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

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SUPPORTING INFORMATION

Materials and Methods

Sample Preparation. The following peptides were used: neuromedin B (H-GNLWATGHF-NH$_2$), neuromedin C (H-GNHWAVGHLM-NH$_2$), neurokinin B (H-DMHDFFVGLM-OH), cholecystokinin (CCK, H-DYMGWMDF-NH$_2$), H-AKPSYP*P*TYK-OH (P* = hydroxyproline), eHWSYGLRPG-NH$_2$ (e = pyroglutamic acid), exorphin C (H-YPISL-OH), H-RRREEEpSEEEAA-OH, H-KRSpYEEHIP-OH, angiotensin I (H-DRVYIHPFHL-OH), H-RRApSVA-OH, H-TSTEPQpYQPGENL-NH$_2$, bradykinin 2-9 (H-PPGFSPFR-OH), substance P-OH (H-RPKPQQFFGLM-OH), and sulfonated cholecystokinin (CCKS, H-DsYMGWMDF-NH$_2$). Most peptides were purchased from Sigma-Aldrich (St. Louis, MO), except H-TSTEPQpYQPGENL-NH$_2$ (which was from Millipore, Billerica, MA) and CCKS (from Advanced Chemtech, Louisville, NY). Bovine milk α- and β-casein were from Sigma-Aldrich. Coumarin tag (7-methoxycoumarin-3-carboxylic acid succinimidyl ester) was from Sigma-Aldrich. The above chemicals were used without further purification. Coumarin tagging reaction was performed according to a published procedure,[1] except for using longer reaction time (1 h). Trypsin (Promega, Madison, WI) digestion of α-/β-casein was performed for 12 h at 37 °C at an enzyme/substrate ratio of 1:50. The tryptic peptides H-FQpSEEQQQTEDELQDK-OH (β-casein 48-63), H-YLGYLEQLLR-OH (α-casein 106-115), H-FALPQYLK-OH (α-casein 189-196), H-TVDMEpSTEVFTK-OH (α-casein 153-164), H-DIGpSEpSTEDQAMEDIK-OH (α-casein 58-73), and H-VPQLEIVPNpSAEER-OH (α-casein

121-134) were subjected to MS/MS. Phosphopeptide enrichment[2] was performed with ZrO_2 microtips (Glygen, Columbia, MD), when necessary. 5-10 µM peptide solutions were prepared in 50/50 (v/v, H_2O/isopropanol) with 0.1 % triethylamine.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). Negatively-charged peptide ions were generated by external electrospray ionization (ESI) at 70 µL/h (Apollo II ion source, Bruker Daltonics, Billerica, MA). All experiments were performed with a 7 Tesla quadrupole (Q)-FT-ICR mass spectrometer (APEX-Q, Bruker Daltonics) as previously described.[3] All data were obtained in negative ion mode. For ESI, N_2 was used as both nebulizing gas (5 L/s) and drying gas (2.5 L/s). The drying gas temperature was set to 200˚C. Briefly, ions produced by ESI were mass-selectively externally accumulated in a hexapole for 0.2-3 s, transferred via high voltage ion optics, and captured in the ICR cell by dynamic trapping. This accumulation sequence was looped three times to improve precursor ion abundance. For MS/MS experiments, mass- selective external accumulation of negatively-charged peptide ions was performed. For negative ion electron capture dissociation (niECD), mass selectively accumulated peptide ions were irradiated for 10-20 s with 4.5-5.5 eV electrons (corresponding to a cathode bias voltage of 6-7 V, see Figure S1) provided by an indirectly heated hollow dispenser cathode.[4] A lens electrode located in front of the hollow cathode was kept 1.5 V more positive than the cathode bias voltage. For isolation of radical species produced from electron capture by negatively-charged peptide ions, correlated harmonic excitation fields (CHEF)[5] was used inside the ICR cell. For MS^3, infrared multiphoton dissociation (IRMPD) was performed inside the ICR cell with a 25 W, 10.6 µm, CO_2 laser (Synrad, Mukilteo, WA). The laser beam was deflected by two mirrors for alignment through a hollow dispenser cathode to the center of the ICR cell. The beam entered the vacuum system through a BaF_2 window. Photon irradiation was performed for 300-700 ms at 7.5-10 W laser power. Collision activated dissociation (CAD) was performed in an external hexapole at a collision cell DC offset of 20-40 V with argon as collision gas. All mass spectra were acquired with XMASS software (version 6.1, Bruker Daltonics) in broadband mode from m/z 200 to 3000 with 256K data points and summed over 10-32 scans.

