Supporting Information

Radical Cascades in Electron Transfer Dissociation (ETD) –

Implications for Characterizing Peptide Disulfide Regio-Isomers

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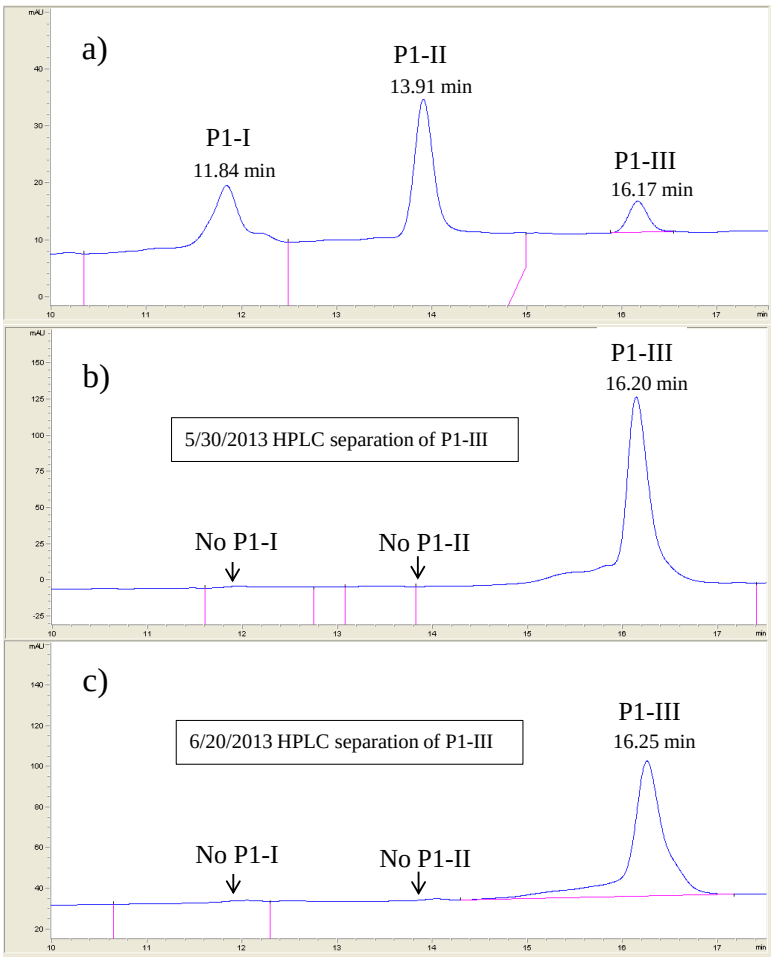
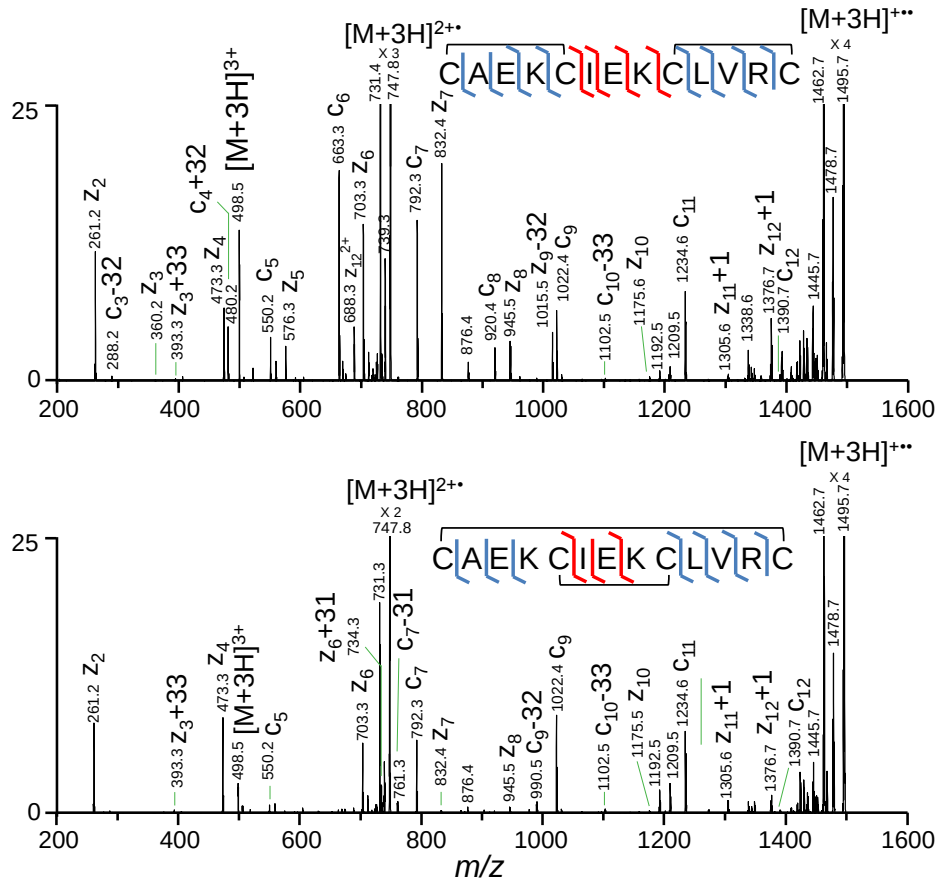
Stability of the peptide regio-isomers

