' and $z^{\bullet}$) observed between niECD and ECD supports related mechanisms and that positively charged sites play a role in niECD. By contrast, c' and $z^{\bullet}$-type product ion pairs are not observed as the major products in other anion-electron reactions such as electron-detachment dissociation[8,23] and electron-induced dissociation.[24] We tested our zwitterion hypothesis by derivatizing peptides to prevent or promote formation of gaseous zwitterionic structures.[11] While the corresponding data supported our proposed mechanism we also found that the presence of a gas-phase zwitterion did not appear to be the only criterion for successful niECD. Here, we report additional structure perturbation and ion mobility-mass spectrometry (IM-MS) experiments for further evaluation of analyte structural requirements in niECD. The current work includes a systematic study of five sets of synthetic peptides with gradually decreasing abilities to form gaseous zwitterions in negative ion mode. Taken together, these data suggest that salt-bridged structures are essential for efficient niECD.

EXPERIMENTAL SECTION

Reagents. Cholecystokinin (CCK, H-DYMGWMDF-NH_2), *S*-methylisothiourea hemisulfate, and 4-amino-1-naphthalenesulfonic acid (ANSA) were purchased from Sigma-Aldrich (St. Louis, MO). Methylated 1,4-diazabicyclo[2.2.2]-octane (mDABCO)-based *N*-hydroxysuccinimide (NHS) ester reagent (Figure S1a) was a generous gift from Dr. Philip Andrews' laboratory at the University of Michigan. Synthetic peptides were purchased from GenicBio (Shanghai, China).

Sample Preparation. mDABCO-based NHS reagent was incubated with CCK at 5:1 molar ratio in HEPES buffer (pH = 7) at room temperature for 20 min. N-terminal guanidination reactions were carried out as previously described[25] with some modifications.[26] Four microliters of 250 mM 1*H*-pyrozole-1-carboxamidine hydrochloride in 10% triethylamine (TEA) was combined with 2 μL of peptide and incubated at 95 °C for 30 min. ANSA derivatization was performed as previously described[27] with a few modifications. Unmodified peptide was dissolved in 25 μL pyridine/HCl coupling buffer. Twenty-five microliters of 1-ethyl-3(3-(dimethylamino)propyl) carbodiimide hydrochloride coupling reagent and 200 μL of 250 mM ANSA were added followed by incubation at room temperature for 1 h and quenching with 50 μL acetic acid. After the reactions, solvents were removed under vacuum. Derivatized as well as synthetic peptides were purified using C18 microtips (Millipore, Billerica, MA) following acidification with formic acid, except for the ANSA-derivatized peptide which was purified with HPLC (4.6 mm, 10 cm, i.d. C18 column). All samples were dissolved in 1:1 isopropanol/water (v/v) with 0.1% trimethylamine except for the ANSA-derivatized peptide, which was dissolved in 1:1 methanol:water with triethylamine, for negative ion mode analysis.

Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry. Peptide samples were directly infused via an external Apollo II electrospray ion source (Bruker Daltonics, Billerica, MA) at a flow rate of 70−300 μL/h in negative ion mode. All experiments were performed with an actively shielded 7 T hybrid quadrupole (Q)-FT-ICR mass spectrometer, either an APEX-Q or SolariX, Bruker Daltonics), as previously described.[11] Briefly, ions generated by electrospray ionization were accumulated in the first hexapole for 0.05 s, mass-selectively accumulated in the second hexapole collision cell for 0.5−4 s, transferred through high voltage ion optics (ApeX) or a hexapole ion guide (SolariX), and captured in an Infinity[28] (Apex) or dynamically harmonized[29] (SolariX) ICR cell. This accumulation sequence was looped one to three times to improve precursor ion abundance.

For niECD experiments, trapped peptide anions were irradiated with electrons originating from an indirectly heated hollow dispenser cathode.[30] The cathode heating current was 1.8 A (Apex) or 1.5 A (SolariX), and the cathode voltage was pulsed to −5 to −6 V (corresponding to ∼4.5 eV electrons[11]) for 5−20 s. A lens electrode located in front of the hollow cathode was kept 1−3 V more positive than the cathode bias voltage. All mass spectra were acquired with XMASS software (version 6.1; ApeX) or FTMSControl (version 2.3.0; SolariX) in broadband mode from m/z 200 up to 3000 with 256 K data points and summed over 10−64 scans.