Data Analysis. Data processing was performed with the MIDAS analysis software.[6] Peaks in MS^n were assigned within 10 ppm error after internal calibration. Typically, internal calibration was performed with precursor ions and their electron-capture species as calibrants.

Calibration of Electron Energy and Determination of niECD Electron Energy Dependence. Electron energy distributions were measured at cathode bias voltages from -4 to -19 V, consistently keeping the extraction lens voltage 1.5 V more positive than the cathode bias voltage. These measurements were performed by monitoring the electron current impinging on a floating cylindrical focusing element on the opposite side of the ICR cell, just outside the magnetic field with a digital multimeter (John Fluke, Everett, MA), as previously described.[7] The floating voltage was generated by a DC power supply (Goodwill Instrument, Taipei, Taiwan). Electron energy distributions were obtained by derivatizing the electron current with respect to the floating voltage. The measured average electron energy as function of cathode bias voltage is shown in Figure S1. Similar to our previous work,[7] and consistent with expected behavior of indirectly heated dispenser cathodes,[8] electron energy distributions were found to be quite narrow: < 0.5 eV at each bias voltage.
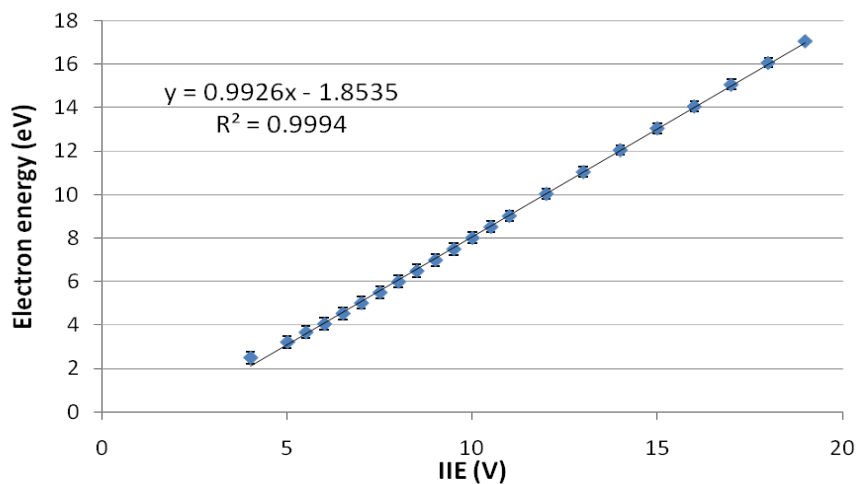
Supplemental Figures and Tables

Figure S1. Measured average electron energy as function of cathode bias voltage (IIE). The electron energy measurement procedure is described above.
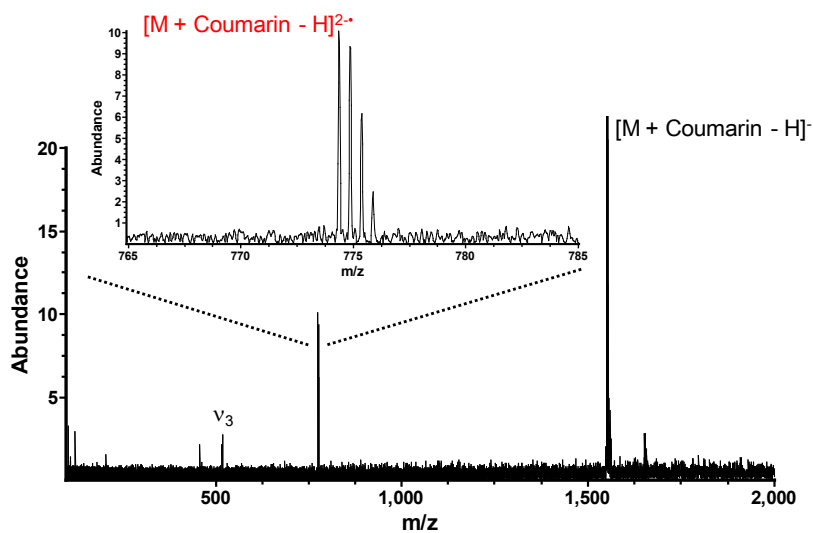

