Online SERS Detection and Characterization of Eight Biologically-Active Peptides Separated by Capillary Zone Electrophoresis

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Abstract

This supplement contains a description of the CZE-ESI-MS experiments, discussion of the results, Figures S1 and S2, and Tables S1-S9. Figure S1 shows (A) the total and (B) the extracted ion electropherograms of the eight peptides resulting from the CZE-ESI-MS experiments. Figure S2 shows the mass spectra of the eight detected peptides. Table S1 summarizes the results of the CZE-ESI-MS experiments and lists each peptide, along with the observed m/z, expected m/z, sample concentration, and estimated LOD. Also included are tables summarizing the observed vibrational bands with assignments based on literature precedents in the SERS spectra of the eight biologically-active peptides shown in Figure 3. They include assignments for the bands observed in the SERS spectrum of Laminin Pentapeptide (Table S2), Bombesin (Table S3), Angiotensin III (Table S4), Somatostatin (Table S5), Amyloid β-Protein (Table S6), Angiotensin I (Table S7), Angiotensin II (Table S8), and Substance P (Table S9).

CZE-ESI-MS Confirmation of Peptide Migration.

A 50 cm (72 μm i.d., 143 μm o.d.) uncoated fused silica capillary (Polymicro Technologies, Phoenix, AZ) was coupled to an Orbitrab Velos mass spectrometer (Thermo Fisher Scientific) via an eletrokinetically pumped sheath-flow electrospray interface.[1] The capillary dimensions and buffer conditions were matched to those used in the CZE-SERS experiments and were not optimized for MS detection. The electrospray ionization (ESI) emitter was generated from a borosilicate glass capillary and pulled (Sutter Instrument, Novato, CA) to an 8 μm i.d. tip. The same separation buffer was used for the CZE-ESI-MS experiments: 10 mM ammonium bicarbonate (pH 8). The sheath liquid was a mixture of 0.1% formic acid and 10% methanol in water to facilitate the electrospray. Voltages were provided by two Spellman CZE 1000R power supplies controlled by a custom LabView program. Electrospray and separation voltages were 1.2 kV and 10.2 kV, respectively, resulting in a 200 V cm^{-1} separation. The sample was introduced into the separation capillary using a 2 s pressure injection. The injection block was identical to that used in the CZE-SERS experiments.

Full MS scans were obtained in the Orbitrap mass analyzer at a resolution of 70,000 (at *m/z* 200) over a range of 380-2000 *m/z*. The maximum allowed ion accumulation time was 250 ms, and the automatic gain control target was $1.00x10^6$. Extracted ion and total ion electropherograms were constructed from the data using MATLAB. The electropherograms were smoothed using a 7 point Savistky-Golay smoothing algorithm and background subtracted using the built-in baseline correction algorithm in MATLAB (msbackadj).