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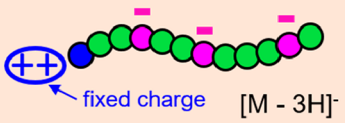
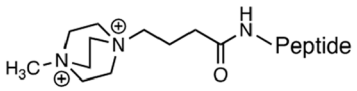
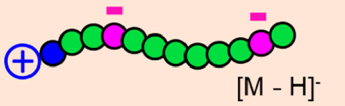
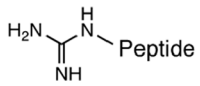
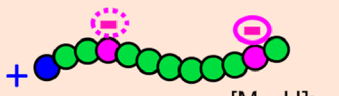
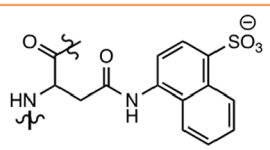
Table 1. Three Strategies for Promoting Formation of Overall Singly Negatively-Charged Gaseous Peptide Anions: N-Terminal Derivatization with a Fixed Positive Charge Tag (Top), N-Terminal Guanidination (Middle), and Carboxylic Acid Derivatization with ANSA (Bottom)[a]

Zwitterion Type	Reagent	Structure	Improved niECD
	mDABCO derivative (++)		✓
	Guanidination (+)		✓
	ANSA (-)		✓

[a]All three approaches enabled niECD of a peptide that did not undergo electron capture in its unmodified form; however, limited structural information was generated.

Data Analysis. Data processing was performed with the MIDAS analysis software[31] (Apex) or Bruker Compass DataAnalysis 5.0 (SolariX). Data were zero-filled once, Hanning apodized, and exported to Microsoft Excel for internal frequency-to-mass calibration with a two-term calibration equation (Apex)[32] or with the default processing in DataAnalysis and external calibration with hirudin niECD fragments (SolariX). Typically, internal calibration was performed with precursor ions and their electron-capture species as calibrants. MS/MS spectral peaks were assigned within 10 ppm error and only if their signal-to-noise ratio was at least 3.

Ion Mobility-Mass Spectrometry. Collision cross section (CCS) values for the synthetic peptides P13−P17 were determined using a Waters Synapt G2 traveling wave ion mobility mass spectrometry (TWIM-MS) instrument.[33,34] Briefly, peptide anions were generated with nano electrospray ionization in negative ion mode. Ions were pulsed from the trap cell into the TWIM cell, pressurized at 3.52 mbar with a mixture of He and N_2 gas (He and N_2 flow rates were 200 mL/min and 90 mL/min, respectively). For TWIM separations, 300 m/s wave velocity and 30, 32, and 34 V wave amplitudes were used. After mobility separations, ions were transferred via the transfer cell into the orthogonal acceleration time-of-flight (oa-ToF) mass analyzer. TWIM arrival time distributions (ATDs) are recorded by synchronizing the oa-ToF acquisition with the gated release of ions from the trap cell into the TWIM ion guide. D,L-Polyalanine was used as reference ions[35] for CCS calibrations at all wave amplitudes and velocity conditions.

TWIM ATDs were extracted using TWIMExtract[36] and fitted with Gaussian functions to determine centroid values. Arrival times were corrected for the mass analyzer flight time using a previously described method.[37] CCSs were calibrated using the corrected arrival times and the reference N_2 CCS values for the D,L-polyalanine ion series (number of alanines = 5−14, charge state 1^-).[35] Blend + radial calibration function[38] was employed to predict highly accurate $^{TW}CCS_{N2}$ values[39] for the synthetic peptides P13−P17 using IMSCal software. A 2% error was incorporated for the uncertainty related to the measured arrival times and reference CCS values. IMSCal provides average and standard deviation of predicted CCS values, calculated by combining the CCS values from all wave amplitude and velocity conditions. Error values are calculated according to the following equation:

$$\text{error} = \sqrt{\text{std dev(CCS values)}^2 + \frac{1}{n}\left(\sum_i^n \text{std\_dev\_ccs}^2\right)}$$

in which CCS values are the average CCS values from IMSCal for each TWIM condition, std_dev_ccs is the standard deviation of the predicted CCS values, i denotes the TWIM condition used for calibration, and n is the number of TWIM conditions.