Figure S2. Electron irradiation (~4.5 eV electrons, 20 s) of singly-deprotonated coumarin-tagged substance P-OH. Electron capture to form a charge-increased peptide is observed.
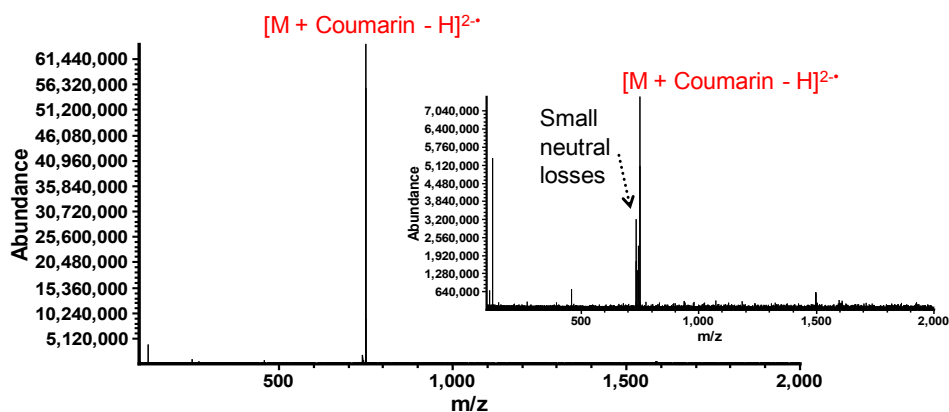
Figure S3. In-cell isolation of charge-increased coumarin-tagged angiotensin I, generated via 20 s irradiation with 4.5 eV electrons of the singly-deprotonated peptide (Figure 1A). Further activation (IRMPD MS^3, 500 ms, 7.5 W laser power) of the isolated charge-increased radical anion only yielded small neutral losses (inset).
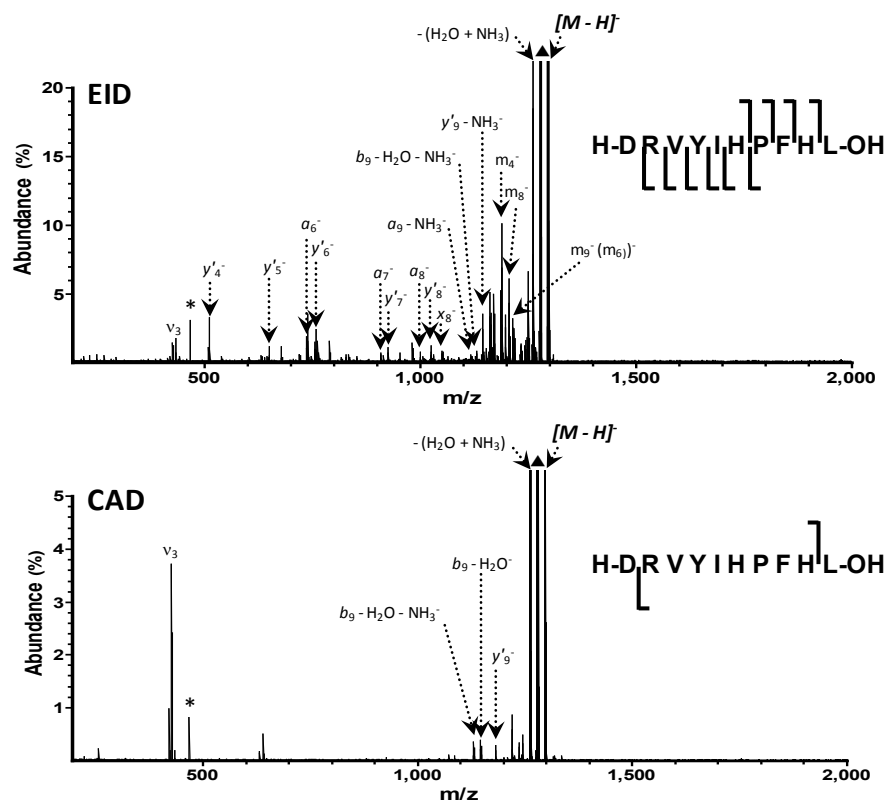

