JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Negative-Ion Electron Capture Dissociation: Radical-Driven Fragmentation of Charge-Increased Gaseous Peptide Anions

Hyun Ju Yoo,[†,§,⊥] Ning Wang,[†,§] Shuyi Zhuang,[†,‡] Hangtian Song,[†] and Kristina Håkansson*[,†]

[†]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055, United States

[‡]Department of Chemistry, Tsinghua University, Beijing 100084, China

Supporting Information

ABSTRACT: The generation of gaseous polyanions with a Coulomb barrier has attracted attention as exemplified by previous studies of fullerene dianions. However, this phenomenon has not been reported for biological anions. By contrast, electron attachment to multiply charged peptide and protein cations has seen a surge of interest due to the high utility for tandem mass spectrometry (MS/MS). Electron capture dissociation (ECD) and electron transfer dissociation (ETD) involve radical-driven fragmentation of charge-reduced peptide/protein cations to yield N−C_α backbone bond cleavage, resulting in predictable $c'/z^\bullet$-type product ions without loss of labile post-translational modifications (PTMs). However, acidic peptides, e.g., with biologically important PTMs such as phosphorylation and sulfonation, are difficult to multiply charge in positive ion mode and show improved ionization in negative-ion mode. We found that peptide anions ($[M - nH]^{n-}$, $n \geq 1$) can capture electrons within a rather narrow energy range (~3.5−6.5 eV), resulting in charge-*increased* radical intermediates that undergo dissociation analogous to that in ECD/ETD. Gas-phase zwitterionic structures appear to play an important role in this novel MS/MS technique, negative-ion electron capture dissociation (niECD).

Gas-phase ion−electron and ion−ion reactions are gaining popularity for peptide activation in tandem mass spectrometry (MS/MS). Electron capture dissociation (ECD)[1] and electron transfer dissociation (ETD)[2] are powerful alternatives to collision-activated dissociation (CAD). Fragmentation patterns observed in electron-mediated MS/MS are complementary to those observed in CAD, frequently providing more extensive peptide sequence information and, importantly, not involving loss of labile post-translational modifications (PTMs). Thus PTM sites can be determined, which is often challenging with CAD. More recently, electron ionization and subsequent extensive dissociation (electron ionization dissociation, EID) has been reported following irradiation of $[M + nH]^{n+}$ ($n \geq 1$) peptide cations with fast electrons (>20 eV).[3] Such irradiation causes double ionization to $[M + nH]^{(n+2)+}$ followed by electron capture to form electronically excited $[M + nH]^{(n+1)+\bullet *}$ ions, which dissociate via both side-chain losses and backbone fragmentation.

ECD, ETD, and EID all involve positively charged precursor ions with at least two charges for ECD/ETD because capture/transfer of an electron reduces total charge by 1, and mass spectrometers cannot detect neutrals. Generation of multiply charged cations is challenging for acidic analytes, including peptides with important PTMs such as phosphorylation and sulfonation. Thus, alternative negative-ion MS/MS techniques are desired. CAD of peptide anions typically results in PTM loss, similar to cation CAD. Further, backbone fragmentation in negative-ion CAD is more complex than in positive-ion mode and not predictable.[4] Electron-based techniques operating in negative-ion mode include electron detachment dissociation (EDD)[5a−c] and negative electron-transfer dissociation (NETD).[6a,6b] The former technique has low fragmentation efficiency, and the latter can result in PTM loss due to energy release from charge reduction. Both EDD and NETD yield backbone $a^\bullet$- and *x*-type product ions but involve structurally uninformative neutral losses as major fragmentation pathways. In addition, both techniques require multiply charged anions as precursors. Metastable atom-activated dissociation,[7a,b] also believed to involve radical-driven dissociation, was recently shown to yield fragmentation complementary to CAD, ECD, and EDD, with little PTM loss for peptide anions.[8]

