Supporting Information for:

Site-Specific Characterization of D-Amino Acid-Containing Peptide Epimers by Ion Mobility Spectrometry

Chenxi Jia, Christopher B. Lietz, Qing Yu, and Lingjun Li*

School of Pharmacy and Department of Chemistry, University of Wisconsin–Madison, WI

Correspondence: Professor Lingjun Li, School of Pharmacy and Department of Chemistry, University of Wisconsin–Madison, 777 Highland Avenue, Madison, Wisconsin 53705-2222

E-mail: lli@pharmacy.wisc.edu

Fax: +1-608-262-5345

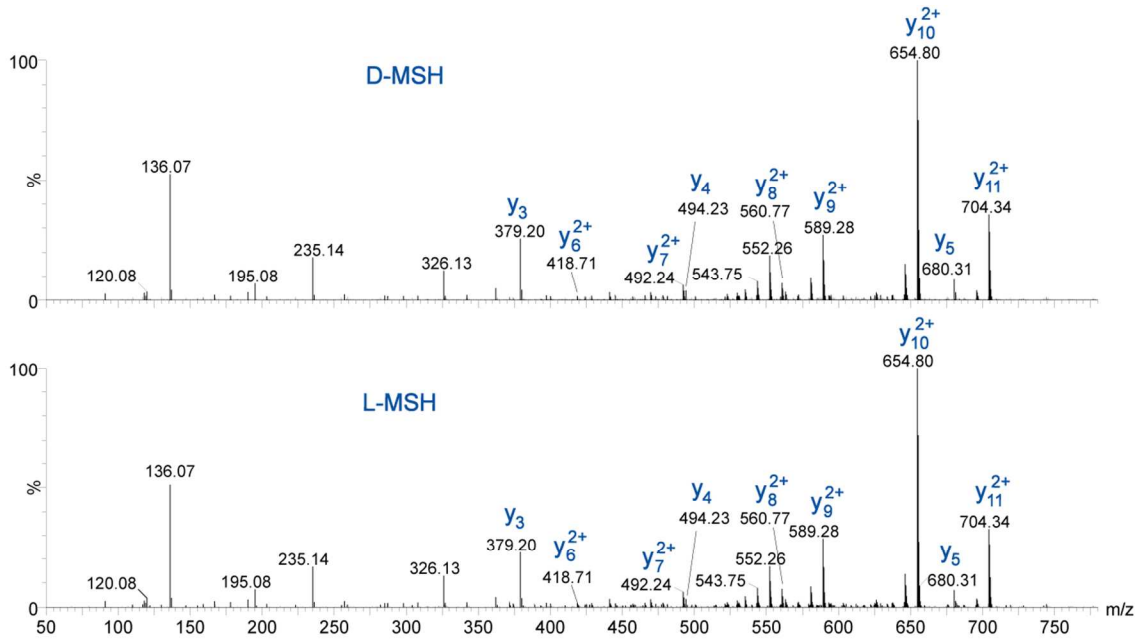

Figure S-1. CID MS/MS spectra of the D- and L-MSH peptides acquired from LC-MS/MS.