Figure S4. Electron induced dissociation (EID,^{9} 9.5 eV electrons, 20 s, 20 scans) and collision activated dissociation (CAD, 30 V collision voltage, 10 scans) of singly-deprotonated angiotensin I for comparison with the niECD spectrum shown in Figure 1B. "▲" indicates H$_2$O loss from an adjacent peak and "*" indicates an electronic noise peak. Lines on peptide sequences indicate backbone fragmentation.
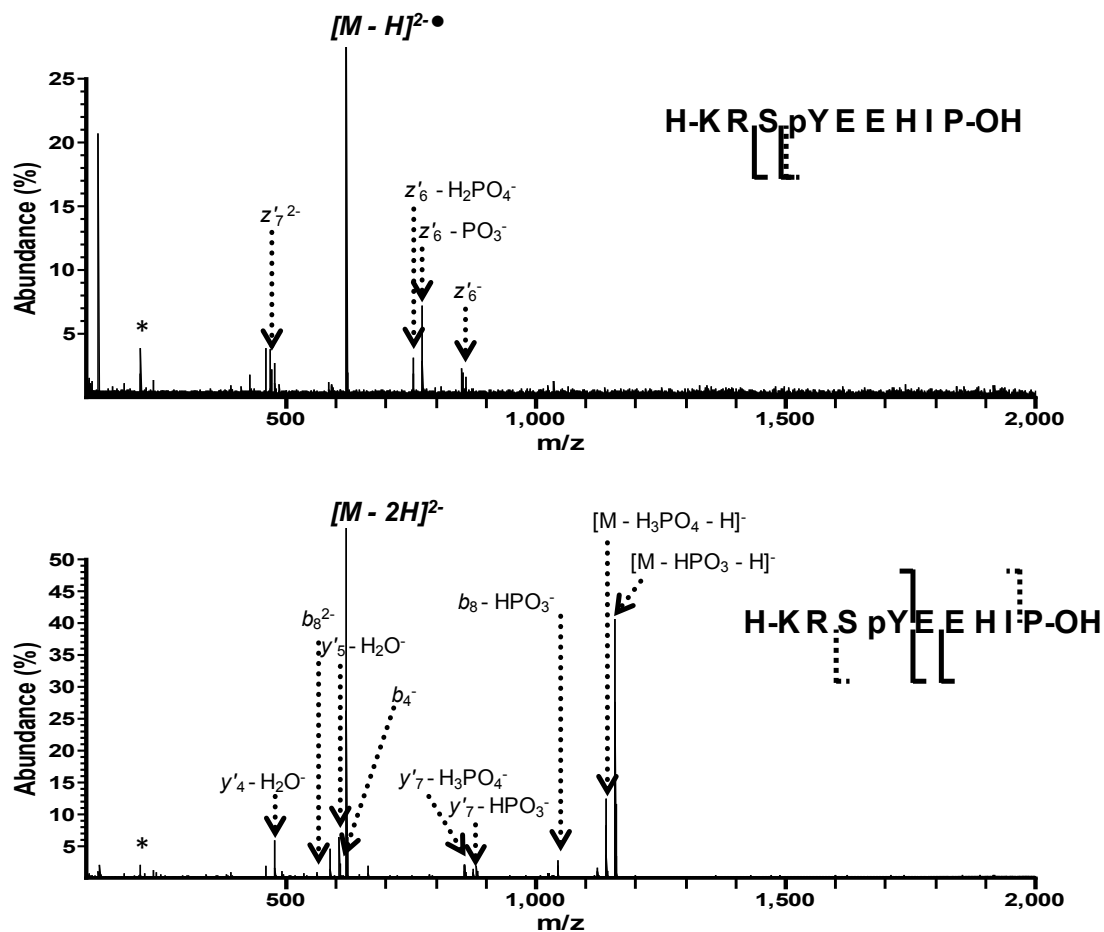
Figure S5. Comparison of IRMPD MS^3 of the radical species, $[M - H]^{2-\bullet}$, with MS/MS of the doubly-deprotonated form, $[M - 2H]^{2-}$, of a tyrosine-phosphorylated peptide. (Top) IRMPD MS^3 of the radical species, $[M - H]^{2-\bullet}$, generated from electron capture by the singly-deprotonated