Electron capture by anionic gaseous peptides appears unlikely due to Coulomb repulsion. However, previous work has shown attachment of 2−3 eV electrons to singly charged fullerene anions to form dianions in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.[9] Electron transfer to unmodified[10] and fluorinated[11] fullerene anions was also observed in high-energy (keV) collisions with atomic and molecular targets. We argued that such a phenomenon may be feasible for peptide anions at a certain mass-to-charge (m/z) ratio and an appropriate electron energy. To test this hypothesis, we started with coumarin-tagged peptides, based on work by O'Connor et al. who showed that coumarin acts as a radical trap in conventional cation ECD.[12] After careful optimization of the electron energy, we observed abundant charge-increased radical species, $[M + \text{coumarin} - H]^{2-\bullet}$, generated from capture of ~4.5 eV electrons (corresponding to a cathode bias voltage of 6 V, see Figure S1) by singly deprotonated coumarin-tagged peptides following 20 s electron irradiation (Figures 1A and S2).

The charge-increased radical species from angiotensin I (Figure 1A) was isolated in the ICR cell to verify that this product is not an artifact at twice the precursor ion ICR frequency (Figure S3). These data demonstrate the feasibility of electron capture by peptide anions, but the generated doubly charged radical anions appeared stable to further dissociation, consistent with the previously observed behavior of coumarin-tagged peptides[12] and peptides containing other electron predators[13a,b] in

Received: August 16, 2011
Published: September 26, 2011

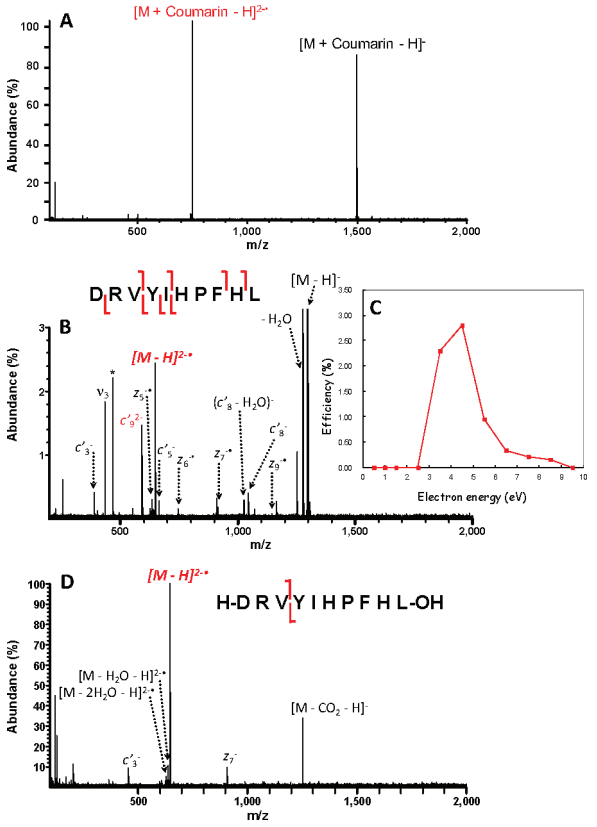

Figure 1. (A) Electron irradiation (~4.5 eV electrons, 20 s, 1 scan) of singly deprotonated coumarin-tagged angiotensin I. (B) niECD of singly deprotonated un-derivatized angiotensin I (~4.5 eV electrons, 20 s irradiation, 5 scans). (C) Abundance change of the charge-increased $[M-H]^{2-\bullet}$ species as a function of electron energy. (D) IRMPD MS^3 (10.6 μm, 300 ms, 7.5 W, 20 scans) of the in-cell-isolated $[M-H]^{2-\bullet}$ species generated upon electron capture by un-derivatized angiotensin I. Charge-increased product/precursor ions are marked in red. ν_3 = third harmonic; * = electronic noise.