RESULTS AND DISCUSSION

Peptide Fixed-Positive Charge Derivatization and Guanidination. In our previous work,[11] we showed that N-terminal trimethylammoniumacetyl (TMAA) derivatization, introducing a fixed positive charge, enabled niECD of peptides recalcitrant to electron capture in their native forms. Such derivatized peptides must undergo double deprotonation to yield an overall singly charged anion, thus ensuring a zwitterionic structure in negative ion mode. However, electron capture at the positively charged site mainly resulted in uninformative neutral trimethylamine loss with only a few low abundance backbone fragment ions. This outcome is similar to reported fragmentation behavior of fixed charge-containing peptides in conventional ECD.[40−42] In stark contrast, tris-(2,4,6-trimethoxyphenyl)phosphonium-acetyl (TMPP-Ac) derivatization of the same peptides did not enable niECD. We speculated that shielding of the phosphonium positive charge by the surrounding phenyl groups may account for this

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(a)

1st	Peptide Sequence
P1	DFEEIPEEYLQ
P2	DFYEIPEEYLQ
P3	DFYEIPLEYLQ
P4	DFYLIPLEYLQ
P5	YFYLIPLEYLQ
P6	YFYLIPLSYLQ

(b)

2nd	Peptide Sequence
P7	DFEEIPEEYLK
P8	DFYEIPEEYLK
P9	DFYEIPLEYLK
P10	DFYLIPLEYLK
P11	YFYLIPLEYLK
P12	YFYLIPLSYLK

(c)

3rd	Peptide Sequence
P13	KLFIDYEEPEE
P14	KLFIDYEEPEY
P15	KLFIDYELPEY
P16	KLFIDYLLPEY
P17	KLFIYYLLPEY
P18	KLFIYYLLPSY

(d)

4th	Peptide Sequence
P19	DFEEIPEEYLK*
P20	DFYEIPEEYLK*
P21	DFYEIPLEYLK*
P22	DFYLIPLEYLK*
P23	YFYLIPLEYLK*
P24	YFYLIPLSYLK*

(e)

5th	Peptide Sequence
P25	K*LFIDYEEPEE
P26	K*LFIDYEEPEY
P27	K*LFIDYELPEY
P28	K*LFIDYLLPEY
P29	K*LFIYYLLPEY
P30	K*LFIYYLLPSY

Figure 1. Five sets of peptides, designed around the hirudin sequence, examined by niECD. In the first series [peptides P1−P6 (a)], acidic residues were gradually replaced with more neutral ones, thus decreasing the probability of gaseous zwitterionic structures. In peptides P7−P12 (b), the C-terminal glutamine was replaced with lysine. For peptides P13−18 (c), this C-terminal lysine was moved to the N-terminus. In peptides P19−24 (d) and P25−P30 (e), these terminal lysine side chains were converted to more basic guanidino groups. Acidic residues are highlighted in purple and basic ones in blue. K* represents guanidinated lysine.

outcome. Another, mDABCO-based, moiety for N-terminal fixed positive charge derivatization[43] includes two intrinsic positive charges, thus peptides need to undergo triple deprotonation to form an overall singly charged anion. Similar to the TMAA tag, addition of the mDABCO-based group enabled niECD for the peptide CCK (Figure S1b). However, again, the major observed dissociation pathways were elimination of the partial or intact mDABCO-based moiety (Figure S1b). No backbone fragments were observed, i.e., TMAA or mDABCO-based derivatization, and while mechanistically informative, it does not present a solution to broaden the scope of niECD.