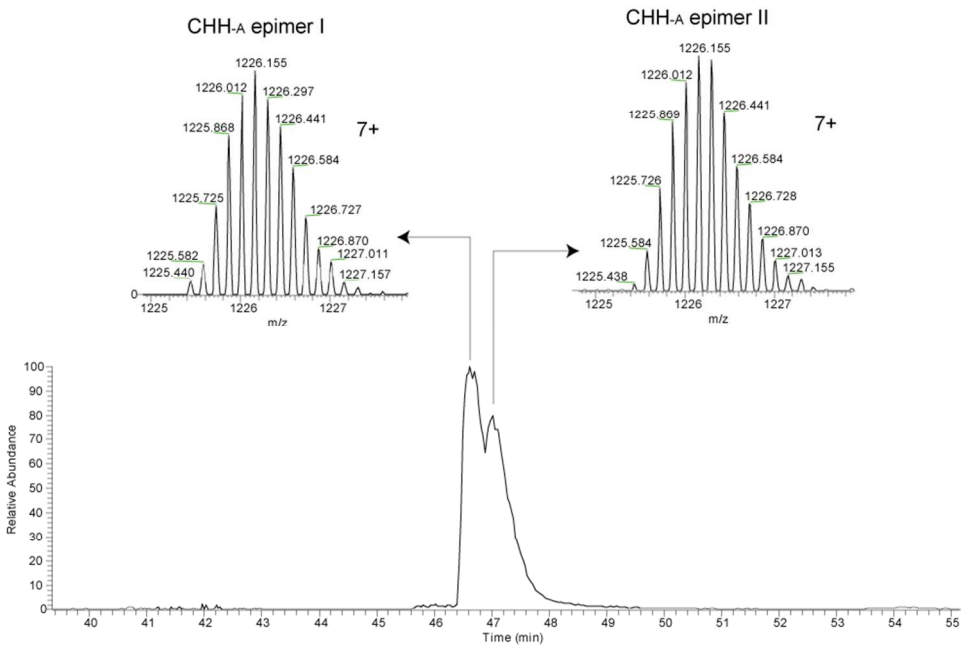

Figure S-2. Extract ion chromatogram of the LC-MS analysis of CHH_{-A} epimers I and II. The two inset panels show the isotopic distributions of the two epimers. They show the same mass, but are eluted at different retention times.

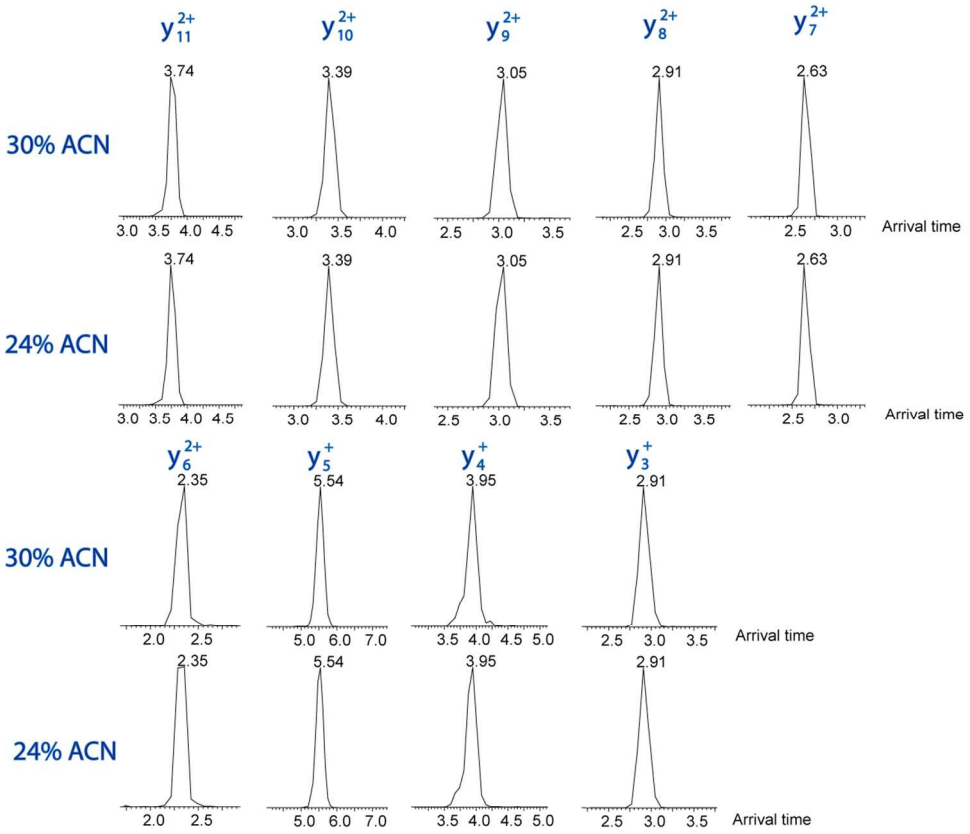

Figure S-3. Arrival time distributions of fragment ions of L-MSH in 30% and 24% acetonitrile/water (0.1% formic acid). The arrival times of these fragment ions are identical under the two solvent conditions.