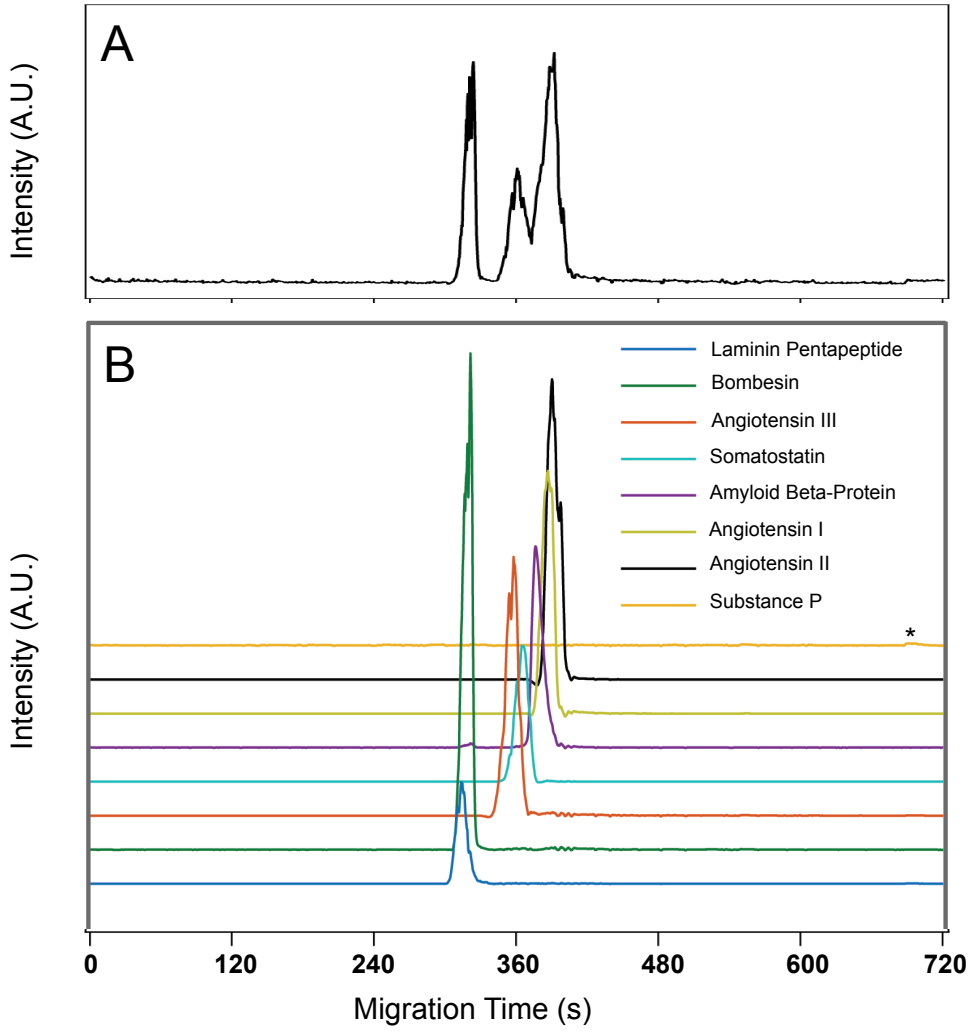

Figure S1. (A) Total and (B) extracted ion electropherograms of the eight peptides resulting from the CZE-ESI-MS experiment showing detection of Laminin Pentapeptide (m/z = 594.3367) at t_{m1} = 318 s, Bombesin (m/z = 810.4175) at t_{m2} = 324 s, Angiotensin III (m/z = 466.2615) at t_{m3} = 361.2 s, Somatostatin (m/z = 819.3678) at t_{m5} = 378 s, Amyloid β-Protein (m/z = 530.7956) at t_{m4} = 367.2 s, Angiotensin I (m/z = 648.8478) at t_{m6} = 388.2 s, Angiotensin II (m/z = 523.775) at t_{m7} = 391.8 s, and Substance P (m/z = 674.3733) at t_{m8} = 695.4 s as noted by the asterisk. The peptide concentration in this mixture is 5.0×10^{-6} M.

CZE-ESI-MS confirmed the identity, the elution order and the migration times of the peptides observed in the CZE-SERS experiments. The capillary dimensions and separation buffer composition were kept identical to those used in the optimized SERS experiments to provide a direct comparison. However, the large inner diameter of the capillary required a lower separation voltage (10.2 kV for CZE-ESI-MS vs. 15 kV for CZE-SERS) in the mass spectrometry experiment, which produced slightly longer migration times when compared to the CZE-SERS experiments; however, the migration order was unaffected. Attempting to increase the voltages to match those used in the CZE-SERS experiments resulted in a breakdown of the electrospray ionization due to the high conductivity of the larger diameter capillary. The total ion count electropherogram in Figure S1A shows co-migration of many of the peptides. This much poorer resolution is in contrast with the SERS and the TPE electropherograms shown in Figure 1. Interestingly, Substance P was detected late in the CZE-ESI-MS experiments and the peak generated has significantly lower signal to noise than the other peptides (Figure S1B). This effect was consistently observed in replicate MS runs. This low ionization efficiency is attributed to the existence of two basic amino acid residues in Substance P (R and K). The experiment was run in a carbonate buffer (PH=8) compatible for both ESI-MS and SERS detection, which may affect the ionization efficiency of Substance P relative to other peptides. Using isotachophoresis to pre-concentrate the sample at its isoelectric point enabled MS detection at nanomolar concentrations.[2]