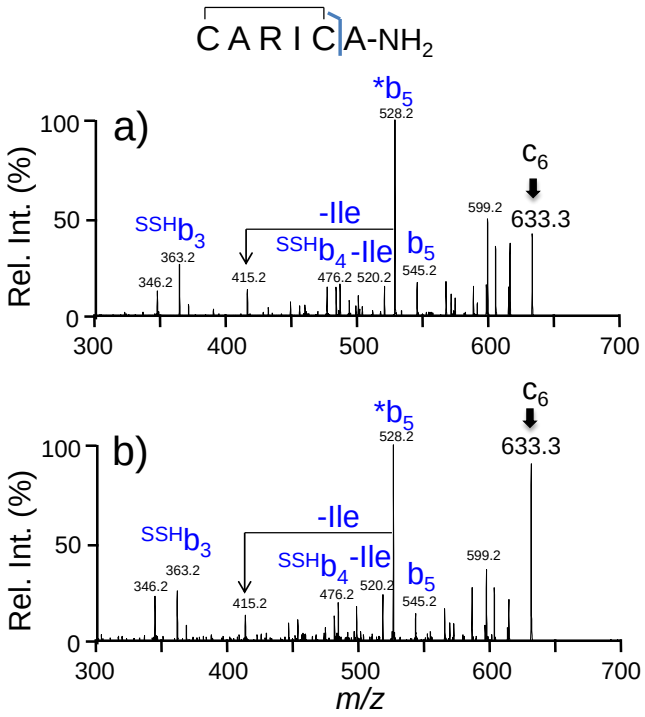
No disulfide bond scrambling was observed for P1 peptides once the two disulfide bonds were generated. The stability of individual regio-isomers was tested by repeated HPLC separations of the purified sample after various periods of storage. Consistent retention time was observed without the detection of other isomers. The data of P1-III were used as an example to illustrate the stability test process. P1-III was first collected after HPLC separation of the oxidation mixture (SI Figure 1a) and vacuum dried. A portion of purified P1-III was redissolved in aqueous solution and immediately subjected to an HPLC run (SI Figure 1b). Note that P1-III was the one with the longest retention time among three regio-isomers. No peak was observed before P1-III, indicating no scrambling to other isomers occurred after vacuum drying. This sample was stored in freezer (-20 ℃) for 20 days and redissolved in aqueous solution for another HPLC run (SI Figure 1c) and no scrambling was observed.