Table S1. Measured CCS_{He} of precursor and fragment ions of D/L-MSH. Standard deviations reflect measurement variation. Absolute errors are approximately 3.5%.

	D-MSH	L-MSH
$[M+3H]^{3+}$	340.3 ± 0.4	347.2 ± 0.4
y_{11}^{2+}	294.1 ± 0.2	294.7 ± 0.1
y_{10}^{2+}	277.4 ± 0.2	277.2 ± 0.1
y_9^{2+}	260.2 ± 0.2	259.0± 0.1
y_8^{2+}	252.7 ± 0.2	252.9 ± 0.1
y_7^{2+}	236.8 ±0.1	237.5 ± 0.3
y_6^{2+}	216.7 ± 0.3	217.6± 0.0
y_5^{+}	173.5 ± 0.1	177.8 ± 0.3
y_4^{+}	143.7 ± 0.0	143.4 ±0.0
y_3^{+}	118.9 ± 0.0	118.8 ±0.0

Table S2. Tryptic peptides of CHHs identified by bottom-up sequencing approaches.[a]

CHH_{-A}

Tryptic peptides	Position	-10logP	mass	m/z	ppm	Elution time /min
*p*QVFDQAC*K	CHH_{-A}[1-8]	84.06	977.43	489.71	-23.7	21.42, 26.69
*p*QVFDQAC*KGVYDRNLFK	CHH_{-A}[1-17]	108.94	2069.99	690.99	-22.3	33.21,35.70
GVYDRNLFK	CHH_{-A}[9-17]	86.12	1110.58	556.28	-24.9	21.82
KLDRVC*EDC*YNLYR	CHH_{-A}[18-31]	124.97	1902.87	635.28	-27.2	20.12
VC*EDC*YNLYR	CHH_{-A}[22-31]	115.62	1390.56	696.27	-25.8	20.99
VC*EDC*YNLYRKPFVATTC*R	CHH_{-A}[22-40]	103.51	2451.11	613.77	-28.1	24.77
KPFVATTC*RENC*YSNWVFR	CHH_{-A}[32-50]	124.26	2434.13	609.52	-27.2	29.83
ENC*YSNWVFR	CHH_{-A} [41-50]	134.61	1373.58	687.78	-22.0	32.14

CHH_{-B}

Tryptic peptides	Position	-10logP	mass	m/z	ppm	Elution time /min
*p*QVFDQAC*K	CHH_{-B}[1-8]	84.06	977.43	489.71	-23.7	21.42, 26.69
*p*QVFDQAC*KGVYDRNLFK	CHH_{-A}[1-17]	108.94	2069.99	690.99	-22.3	33.21, 35.70
GVYDRNLFK	CHH_{-B} [9-17]	86.12	1110.58	556.28	-24.9	21.82
LNRVC*EDC*YNLYR	CHH_{-B}[19-31]	135.91	1773.79	592.25	-27.0	20.99
VC*EDC*YNLYRKPFIVTTC*R	CHH_{-B}[22-40]	115.93	2493.16	624.28	-26.0	27.94
KPFIVTTC*R	CHH_{-B}[32-40]	111.98	1120.60	561.29	-23.6	17.61
KPFIVTTC*RENC*YSNRVFR	CHH_{-B}[32-50]	35.36	2446.19	612.54	-29.8	22.48
ENC*YSNRVFR	CHH_{-B} [41-50]	45.18	1343.60	448.86	-27.8	16.78

[a] The two CHHs share parts of sequences, so some tryptic peptides are the same in the two tables.

Table S3. The ions used for collision cross section calibration.