An alternative approach to promoting gaseous zwitterionic structures in negative ion mode is to introduce a highly basic group while ensuring at least two acidic groups are also present. The peptide CCK (Figure S1b) only contains one moderately basic site (the N-terminus) and two aspartic acid residues (the C-terminus is amidated). Guanidination of N-terminal amino groups has shown utility for characterization of the N-terminome.[25] We used this chemistry to convert the CCK N-terminal α-amino group to a guanidino group, hypothesizing that the corresponding increase in basicity would enhance N-terminal protonation and thus increase the probability of zwitterion formation in negative ion mode. As shown in Figure S2, N-terminal guanidination indeed enabled niECD for the CCK peptide, generating a charge-increased radical intermediate, one *c*-, one *z*-, and two *y*-type backbone fragments. Loss of the guanidino group was not observed.

A third approach to zwitterion formation may be to decrease the pK_a of acidic sites. To this effect, we derivatized the CCK aspartic acids with ANSA, which contains a more acidic sulfonate group. Following our 1 h reaction, the main product

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was addition of one ANSA tag. Collision-induced dissociation of this derivatized peptide showed fragment ions consistent with a mixture of isomers in which ANSA is added to either the aspartic acid residue in position 1 or 7 in the CCK sequence (Figure S3, top). The niECD spectrum of ANSA-CCK is shown in Figure S3, bottom. Similar to mDABCO-based derivatization and guanidination, ANSA tagging enabled niECD of CCK. Addition of the sulfonate moiety resulted in the observation of a doubly charged *c*-type ion as well as one singly charged *y* ion. These derivatization results are summarized in Table 1.

niECD of Synthetic Peptide Series. One challenge in interpreting niECD data for the natural peptides examined to date is that they frequently contain multiple possible protonation and deprotonation sites. For example, the sulfopeptide hirudin (H-DFEEIPEEsYLQ−OH), which undergoes highly favorable niECD,[11] has one aspartic acid residue, four glutamic acid residues, one sulfate group, and the C-terminus as likely deprotonation sites. With the N-terminus constituting the most likely protonation site, only two of these seven acidic sites need to be deprotonated for an overall singly charged anion. While Columb repulsion renders some pairs of deprotonation sites less likely, prediction of charge location is highly challenging. In order to explore a more systematic approach, niECD was applied to several series of synthetic peptides in which the number and positions of charges are more carefully controlled. These peptide series were designed around the hirudin sequence. Peptide lengths were chosen not to disfavor niECD due to excessive increase in charge density upon electron capture. Three sets of peptides were synthesized and further modified via guanidination for a total of five sets. All sequences are listed in Figure 1.

In the first set of six synthetic peptides (Figure 1a), the peptide P1 has the same sequence as hirudin but without the sulfate group. This peptide contains six acidic sites for possible deprotonation and the N-terminus for protonation. Thus, it still has a high probability of being zwitterionic in the gas phase. Consistently, singly deprotonated P1 showed favorable niECD with a fragmentation efficiency of 50% (Figure 2a). For efficiency calculations, product ion peak abundances were normalized to charge, summed, and divided by the precursor ion abundance in the same spectrum. Experiments were performed in triplicate for all samples. In the peptide P2, one glutamic acid residue was replaced with a less acidic tyrosine residue. This peptide still contains several acidic sites and should readily form a zwitterion structure in the gas phase; however, compared with P1, statistically the zwitterion probability for P2 should be lower. Accordingly, the P2 niECD efficiency was 41% (Figure 3). For the peptides P3−P6, the probability of zwitterionic structures was further reduced by gradually replacing acidic residues with more neutral ones. The replacement residues were chosen to maintain peptide mass relatively constant and thus eliminate this parameter from influencing the experiments. Consistent with the hypothesis, niECD efficiency further decreased from P3 to P6 (Figure 3). The peptide P6 is entirely composed of neutral residues; however, it still undergoes a low level (1% efficiency) of niECD (Figure 2b).