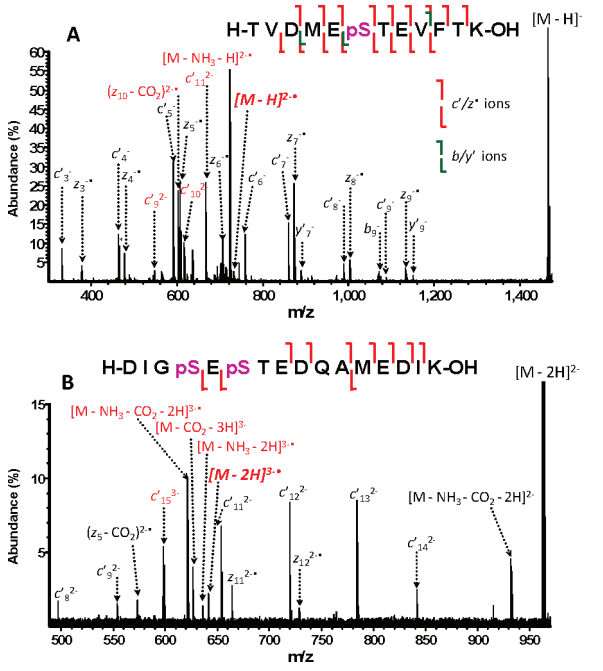

Figure 2. niECD of α-casein tryptic phosphopeptides. (A) 10 s irradiation of a singly deprotonated, singly phosphorylated peptide (~4.5 eV electrons, 10 scans). (B) 20 s irradiation of a doubly deprotonated, doubly phosphorylated phosphopeptide (~5.5 eV electrons, 10 scans).

conventional ECD, and with the previously observed fullerene dianions.[9] Further activation of the generated $[M+\text{coumarin}-H]^{2-\bullet}$ radical species through infrared multiphoton dissociation (IRMPD[14]) mainly resulted in ejection of small, structurally uninformative neutrals (Figure S3, inset).

Following our discovery that ~4.5 eV electrons can be captured by coumarin-tagged peptide anions, we applied electron irradiation to unmodified peptide anions. Figure 1B shows electron irradiation of singly deprotonated angiotensin I without a coumarin tag. Similar to the coumarin-tagged species (Figure 1A), a charge-increased radical anion, $[M-H]^{2-\bullet}$, is observed, but, in contrast to the coumarin-tagged species, several c'- and $z^\bullet$-type fragments (Zubarev nomenclature)[15] from backbone $N-C_\alpha$ bond cleavage are also detected. We termed this phenomenon negative-ion electron capture dissociation (niECD). Remarkably, a doubly charged c'_9 ion is observed from the singly charged precursor ion. Electron-induced dissociation at higher electron energy[16] (~9.5 eV, Figure S4A) does not yield any charge-increased product ions or c/z-type fragments, and such fragments are also absent in CAD of the same species (Figure S4B), indicating that niECD proceeds through a unique mechanism related to that of conventional peptide ECD/ETD (which also yield c/z-type product ions). A rather narrow electron energy range (~3.5–6.5 eV) appears acceptable for niECD (Figure 1C).

To further investigate the observed gas-phase chemistry, the charge-increased species, $[M-H]^{2-\bullet}$, generated from electron irradiation of singly deprotonated un-derivatized angiotensin I was isolated in the ICR cell and activated in an MS^3 experiment via IRMPD (Figure 1D). Two major backbone fragments are observed in the form of complementary c_3 and z_7 ions. The different outcomes in direct niECD (Figure 1B) and MS^3 involving electron irradiation followed by IRMPD of the isolated, charge-increased $[M-H]^{2-\bullet}$ species are likely due to the different time scales of the two experiments. Lin et al. previously demonstrated that several radical intermediates with different lifetimes exist in conventional cation ECD.[17] Nevertheless, c/z-type ions were observed in both MS^2 and MS^3. A comparison between MS^3 of the charge-increased $[M-H]^{2-\bullet}$ radical species and direct IRMPD (MS^2) of even-electron $[M-2H]^{2-}$ precursor ions is shown in Figure S5 for the peptide H-KRSpYEEHIP-OH. Very different product ion spectra result, with solely z-type ions observed for radical precursors and mainly b- and y-type ions for even-electron precursor ions.