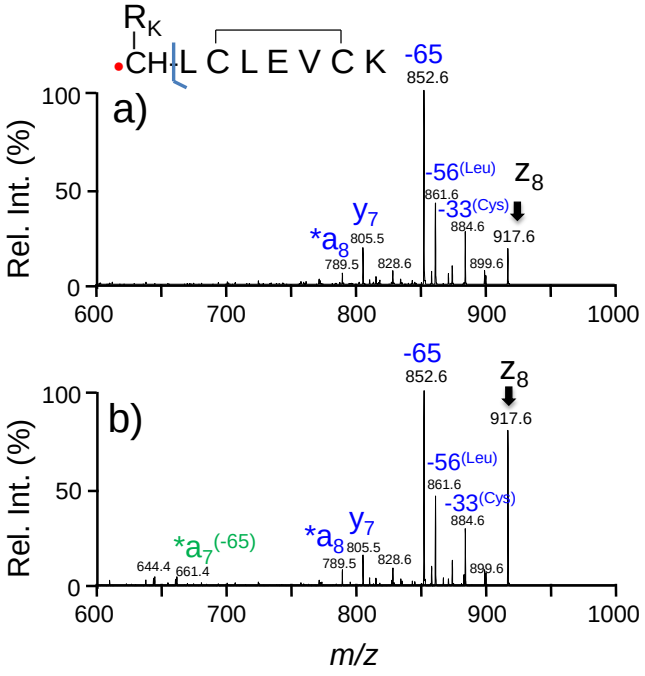
SI Figure 1. HPLC separation chromograph of: a) mixture of three regio-isomers of P1 after the oxidation reactions, b) P1-III redissolved in 20 µL of water right after being vacuum dried; c) P1-III redissolved in 20 µL of water after storage at -20 ℃ freezer for 20 days. All separations were carried out under the same condition: B: 20-35% over 30 min at 0.65 mL/min flow rate (solvent A: 0.1% TFA in water, B: 0.085% TFA in acetonitrile).



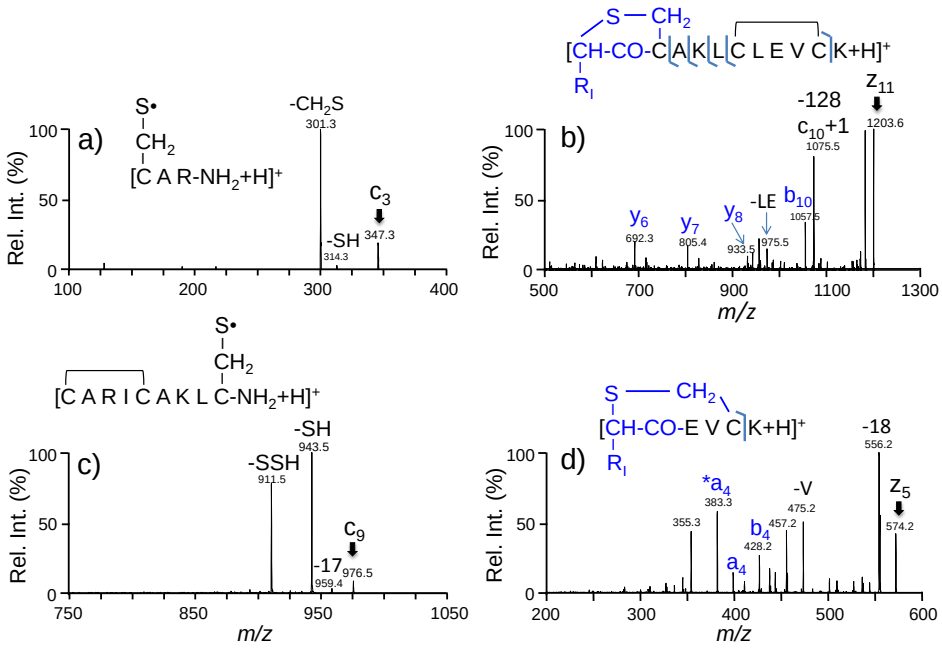
SI Figure 2. ETD of $[M+3H]^{3+}$ of a) P2-I; b) P2-II. ETD reaction time was 100 ms, without supplementary activation.