A plausible zwitterionic structure could have a protonated N-terminus, a deprotonated C-terminus, and a deprotonated tyrosine residue. Tyrosine deprotonation has been reported to occur at high pH,[44] i.e., under the conditions employed here. An alternative deprotonation site may be a backbone amide

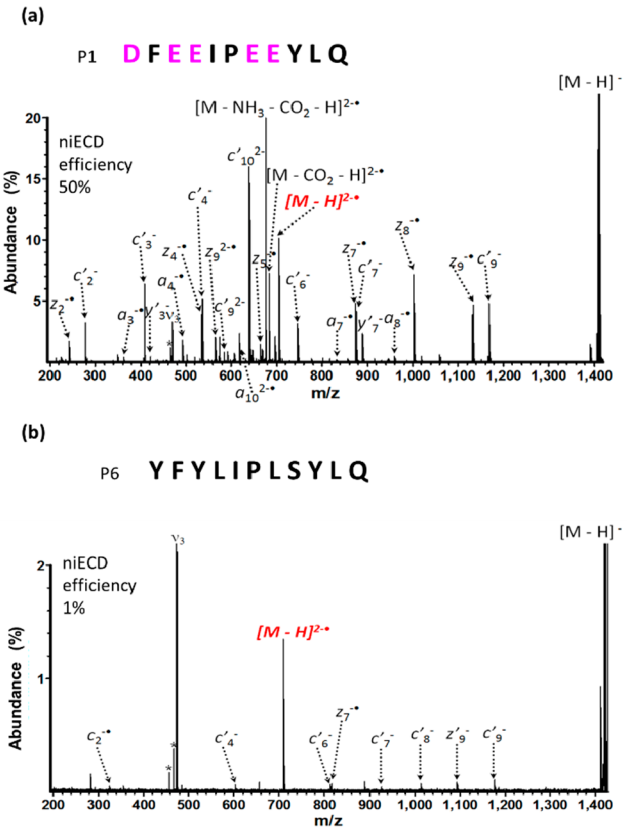

Figure 2. niECD (4.5 eV, 16 scans, 5 s irradiation, Bruker Apex-Q FT-ICR mass spectrometer) MS/MS spectra of singly deprotonated P1 (a) and P6 (b). niECD efficiency decreased dramatically from P1 to P6. The charge increased radical intermediates are highlighted in red.

group.[23] The lower probability of tyrosine or backbone deprotonation, however, compared with more acidic residues would explain the lower P6 niECD efficiency. The dramatic difference in niECD fragmentation efficiency between P1 and P6 (Figure 2) is also reflected in the resulting peptide sequence coverage (75% for P1 vs 40% for P6). The overall trend in niECD efficiency for the peptides P1−P6 (Figure 3) correlates well with the zwitterion mechanism.

Following manipulation of peptide acidic sites, the effect of basic sites were investigated. A second series of peptides, P7−P12 (Figure 1b), was synthesized, replacing the C-terminal glutamine residue in P1−P6 with lysine. With the added basic amino acid residue, these peptides have more available sites for protonation and gaseous zwitterions may form more readily. The niECD efficiencies of P7−P12 are shown in Figure 3. Similar to P1−P6, niECD efficiency drops gradually as the number of acidic sites decreases. Unexpectedly, however, the niECD efficiency of P7−P12 was lower overall compared with P1−P6, again suggesting that a general zwitterion structure is not the only requirement for niECD. To investigate whether the peptide sequence plays a role, the lysine residue was moved to the N-terminus to generate the peptides P13−P18 (Figure 1c). In this third series of synthetic peptides, P13 and P14 showed exceptionally high niECD efficiency (94 and 72%, respectively). On the other hand, P15−P18 were virtually recalcitrant to niECD with P15−P17 showing significantly lower efficiency than P3−P5 and P9−P11 (Figure 3). The remarkable change in behavior for the peptide P15 compared