Polyalanine Standards

n	mass (Da)	charge	m/z	Ω_{He} (Å^2)
11	799.42	2	400.72	197
12	870.46	2	436.24	208
13	941.49	2	471.75	220
14	1012.53	2	507.27	232
15	1083.57	2	542.79	243
16	1154.60	2	578.31	255
17	1225.64	2	613.83	265
18	1296.68	2	649.35	276
19	1367.72	2	684.87	287
20	1438.75	2	720.38	297
21	1509.79	2	755.90	308
22	1580.83	2	791.42	317
23	1651.86	2	826.94	327
24	1722.90	2	862.46	337
25	1793.94	2	897.98	348
26	1864.98	2	933.50	358
21*	1509.79	3	504.27	361
22	1580.83	3	527.95	373
23	1651.86	3	551.63	386
24	1722.90	3	575.31	399
25	1793.94	3	598.99	412
26	1864.98	3	622.67	425
27	1936.01	3	646.35	438
28	2007.05	3	670.02	452
29	2078.09	3	693.70	465
30	2149.12	3	717.38	479
31	2220.16	3	741.06	490
32	2291.20	3	764.74	502
33	2362.24	3	788.42	516

* The ion was not detected at all wave velocities.

EXPERIMENTALS

Collision Cross Section Measurement

Gas-phase helium collision cross section values (CCS_{He}) for all of the ions described in the manuscript were measured on the Synapt G2 travelling-wave ion mobility mass spectrometer with nitrogen buffer gas. CCS_{He} calibration was performed using combined methods from Bush et al.[1] and Ruotolo et al.[2] to obtain accurate measurements. Polyalanine peptides (Sigma Aldrich) were dissolved in 49.5/49.5/1 water/ACN/formic acid at a concentration of 10 µg/mL. A separate calibration spectrum was acquired for each of the following wave velocities (m/s): 600, 700, and 800. The wave height was kept constant at 40 V. Following acquisitions, the log of the arrival times (t_D) were plotted against the log of the reduced CCS todetermine the constants needed to calculate unknown Ω_{He}. The equations in the following paragraph were obtained from previously published sources.[2-3] In travelling wave ion mobility, t_D and CCS_{He} (Ω_{He}) are non-linearly related by Equation 1:

$$\Omega_{He} = \frac{ze}{16}\left[\frac{18\pi}{k_b T}\left(\frac{1}{m}+\frac{1}{M_{He}}\right)\right]^{1/2}\frac{760}{P}\frac{T}{273.2}\frac{1}{NL}At_D{}^{B} \quad (1)$$

The variables z and e make up the charge of the analyte, T is the temperature of the drift gas, M_{He} is the mass of the helium drift gas, m is the mass of the analyte, P is the pressure inside the drift cell, N is the number density of drift gas molecules, L is the length of the drift cell, and k_b is Boltzmann's constant. A and B are constants that arise from the non-uniformity of the travelling-wave electric field and must be empirically determined by calibration.

The t_D for each calibration standard was converted to corrected arrival time (t_D') by Equation 2 to account for the *m*/*z*-dependent travel time through the Synapt G2's ion optics:

$$t_D{}' = t_D - \left(\frac{C\sqrt{m/z}}{1000}\right) \quad (2)$$

C is the delay constant set by the MS control software. The reduced collision cross section (Ω') was normalized for mass and charge contributions and calculated by Equation 3:

$$\Omega' = \frac{\Omega_{He}}{z\left(\frac{1}{m}+\frac{1}{M_{He}}\right)^{1/2}} \quad (3)$$

Plotting the natural log of Ω' versus the natural log of t_D' yielded a linear best-fit line, the slope of which is B from Equation 1. From here, the doubly corrected arrival time (t_D'') was calculated by Equation 4:

$$t_D{}'' = z\left(t_D{}'\right)^B \left(\frac{1}{m}+\frac{1}{M_{He}}\right)^{1/2} \quad (4)$$

A final plot was constructed with t_D'' on the x-axis and Ω_{He} on the y-axis. The equation of the best-fit line was then used to calculate the Ω_{He} of the unknown peptides and peptide fragments.

References

(1) Bush, M. F.; Campuzano, I. D.; Robinson, C. V. *Anal Chem* **2012**, *84*, 7124.

(2) Ruotolo, B. T.; Benesch, J. L.; Sandercock, A. M.; Hyung, S. J.; Robinson, C. V. *Nat Protoc* **2008**, *3*, 1139.

(3) Smith, D. P.; Knapman, T. W.; Campuzano, I.; Malham, R. W.; Berryman, J. T.; Radford, S. E.; Ashcroft, A. E. *Eur J Mass Spectrom (Chichester, Eng)* **2009**, *15*, 113.