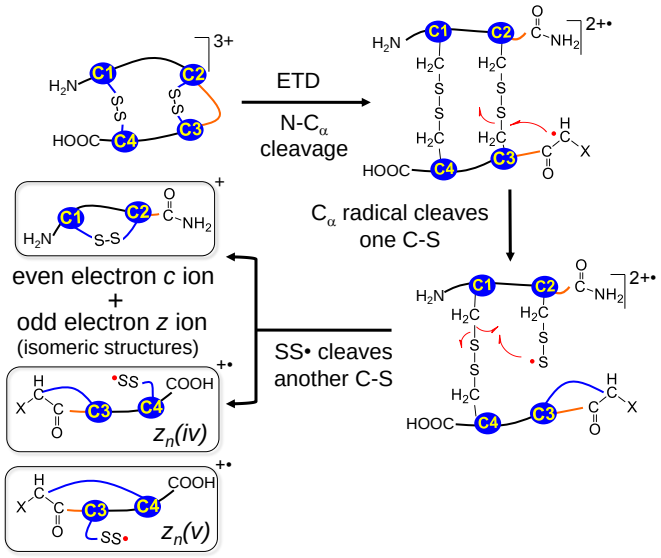
SI Figure 3. MS^3 CID of c_6 from ETD of $[M+3H]^{3+}$ from a) P1-I; b) P1-III.



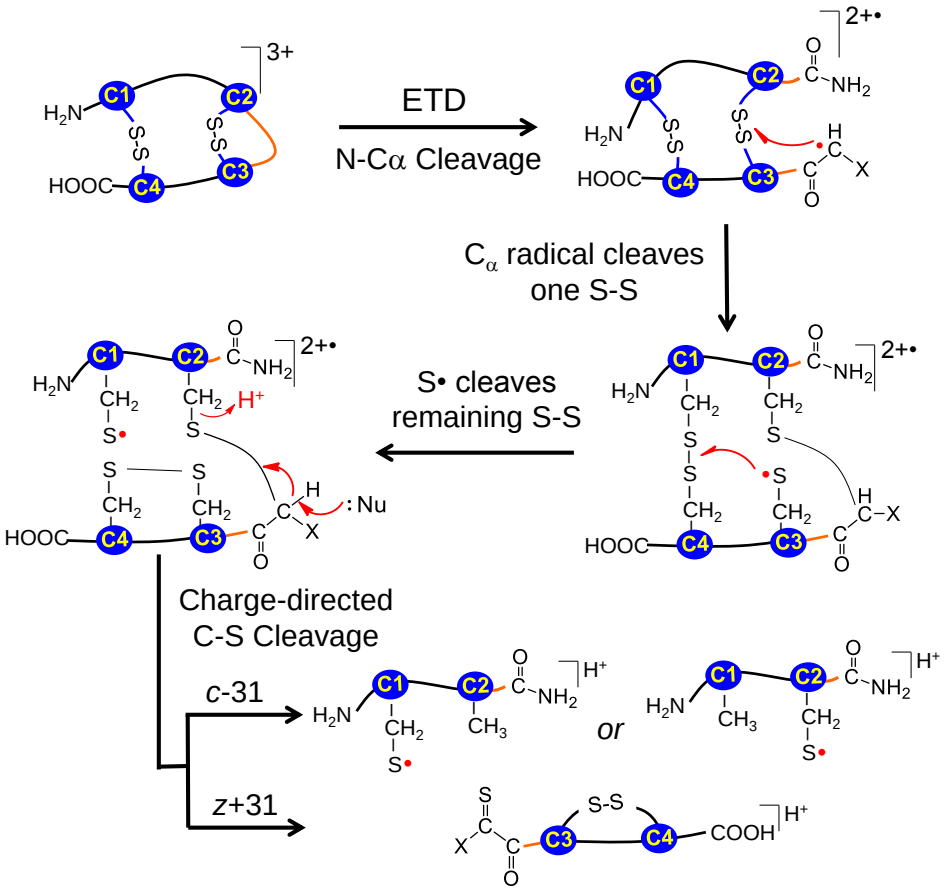
SI Figure 4. MS^3 CID of z_8 from ETD of $[M+3H]^{3+}$ from a) P1-I; b) P1-III.