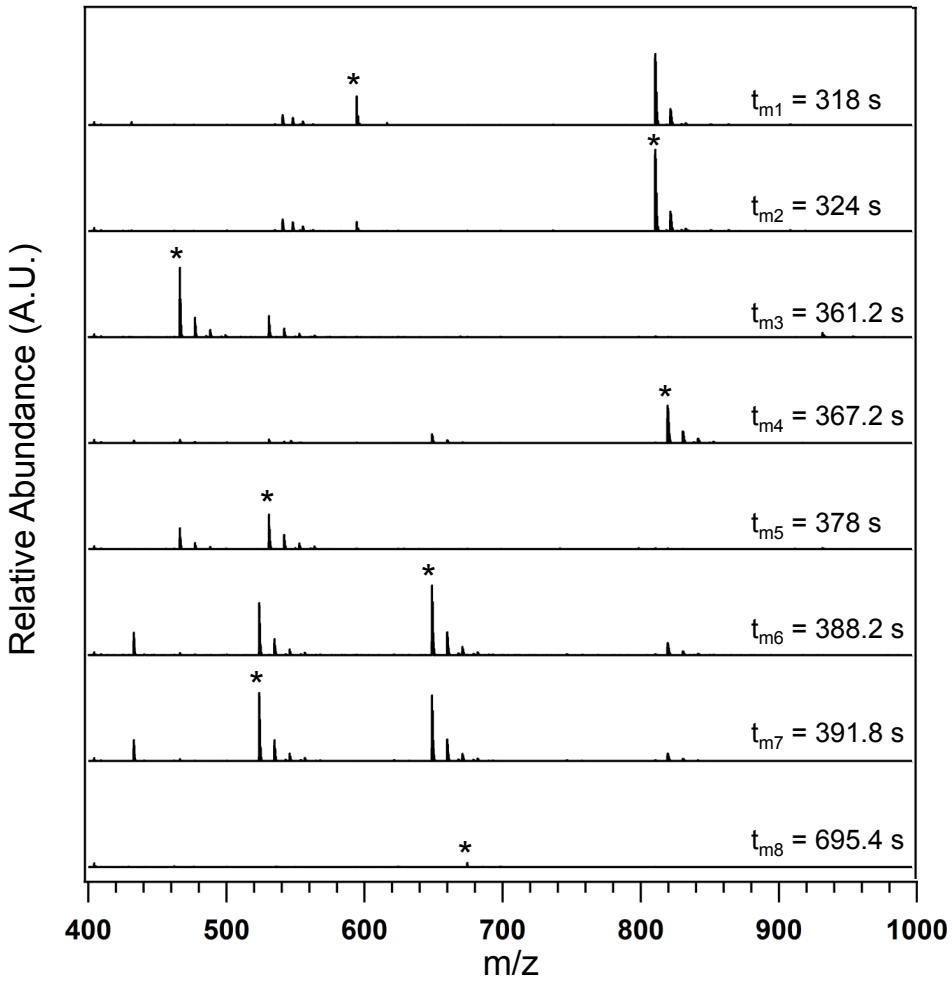

Figure S2. Mass spectra of the eight peptides resulting from the CZE-ESI-MS experiments extracted at t_{m1} = 318 s (Laminin Pentapeptide, m/z = 594.3367), t_{m2} = 324 s (Bombesin, m/z = 810.4175), t_{m3} = 361.2 s (Angiotensin III, m/z = 466.2615), t_{m4} = 367.2 s (Somatostatin, m/z = 819.3678), t_{m5} = 378 s (Amyloid β-Protein, m/z = 530.7956), t_{m6} = 388.2 s (Angiotensin I, m/z = 648.8478), t_{m7} = 391.8 s (Angiotensin II, m/z = 523.775), and t_{m8} = 695.4 s (Substance P, m/z = 674.3733). The asterisks indicate the ions used for identification of each peptide.

Table S1. Tabulated results from the CZE-ESI-MS experiment including the observed m/z, predicted m/z, sample concentration, and estimated LOD for each of the eight detected peptides.

Peptide Name	S/N	Observed m/z	Predicted m/z	Sample Conc (uM)	Estimated LOD (nM)
Laminin Pentapeptide (+1)	1900	594.336	594.336	5.00	7.7
Bombesin (+2)	2300	810.416	810.415	5.00	6.5
Angiotensin III (+2)	1600	466.261	466.262	5.00	9.7
Somatostatin (+2)	4300	819.365	819.881	5.00	3.5
Amyloid Beta-Protein (+2)	4700	530.794	530.795	5.00	3.2
Angiotensin I (+2)	6500	648.845	648.847	5.00	2.3
Angiotensin II (+2)	8199	523.774	523.775	5.00	1.8
Substance P (+2)	5	674.3733	674.372	5.00	3000

Table S2. Observed vibrational bands with assignments in the SERS spectra of Laminin Pentapeptide shown in Figure 5a. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
693	COO^- deformation[3]	
854	Fermi resonance between ring breathing and out-of-plane ring bend overtone[4, 5]	Tyr
1135	NH_3^+ deformation[6]	
1204	C_β-C_γ stretching[7-9]	Tyr
1249	Amide III vibration[10]	
1293	CH_2 wagging[6]	
1344	C-H deformation[6]	
1361	CH_2 scissoring[11]	
1444	CH_2 scissoring[12]	
1631	Amide I vibration[10, 12]	

Table S3. Observed vibrational bands with assignments in the SERS spectra of Bombesin shown in Figure 5b. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
674	C-S stretching[13, 14]	Met
693	COO^- deformation[3]	
843	C-C stretching[6]	
915	C-COO^- stretching[6]	
1040	C-N stretching[10]	
1173	NH_3^+ deformation[15]	
1225	Combined N-H bending and C-N stretching[6]	
1245	Amide III vibration[10]	
1324	CH_2 wagging[6]	
1344	CH_3 symmetric deformation[6]	Pyr
1481	CH_3 asymmetric bending[12]	Pyr
1537	Amide II vibration[4]	
1547	Indole vibration[4, 7, 9, 16]	Trp
1579	COO^- asymmetric stretching[6]	
1586	Amide II vibration[10]	
1631	Amide I vibration[10, 12]	
1657	Amide I vibration (NH_2)[12]	