niECD of α-casein tryptic phosphopeptides is shown in Figure 2. For a singly deprotonated serine-phosphorylated peptide (Figure 2A), ~4.5 eV electron irradiation yields an ammonia-deficient charge-increased radical, $[M-NH_3-H]^{2-\bullet}$, as the major product along with three doubly charged and many singly charged c and z ions. For doubly charged precursor ions (e.g., the doubly deprotonated and doubly phosphorylated α-casein tryptic peptide shown in Figure 2B), the optimum niECD electron energy is slightly higher than for singly charged precursor ions, ~5.5 rather than ~4.5 eV, consistent with increased Coulomb repulsion. The fragmentation efficiency is lower for doubly charged precursor ions; however, a charge-increased

dx.doi.org/10.1021/ja207736y | J. Am. Chem. Soc. 2011, 133, 16790–16793

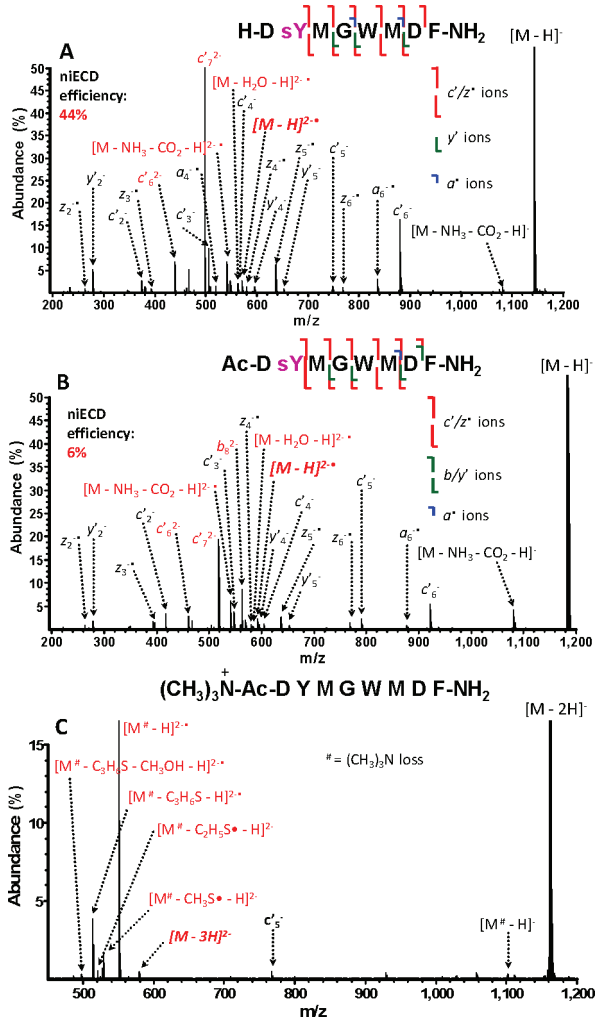

Figure 3. (A) niECD of sulfonated cholecystokinin (CCKS; ~4.5 eV electrons, 20 s, 10 scans). (B) niECD of N-terminally acetylated CCKS under conditions identical to those in (A). (C) niECD of trimethylammonium-derivatized CCK (~4.5 eV electrons, 10 s, 32 scans). Charge-increased product ions are highlighted in red. Fragmentation efficiency was calculated as previously described.[3]

triply charged radical, $[M - 2H]^{3-\bullet}$, is observed along with four other charge-increased products and many doubly charged c/z ions. Phosphate loss is absent for both phosphopeptides.