SI Figure 5. MS^3 CID of a) c_3, b) z_{11}, c) c_9 and d) z_5 from ETD of $[M+3H]^{3+}$ from P1-II.



SI Scheme 1. Alternative radical cascades pathway for the formation of cyclic *c* and *z*• ion between C2 and C3 in ETD of P1-II.



SI Scheme 2. Proposed pathway for the formation of *c*-31/*z*+31 ion between C2 and C3 in ETD of P1-II.

SI Table 1. Theoretical prediction of the backbone fragments (*c* and *z* ions, 1+) resulting from ETD of fully oxidized P1 peptides. The prediction is based on homolytic cleavage of disulfide bond and carried out with ProSight PTM developed by the Kelleher Group at Northwestern University.

Ion	Theoretical *m/z*	Ion	Theoretical *m/z*
c_1	120.0	z_1	131.1
c_2	191.1	z_2	233.1
c_3	347.2	z_3	332.2
c_4	460.3	z_4	461.2
c_5	562.3	z_5	574.3
c_6	633.3	z_6	676.3
c_7	761.4	z_7	789.4
c_8	874.5	z_8	917.5
c_9	976.5	z_9	988.5
c_{10}	1089.6	z_{10}	1090.5
c_{11}	1218.6	z_{11}	1203.6
c_{12}	1317.7	z_{12}	1359.7
c_{13}	1419.7	z_{13}	1430.8
c_{14}	1547.8	z_{14}	1532.8

SI Table 2. Assigned peak list for ETD of $[M+3H]^{3+}$ (*m/z* 517.1) of P1-I. Relative ion intensities (%) are normalized to the most abundant product ion peak within each spectrum.

ExpPeak *m/z*	Charge State	Relative Intensity	Assignment
331.3	1+	0.3	z_3-1
347.3	1+	0.6	c_3
461.3	1+	0.2	z_4
494.3	1+	1	z_4+33
517.1	3+	82	$[M+3H]^{3+}$
562.3	1+	2.2	c_5
574.4	1+	2.2	z_5
633.4	1+	9	c_6

676.5	1+	0.6	z_6
697.0	2+	6.5	
710.5	2+	4	c_{13}
732.5	2+	2	
759.1	2+	100	$[M+3H]^{2+\bullet}$-SH
761.5	1+	9.5	c_7
767.0	2+	9.5	$[M+3H]^{2+\bullet}$-NH_3
775.5	2+	64	$[M+3H]^{2+\bullet}$
789.5	1+	8	z_7
805.5	1+	0.7	y_7
867.6	1+	0.8	
874.6	1+	0.9	c_8
884.6	1+	1.2	
917.6	1+	8.2	z_8
933.5	1+	0.4	y_8
976.6	1+	2.2	c_9
988.6	1+	2.8	z_9
1056.7	1+	0.9	c_{10}-33
1089.6	1+	0.2	c_{10}
1090.6	1+	0.3	z_{10}
1203.6	1+	0.7	z_{11}
1219.6	1+	0.4	c_{11}+1
1359.7	1+	0.2	z_{12}
1393.7	1+	1.2	
1420.7	1+	0.4	c_{13}+1
1431.7	1+	0.3	z_{13}+1
1500.9	1+	1.2	
1517.8	1+	12	$[M+3H]^{+\bullet\bullet}$-SH
1533.8	1+	3.2	$[M+3H]^{+\bullet\bullet}$-NH_3
1550.8	1+	5.5	$[M+3H]^{+\bullet\bullet}$

SI Table 3. Assigned peak list for ETD of $[M+3H]^{3+}$ (*m/z* 517.1) of P1-II. Relative ion intensities (%) are normalized to the most abundant product ion peak within each spectrum.