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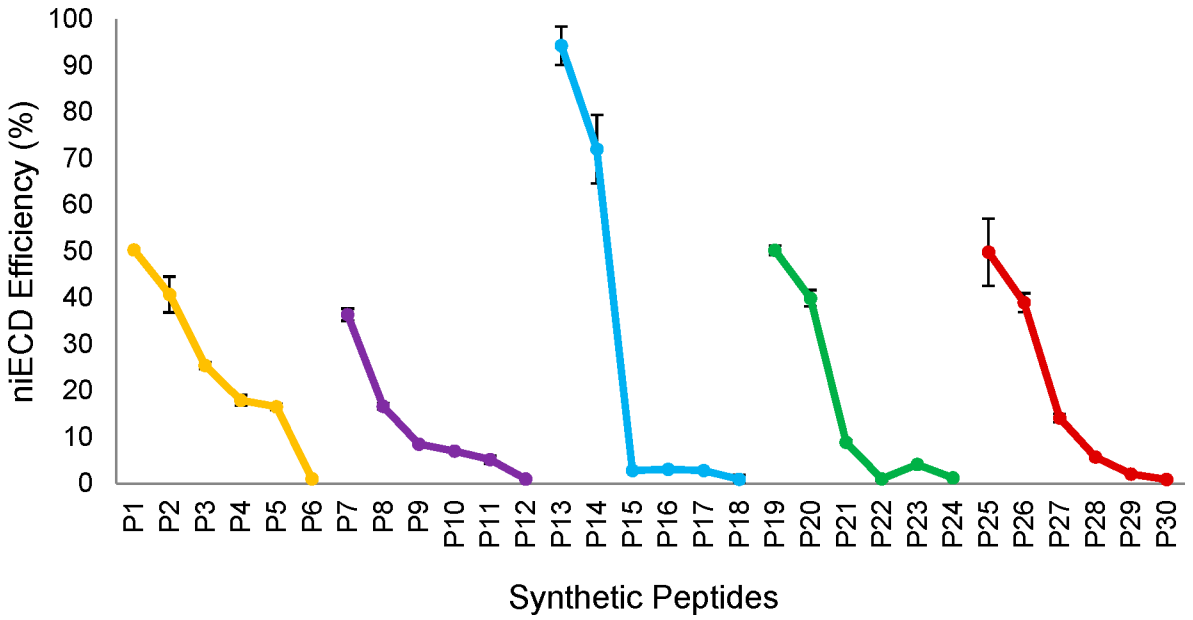

Figure 3. niECD efficiency for all five sets of synthetic peptides: the three sets of synthetic peptides P1−P6 (yellow), P7−P12 (purple), and P13−P18 (blue) and the guanidinated version of the latter two sets: P19−24 (green), and P25−30 (red). All niECD experiments were performed under the same conditions: 4.5 eV electron irradiation was applied for 5 s and the spectra were collected for 16 scans. Error bars were generated from triplicate measurements.

with P14, which only differs by one amino acid residue (glutamic acid to leucine substitution), again suggests additional peptide structural effects in niECD.

Another two sets of peptides were generated by guanidinating the peptides P7−12 and P13−18 to yield the peptides P19−P24 and P25−P30 containing C-terminal and N-terminal arginine-like residues, respectively (Figure 1d, e). Several, but not all, of these peptides showed improved niECD efficiency upon guanidination, including P7, P8, P15, and P16 (Figure 3). Overall, the niECD efficiency trends for the peptides P19−P24 and P25−P30 showed similar decreasing behavior as the previous peptide series, consistent with the zwitterion mechanism (Figure 3). Exceptions to the niECD efficiency increase upon guanidination included the peptides P13 and P14, which showed exceptionally high niECD efficiency prior to guanidination (Figure 3). Thus, the gas-phase structures of these two peptides are of particular interest.

Determination of Gas-Phase Collision Cross Sections for the Peptides P13−P17. One strategy for gaining information about the structures of analyte ions in the gas phase is to determine their orientationally averaged collision cross sections (CCSs) with ion mobility experiments.[45] IM separates ions according to their size and charge and the transit time through an IM cell can be converted to a CCS value.[46] We utilized a traveling wave IM (TWIM)−MS platform[33,34] to determine CCS values for the singly deprotonated peptides P13−P17 (Figure 4 and Table S1) with an improved calibration function.[38]

Peptide CCS values increase in the order P13, P14, P16, P15, and P17. Intriguingly, this increase in CCS values is inversely correlated with niECD efficiency for these peptides. The differences in CCS values mainly reflect the various conformations of the peptides P13−P17 as they have similar m/z ratios. Previous studies using IM experiments and computational modeling have shown that compact peptide gas phase structures are often a result of electrostatic and salt bridge interactions.[47−50] Thus, the compact structures