Figure 3A shows niECD of a tyrosine-sulfonated peptide (cholecystokinin, CCKS). Sulfonation is even more labile in the gas phase than phosphorylation and frequently lost in positive-ion mode, even without ion activation. Thus, negative mode, in which sulfotyrosine is stable and shows higher ionization efficiency, is preferred compared to positive mode analysis. EDD has shown some success for sulfonate localization in sulfopeptides;[5] however, backbone fragmentation competes with neutral loss of CO_2 and SO_3. In niECD (Figure 3A), no sulfonate loss occurs, and virtually complete sequence coverage is observed.

Additional examples of phosphopeptide niECD and comparison to anion CAD are shown in Table 1. In all cases, niECD provides significantly more extensive peptide sequence coverage than CAD, and both serine and tyrosine phosphorylation are retained. The only phosphopeptide we analyzed that did not undergo niECD (or electron capture by the singly deprotonated anion) had the sequence H-RRApSVA-OH. This resistance to niECD is likely due to the smaller molecular weight and thus decreased favorability for accommodating two negative charges in the gas phase.

Table 1. Comparison of niECD and CAD for Phosphopeptide Anions[a]

z	m/z	niECD	CAD
-1	1236.541	H-K R S pY E E H I P-OH	H-K R S pY E E H I P-OH
-1	1568.634	H-R R R E E E pS E E E A A-OH	H-R R R E E E pS E E E A A-OH
-1	1540.632	H-T S T E P Q pY Q P G E N L-NH_2	H-T S T E P Q pY Q P G E N L-NH_2
-1	1658.780 (α-casein 121-134)	H-V P Q L E I V P N pS A E E R-OH	H-V P Q L E I V P N pS A E E R-OH
-2	1029.403 (β-casein 48-63)	H-F Q pS E E Q Q Q T E D E L Q D K-OH	H-F Q pS E E Q Q Q T E D E L Q D K-OH

[a] Backbone N−C_α bond cleavages to yield $c'/z^\bullet$ ions are indicated with red lines, and backbone amide bond cleavages to yield b/y' ions are indicated with green lines. Dashed lines indicate accompanying phosphate loss. Lack of indicated fragments in CAD is due to extensive neutral losses (e.g., HPO_3, H_3PO_4, and H_2O).

The niECD outcome of several unmodified peptides is summarized in Table S1. In contrast to phosphopeptides (Figure 2, Table 1), for which all but one short peptide showed extensive fragmentation in niECD, several singly deprotonated non-phosphopeptides did not capture electrons, including the larger (>1 kDa) peptides cholecystokinin, neurokinin B, substance P-OH, neuromedin C, and neuromedin B. One common characteristic of these five peptides is a lack of either strongly basic or strongly acidic residues, thus reducing the probability of gas-phase zwitterionic structures. In addition, previous work has shown that such structures are favored for phosphopeptides,[18a,b] which also undergo favorable niECD (Figure 2, Table 1). These observations, along with the striking similarity of niECD spectra to cation ECD/ETD spectra, suggest that zwitterionic structures may play an important role for successful niECD, with electron capture either occurring at or being directed by the positively charged site.[19a−c] Work by Vasil'ev et al. involving electron capture by neutral gaseous peptides[20] showed somewhat different product ion spectra, with a larger variety of fragment types compared to niECD, further suggesting that charged sites may play a role in niECD. Furthermore, recent computational work proposes that singly deprotonated angiotensin II is zwitterionic.[21]

To test this zwitterion hypothesis, we performed several experiments with the goal to either prevent or promote gas-phase peptide zwitterion formation. Figure 3B shows niECD of N-terminally acetylated CCKS. This sulfopeptide showed highly favorable niECD in its unmodified form (Figure 3A); however, it does not contain any basic residues. Thus, the most basic site is the N-terminus, and zwitterion formation should be less favorable upon acetylation. Consistently, niECD efficiency of N-terminally acetylated CCKS is significantly lower than that of unmodified CCKS (Figure 3A,B). However, electron capture and fragmentation still occur for the acetylated species, possibly due to tryptophan protonation.