peptide, $[M - H]^-$ (7.5 W laser power for 300 ms, 5 scans). (Bottom) IRMPD MS^2 of the doubly-deprotonated peptide, $[M - 2H]^{2-}$, (7.5 W laser power for 700 ms). "*" indicates electronic noise peaks. Lines on peptide sequences indicate backbone fragmentation with (dashed lines) and without (solid lines) phosphate loss. Clearly, very different product ion types are observed from the radical and even-electron precursor ions, respectively.
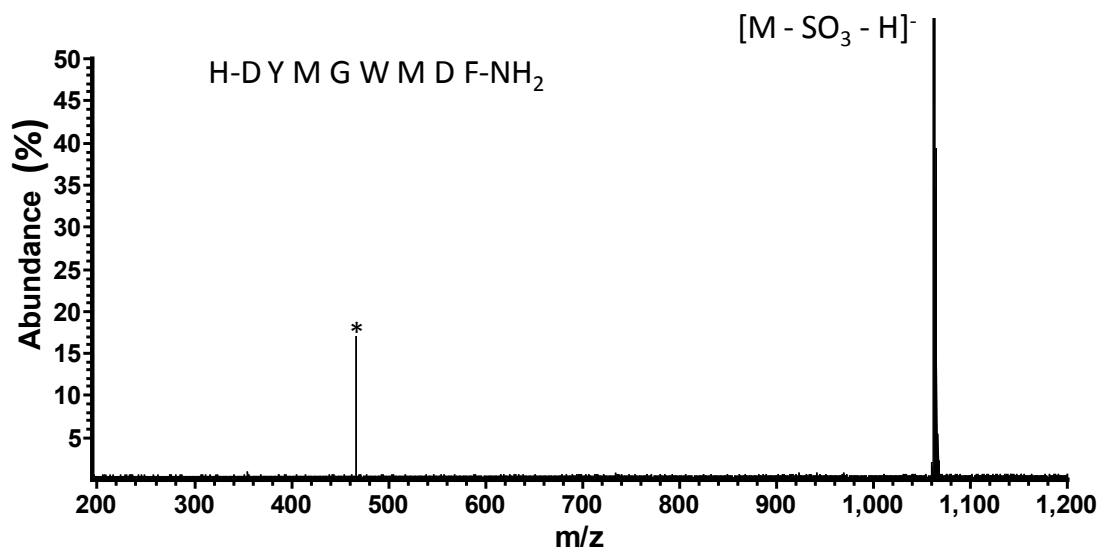
Figure S6. Electron irradiation (4.5 eV, 20 s, 10 scans) of gas-phase desulfonated CCKS (via in source nozzle-skimmer dissociation followed by quadrupole isolation). No electron capture is observed. "*" indicates an electronic noise peak.
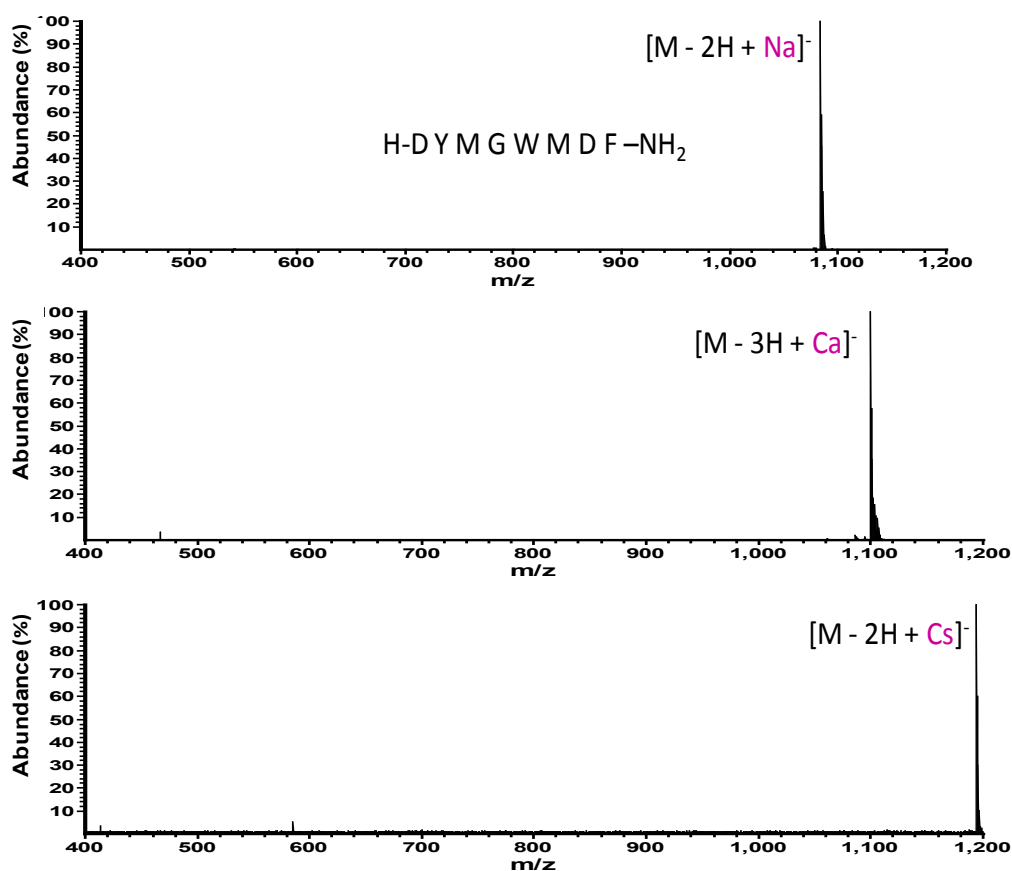