ExpPeak *m/z*	**Charge State**	**Relative Intensity**	**Assignment**
331.3	1+	2.5	z_3-1
347.3	1+	0.5	c_3

461.3	1+	0.3	z_4
494.3	1+	2.2	z_4+33
517.1	3+	50	$[M+3H]^{3+}$
556.4	1+	1.5	
562.3	1+	0.8	c_5
574.4	1+	1.6	z_5
602.4	1+	4.5	c_6-31
633.4	1+	7	c_6
676.5	1+	0.1	z_6
697.0	2+	4.5	
710.5	2+	2.2	c_{13}
759.1	2+	100	$[M+3H]^{2+\bullet}$-SH
761.5	1+	7.5	c_7
767.0	2+	13	$[M+3H]^{2+\bullet}$-NH_3
775.5	2+	70	$[M+3H]^{2+\bullet}$
789.5	1+	4.1	z_7
805.5	1+	0.4	y_7
821.4	1+	0.7	
867.6	1+	1.8	
874.6	1+	0.1	c_8
899.5	1+	1.9	
917.6	1+	6.5	z_8
948.5	1+	5.8	z_8+31
976.6	1+	1.8	c_9
988.6	1+	1	z_9
1056.7	1+	2.8	c_{10}-33
1089.6	1+	0.3	c_{10}
1090.6	1+	0.4	z_{10}
1203.6	1+	1	z_{11}
1219.6	1+	5	c_{11}+1
1349.7	1+	2.4	
1359.7	1+	0.4	z_{12}
1393.7	1+	2.5	
1419.7	1+	0.8	c_{13}+1
1431.7	1+	0.2	z_{13}+1
1490.8	1+	2	
1500.9	1+	5	
1517.8	1+	36	$[M+3H]^{+\bullet\bullet}$-SH
1533.8	1+	11	$[M+3H]^{+\bullet\bullet}$-NH_3
1550.8	1+	21	$[M+3H]^{+\bullet\bullet}$

SI Table 4. Assigned peak list for ETD of $[M+3H]^{3+}$ (*m/z* 517.1) of P1-III. Relative ion intensities (%) are normalized to the most abundant product ion peak within each spectrum.

ExpPeak *m/z*	Charge State	Relative Intensity	Assignment
301.3	1+	0.7	
314.4	1+	0.7	c_3-33
331.3	1+	2.3	z_3-1
347.3	1+	2.4	c_3
461.3	1+	0.7	z_4
447.3	1+	1.2	
494.3	1+	0.6	z_4+33
517.1	3+	53	$[M+3H]^{3+}$
562.3	1+	0.7	c_5
574.4	1+	1.2	z_5
602.4	1+	3.5	c_6-31
633.4	1+	5	c_6
644.4	1+	0.5	
664.5	2+	1	
676.5	1+	0.2	z_6
697.0	2+	1.4	
710.5	2+	2	c_{13}
759.1	2+	100	$[M+3H]^{2+\bullet}$-SH
761.5	1+	6	c_7
767.0	2+	26	$[M+3H]^{2+\bullet}$-NH_3
775.5	2+	50	$[M+3H]^{2+\bullet}$
789.5	1+	5.5	z_7
821.4	1+	1	
867.6	1+	1	
874.6	1+	0.3	c_8
917.6	1+	5	z_8
948.5	1+	3.4	z_8+31
976.6	1+	1.4	c_9
988.6	1+	0.7	z_9
1045.6	1+	1.6	
1056.7	1+	0.7	c_{10}-33
1089.6	1+	0.5	c_{10}
1090.6	1+	0.5	z_{10}
1203.6	1+	4.5	z_{11}

1219.6	1+	3.5	c_{11}+1
1236.6	1+	0.5	z_{11}+33
1349.7	1+	1.1	
1359.7	1+	0.3	z_{12}
1419.7	1+	0.4	c_{13}+1
1431.7	1+	0.2	z_{13}+1
1500.9	1+	5.5	
1517.8	1+	34	$[M+3H]^{+\bullet}$-SH
1533.8	1+	12	$[M+3H]^{+\bullet}$-NH_3
1550.8	1+	10	$[M+3H]^{+\bullet}$