Intriguingly, non-sulfonated CCKS (CCK) does not undergo niECD (Table S1). Similarly, gas-phase desulfonation of CCKS via nozzle−skimmer dissociation inside the electrospray ion source eliminates electron capture by the resulting CCK-like product (Figure S6). Addition of metal ions (Na^+, Ca^{2+}, and Cs^+), which may promote zwitterion formation,[22] did not enable electron capture by CCK (Figure S7). Neither did N-terminal

tris(2,4,6-trimethoxyphenyl)phosphonium-acetyl (TMPP-Ac) derivatization, which introduces a fixed positive charge and thereby forces observed singly charged anions to have two deprotonation sites (Figure S8). By contrast, introduction of a fixed positive charge in the form of an N-terminal quaternary amine did enable niECD of CCK (Figure 3C). Quaternary amine derivatization also rescued niECD ability of substance P-OH (Figure S9). However, the presence of the fixed charge site altered the fragmentation behavior in both cases, similar to reported behavior of fixed charge-containing peptides in conventional ECD/ETD.[23a−d] The lack of success for TMPP-Ac derivatization or metal adduction may be explained by effective shielding of the positively charged site by the aromatic groups surrounding the phosphonium, or by the peptide carbonyls wrapping around the metal ion. Thus, the presence of a gas-phase zwitterion does not appear to be the only criterion for successful niECD. The particular gas-phase zwitterion structure is likely also crucial: the influence of peptide gas-phase structure has been extensively studied in conventional cation ECD and is known to have a profound influence on fragmentation behavior.[24a−d]

In summary, we show that peptide anions can capture ~3.5−6.5 eV electrons, resulting in radical species with increased charge and yielding peptide backbone bond fragmentation (niECD) analogous to that observed in cation ECD/ETD, including PTM retention and higher sequence coverage compared to CAD. Increased charge improves signal-to-noise ratios in FT-ICR MS because the generated image current is proportional to the charge state.[3] The presence of a coumarin radical trap improved electron capture efficiency but limited fragmentation, presumably due to decreased radical mobility. niECD allows localization of PTMs and *de novo* sequencing for acidic peptides that show improved ionization in negative-ion mode compared to positive-ion mode, e.g., phospho- and sulfopeptides. Further, niECD is compatible with (but not limited to) singly charged peptides, which allows coupling with matrix-assisted laser desorption/ionization.

■ ASSOCIATED CONTENT

Supporting Information. Methods figures, and table. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
kicki@umich.edu

Present Addresses
[⊥]Analytical Chemistry Core Lab, ASAN Medical Center, 88, Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, Korea

Author Contributions
[§]These authors contributed equally to this work.

■ ACKNOWLEDGMENT

This work was supported by an NSF Career Award (CHE-05-47699). H.J.Y. and H.S. were partially supported by George Ashworth Analytical Chemistry Fellowships. S.Z. was supported by a Summer Undergraduate Research Exchange Program sponsored by Pfizer Global R&D and Tsinghua University School of Life Sciences. R. A. Zubarev and K. E. Hersberger are thanked for valuable discussions.

■ REFERENCES

(1) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3265.

(2) Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9528.

(3) Fung, Y. M. E.; Adams, C. M.; Zubarev, R. A. *J. Am. Chem. Soc.* **2009**, *131*, 9977.

(4) Bowie, J. H.; Brinkworth, C. S.; Dua, S. *Mass Spectrom. Rev.* **2002**, *21*, 87.

(5) (a) Budnik, B. A.; Haselmann, K. F.; Zubarev, R. A. *Chem. Phys. Lett.* **2001**, *342*, 299. (b) Kweon, H. K.; Hakansson, K. *J. Proteome Res.* **2008**, *7*, 745. (c) Wolff, J. J.; Chi, L.; Linhardt, R. J.; Amster, I. J. *Anal. Chem.* **2007**, *79*, 2015.

(6) (a) Coon, J. J.; Shabanowitz, J.; Hunt, D. F.; Syka, J. E. P. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 880. (b) Huzarska, M.; Ugalde, I.; Kaplan, D. A.; Hartmer, R.; Easterling, M. L.; Polfer, N. C. *Anal. Chem.* **2010**, *82*, 2873.