Figure S7. Electron irradiation (4.5 eV, 20 s, 10 scans) of metal-adducted CCK. No electron capture is observed.
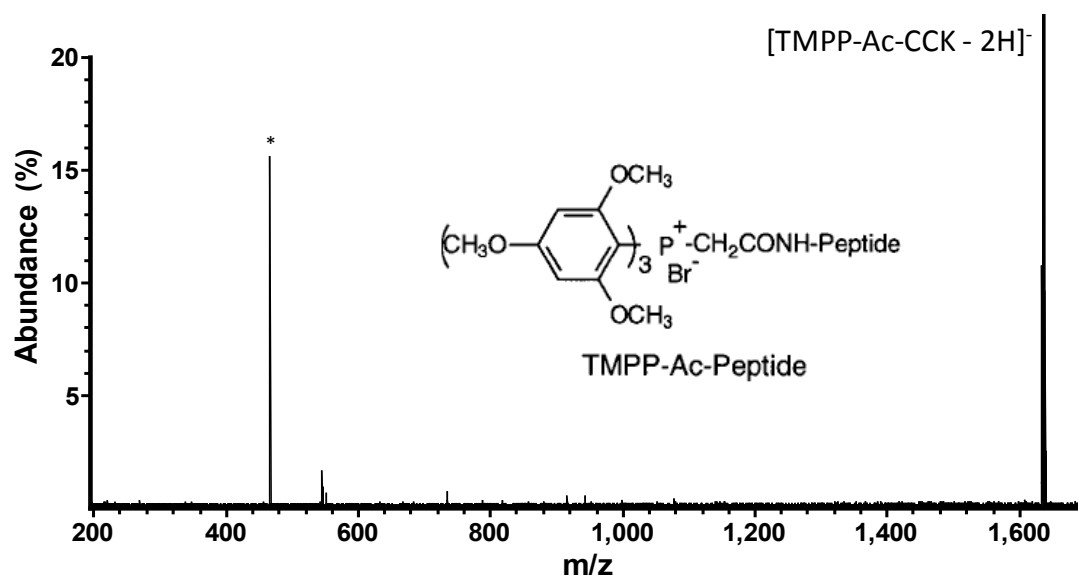
Figure S8. Electron irradiation (4.5 eV, 10 s, 10 scans) of tris(2,4,6-trimethoxyphenyl)phosphonium-acetyl (TMPP-Ac)-CCK (see inset for structure). No electron capture is observed.
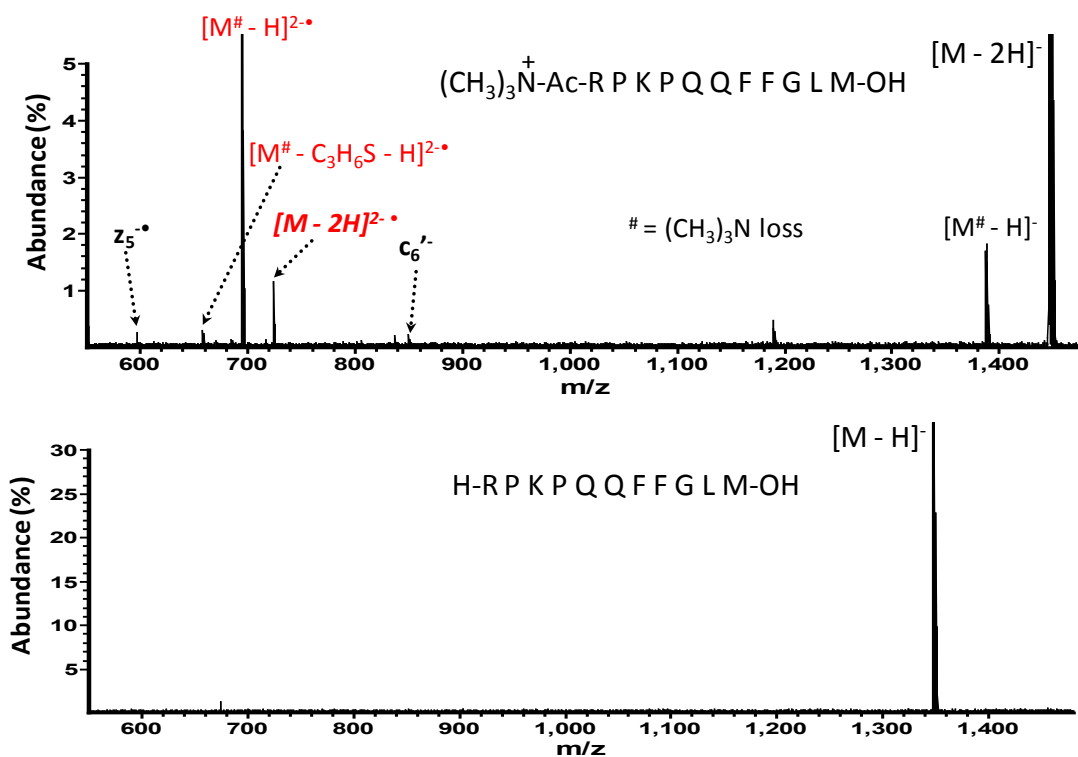