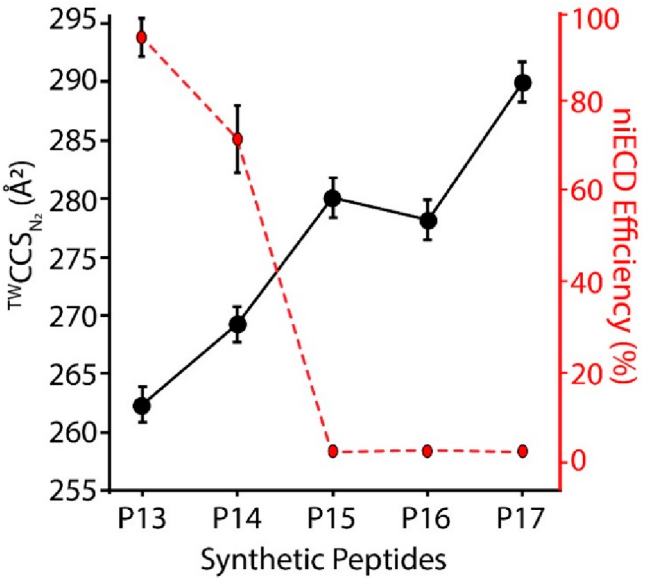

Figure 4. CCS values for the singly deprotonated peptides P13−P17, determined using TWIM-MS (left ordinate, black), and niECD efficiencies for the same five peptides (right ordinate, red).

observed for P13 and P14 (Figure 4) are likely due to the presence of salt bridge interactions between the positive charge on either the N-terminus or lysine residue and negatively charged side chains of aspartic acid or glutamic acid residues (Figure 1c). The higher CCS values for the peptides P15−P17 may be explained by their lower number of acidic residues (Figure 1c), thus resulting in structures that do not contain significant salt bridge stabilizing interactions. The importance of intramolecular salt bridges for effective niECD was proposed by Schneeberger and Breuker who showed that replacement of H^+ with Na^+ or K^+ in a phosphopeptide anion prevents dissociation.[51] Our CCS data support their hypothesis and can

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explain why some peptides that are likely zwitterionic in the gas phase do not undergo effective niECD.

The absence of salt-bridge interactions in the peptides P15−P17 results in niECD efficiency of < ∼3%. Similarly, the doubly deprotonated but singly charged TMPP-Ac derivatized peptide[11] must be a zwitterion due to the positively charged phosphonium center; however, the three phenyl groups surrounding the positive center likely sterically prevent salt bridge formation in derivatized peptides, thus explaining its resistance to niECD.

CONCLUSIONS

Our previously proposed niECD zwitterion mechanism was investigated by altering peptide chemical functionalities through derivatization and by systematically varying the sequence to yield peptides with different probabilities of zwitterion structures. Similar to our previous TMAA derivatization, addition of an mDABCO-based tag, ANSA tag, as well as N-terminal guanidination, which all promote zwitterion probability in negative ion mode, enabled niECD of a peptide that did not undergo electron capture in its unmodified form. For five sets of synthetic peptides, niECD efficiency decreased with decreasing zwitterionic propensity, again correlating with our proposed mechanism. However, fixed-positive-charge derivatization did not consistently rescue the ability of a peptide to undergo niECD.[11] Additionally, unexpectedly high niECD efficiency increase or decrease were observed for some synthetic peptides. For the corresponding peptides, we found that gas-phase CCS values are inversely correlated with niECD efficiency, suggesting that compact structures are required for effective niECD in addition to zwitterionic character. We further propose that salt-bridged structures likely dominate these compact ion populations, a hypothesis that explains the failure of TMPP-Ac derivatization to rescue niECD and that is consistent with the recently reported requirement for proton charge carriers in niECD.[51]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.4c00030.

niECD spectra of a peptide modified at its N-terminus via mDABCO-based tagging and guanidination, respectively; CID and niECD spectra of an ANSA-derivatized peptide; and collision cross section values for the peptides P13−P17 (PDF)

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Notes

The authors declare no competing financial interest.

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