Table S4. Observed vibrational bands with assignments in the SERS spectra of Angiotensin III shown in Figure 5c. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
693	COO^- deformation[3]	
854	Fermi resonance between ring breathing and out-of-plane ring bending overtone[4, 5]	Tyr
912	C-COO^- stretching[6]	
958	C-C stretching[12]	
1204	Combined ring breathing and C_β-C_γ stretching[7-9]	Tyr and Phe
1249	Amide III vibration[10]	
1288	Imidazole C-H in-plane bending[17]	Tyr
1444	CH_2 scissoring[12]	Arg
1482	Combined imidazole ring stretching and imidazole C_1-H in-plane bending[18]	Tyr
1602	Ring C-C stretching[10]	Phe
1631	Amide I vibration[10, 12]	

Table S5. Observed vibrational bands with assignments in the SERS spectra of Somatostatin shown in Figure 5d. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
674	C-S stretching[13, 14]	Cys-Cys
693	COO^- deformation[3]	
1256	Amide III vibration[10]	
1344	CH_3 symmetric deformation[6]	Ala
1378	CH_2 scissoring[11]	
1454	CH_2 scissoring[12]	
1520	NH_3^+ deformation[6]	
1534	Amide II vibration[4]	
1547	Indole vibration[4, 7, 9, 16]	Trp
1602	Ring C-C stretching[10]	Phe
1631	Amide I vibration[10, 12]	

Table S6. Observed vibrational bands with assignments in the SERS spectra of Amyloid β-Protein shown in Figure 5e. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
674	C-S stretching[13, 14]	Met
962	C-C stretching[12]	
1180	NH_3^+ deformation[15]	
1290	CH_2 wagging[6]	
1458	CH_2 scissoring[12]	
1481	CH_3 asymmetric bending[12]	Ile and Met
1514	NH_3^+ deformation[6]	
1579	COO^- asymmetric stretching[6]	
1586	Amide II vibration[10]	
1631	Amide I vibration[10, 12]	

Table S7. Observed vibrational bands with assignments in the SERS spectra of Angiotensin I shown in Figure 5f. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
915	C-COO^- stretching[6]	
948	C-C stretching[12]	
1004	Symmetric ring breathing mode[6, 10, 19]	Phe
1204	Combined ring breathing, C_β-C_γ stretching and imidazole ring deformation[7-9, 18]	Phe, Tyr and His
1249	Amide III vibration[10]	
1288	Imidazole C-H in-plane bending[17]	Tyr and His
1344	CH_3 symmetric deformation[6]	Leu
1482	Combined imidazole ring stretching and imidazole C_1-H in-plane bending[18]	Tyr and His
1517	NH_3^+ deformation[6]	
1547	Amide II vibration[4]	
1602	Ring C-C stretching[10]	Phe
1631	Amide I vibration[10, 12]	

Table S8. Observed vibrational bands with assignments in the SERS spectra of Angiotensin II shown in Figure 5g. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
622	COO^- deformation[12]	
693	COO^- deformation[3]	
915	C-COO^- stretching[6]	
948	C-C stretching[12]	
1004	Symmetric ring breathing mode[6, 10, 19]	Phe
1225	Combined N-H bending and C-N stretching	
1249	Amide III vibration[10]	
1288	Imidazole C-H in-plane bending[17]	Tyr and His
1327	CH deformation[6]	Phe
1361	CH_2 scissoring[11]	Phe, Pro, Asp and Arg
1482	Combined imidazole ring stretching and imidazole C_1-H in-plane bending[18]	Tyr and His
1534	Amide II vibration[4]	
1573	COO^- asymmetric stretching[6]	
1602	Ring C-C stretching[10]	Phe
1631	Amide I vibration[10, 12]	

Table S9. Observed vibrational bands with assignments in the SERS spectra of Substance P shown in Figure 5h. Bands allowing identification of this peptide are highlighted in red.

Peak Position (cm^{-1})	Proposed Band Assignment	AA Contribution
947	C-C stretching[12]	
1004	Symmetric ring breathing mode[6, 10, 19]	Phe
1135	NH_3^+ deformation[6]	
1170	NH_3^+ deformation[15]	
1269	Amide III vibration[10]	
1327	CH deformation[6]	Phe
1344	CH_3 symmetric deformation[6]	Met
1361	CH_2 scissoring[11]	Arg and Met
1484	CH_3 asymmetric bending[12]	Arg and Met
1527	Amide II vibration[4]	
1602	Ring C-C stretching[10]	Phe
1631	Amide I vibration[10, 12]	

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