Figure S9. Electron irradiation (4.5 eV, 10 s, 32 scans) of substance P-OH with (top) and without (bottom) an N-terminal trimethylammonium fixed charge tag. The underivatized peptide does not undergo niECD whereas the presence of the tag allows electron capture and detection of $c'/z^•$-type ions. Similar to trimethylammonium-derivatized CCK (Fig. 3C), trimethylammonium loss is dominant.

Table S1. Electron Irradiation of a Selection of Unmodified Peptides[a]

z	m/z	Peptide name	Peptide sequence	$[M-H]^{2-\bullet}$ observed?
-1	590.320	Exorphin C	H-Y P I S L-OH	No
-1	902.452	Bradykinin 2-9	H-P P G F S P F R-OH	No
-1	977.547	α-casein 189-196	H-F A L P Q Y L K-OH	No
-1	1061.387	Cholecystokinin (CCK)	H-D Y M G W M D F-NH$_2$	No
-1	1209.507	Neurokinin B	H-D M H D F F V G L M-OH	No
-1	1346.704	Substance P-OH	H-R P K P Q Q F F G L M-OH	No
-1	1118.532	Neuromedin C	H-G N H W A V G H L M-NH$_2$	No
-1	1131.504	Neuromedin B	H-G N L W A T G H F M-NH$_2$	No
-1	1180.566		e H W S Y G L R P G-NH$_2$	Yes
-1	1265.690	α-casein 106-115	H-Y L G Y L E Q L L R	Yes
-1	1277.567		H-A K P S Y P* P* T Y K-OH	Yes
-1	1294.669	Angiotensin I	H-D R V Y I H P F H L-OH	Yes

[a] Contrary to phospho- and sulfopeptides, electron capture was not consistently observed. e = pyroglutamic acid, P* = hydroxyproline.

References

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