(7) (a) Misharin, A. S.; Silivra, O. A.; Kjeldsen, F.; Zubarev, R. A. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2163. (b) Berkout, V. D. *Anal. Chem.* **2009**, *81*, 725.

(8) Cook, S. L.; Jackson, G. P. *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1088.

(9) Hartig, J.; Blom, M. N.; Hampe, O.; Kappes, M. M. *Int. J. Mass Spectrom.* **2003**, *229*, 93.

(10) Liu, B.; Hvelplund, P.; Nielsen, S. B.; Tomita, S. *Phys. Rev. Lett.* **2004**, *92*, 168301.

(11) Boltalina, O. V.; Hvelplund, P.; Larsen, M. C.; Larsson, M. O. *Phys. Rev. Lett.* **1998**, *80*, 5101.

(12) Belyayev, M. A.; Cournoyer, J. J.; Lin, C.; O'Connor, P. B. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1428.

(13) (a) Jones, A. W.; Mikhailov, V. A.; Iniesta, J.; Cooper, H. J. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 268. (b) Sohn, C. H.; Chung, C. K.; Yin, S.; Ramachandran, P.; Loo, J. A.; Beauchamp, J. L. *J. Am. Chem. Soc.* **2009**, *131*, 5444.

(14) Bomse, D. S.; Woodin, R. L.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1979**, *101*, 5503.

(15) Kjeldsen, F.; Haselmann, K.; Budnik, B. A.; Jensen, F.; Zubarev, R. A. *Chem. Phys. Lett.* **2002**, *356*, 201.

(16) Kalli, A.; Grigorean, G.; Hakansson, K. *J. Am. Soc. Mass Spectrom.* **2011**, DOI: 10.1007/s13361-011-0233-6.

(17) Lin, C.; O'Connor, P. B.; Cournoyer, J. J. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1605.

(18) (a) Creese, A. J.; Cooper, H. J. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1263. (b) Woods, A. S. *J. Proteome Res.* **2004**, *3*, 478.

(19) (a) Simons, J. *Chem. Phys. Lett.* **2010**, *484*, 81. (b) Syrstad, E. A.; Turecek, F. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 208. (c) Zubarev, R. A.; Kruger, N. A.; Fridriksson, E. K.; Lewis, M. A.; Horn, D. M.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **1999**, *121*, 2857.

(20) Vasil'ev, Y. V.; Figard, B. J.; Morre, J.; Deinzer, M. L. *J. Chem. Phys.* **2009**, *131*, 044317.

(21) Marchese, R.; Grandori, R.; Carloni, P.; Raugei, S. *PLoS Comput. Biol.* **2010**, *6*, e1000775.

(22) Jockusch, R. A.; Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1999**, *103*, 9266.

(23) (a) Xia, Y.; Gunawardena, H. P.; Erickson, D. E.; McLuckey, S. A. *J. Am. Chem. Soc.* **2007**, *129*, 12232. (b) Chamot-Rooke, J.; van der Rest, G.; Dalleu, A.; Bay, S.; Lemoine, J. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1405. (c) Chung, T. W.; Moss, C. L.; Zimnicka, M.; Johnson, R. S.; Moritz, R. L.; Turecek, F. *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 13. (d) Li, X. J.; Cournoyer, J. J.; Lin, C.; O'Connor, P. B. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1514.

(24) (a) Mihalca, R.; Kleinnijenhuis, A. J.; McDonnell, L. A.; Heck, A. J. R.; Heeren, R. M. A. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1869. (b) Robinson, E. W.; Leib, R. D.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1469. (c) Ben Hamidane, H.; He, H.; Tsybin, O. Y.; Emmett, M. R.; Hendrickson, C. L.; Marshall, A. G.; Tsybin, Y. O. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1182. (d) Breuker, K.; Oh, H. B.; Lin, C.; Carpenter, B. K.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14011.