Supplemental Information for

Diethylpyrocarbonate Labeling for the Structural Analysis of Proteins: Label Scrambling in Solution and How to Avoid it

Yuping Zhou and Richard W. Vachet*
Department of Chemistry, University of Massachusetts, Amherst

Table S1. Solvent accessible surface area (SASA) for DEPC-modifiable amino acids and the modification percentages of DEPC-modified β-2-microglobulin under different conditions. All the experiments were repeated three times, and the means and standard deviations are reported.

residues	% SASA ratio[a]			TCEP		without TCEP	TCEP/iodoacetamide
	average	max	min	2 h	2 + 24 h	2 h	2 h
C25	0.1±0.1	0.5	0	52 ± 2	91 ± 7	N.D.[b]	N.D.
C80	0.02±0.06	0.2	0	15 ± 5	100 ± 0	N.D.	N.D.
N-terminus	80±10	98.0	63.0	2.94 ± 0.07	3 ± 1	2.6 ± 0.8	2.4 ± 0.4
K6	80±3	85.4	75.4	0.3 ± 0.1	0.4 ± 0.2	0.21 ± 0.03	0.20 ± 0.07
K19	69±7	81.2	59.4	3.4 ± 0.4	1.0 ± 0.2	3.6 ± 0.5	4.1 ± 0.1
K41	14±4	23.5	7.6	2.1 ± 0.9	2.8 ± 0.8	2.0 ± 0.5	2.6 ± 0.7
K48	80±12	100	63.6	6.8 ± 0.8	15 ± 4	5 ± 1	8 ± 2
K75	90±10	100	72.0	0.31 ± 0.03	0.4 ± 0.1	0.2 ± 0.2[c]	0.4 ± 0.2
K91	62±9	74.8	43.1	2.0 ± 0.5	1.75 ± 0.03	1.4 ± 0.3	1.4 ± 0.3
K94	70±10	87.6	49.2	2.9 ± 0.7	2.62 ± 0.05	2.0 ± 0.5	2.1 ± 0.5
H13	51±9	66.8	38.6	35 ± 5	9 ± 1	41 ± 7	42.7 ± 0.7
H31	30±11	49.3	10.2	3.3 ± 0.6	0.7 ± 0.2	3.8 ± 0.7	7 ± 1
H51	55±6	61.9	40.3	10.2 ± 0.1	N.D.	6 ± 1	10 ± 1
S11	10±12	44.1	0.0	4 ± 1	4 ± 4[c]	13.3 ± 0.4	8 ± 1
S20	70±11	90.9	42.5	0.36 ± 0.09	0.5 ± 0.5[c]	1.24 ± 0.03	0.7 ± 0.1
S28	20±3	27.3	10.9	0.27 ± 0.04	0.5 ± 0.2	0.30 ± 0.07	0.31 ± 0.03
S33	66±4	70.8	53.5	1.21 ± 0.09	1.1 ± 0.5	2.4 ± 0.8	1.3 ± 0.3
S52	44±9	70.5	24.6	N.D.	N.D.	N.D.	N.D.
S55	56±5	66.5	46.1	2 ± 1	2.8 ± 0.4	5 ± 2	3.4 ± 0.6
S57/K58	35±9/96±5	52.5/100	19.3/84.4	3 ± 1	5 ± 2	3 ± 2	3.3 ± 0.6
S61	27±9	49.8	14.8	N.D.	N.D.	N.D.	N.D.
S88	90±10	100	74.4	N.D.	N.D.	N.D.	N.D.
Y10	42±9	54.0	24.9	0.13 ± 0.06	N.D.	0.07 ± 0.03	0.065 ± 0.008
Y26	26±3	32.3	20.9	N.D.	N.D.	N.D.	N.D.
Y63	27±4	36.5	17.9	N.D.	N.D.	N.D.	N.D.
Y67/T68	22±3/2±2	27.3/5.2	15.3/0.0	0.57 ± 0.02	0.5 ± 0.1	2 ± 1	1.6 ± 0.8
Y78	6±2	10.8	3.5	0.10 ± 0.02	0.1 ± 0.1 [c]	1.8 ± 0.9	0.7 ± 0.3
T4	84±5	92.1	75.8	N.D.	N.D.	N.D.	N.D.
T71	36±6	50.0	28.2	N.D.	N.D.	N.D.	N.D.
T73	61±6	74.2	49.6	N.D.	N.D.	N.D.	N.D.

[a] The PDB ID for β-2-microglobulin that was used to determine SASA was 1JNJ. This PDB ID corresponds to a collection of 20 protein conformers obtained from NMR measurements. SASA values were calculated using GETAREA 1.1 [1]. The average SASA values, as well as the maximum and minimum values, are reported. 1.4 Å was used as the probe radius. GETAREA calculates SASA percentages as the ratio of the SASA of the side chain in the protein to the SASA of the side chain (X) in the hypothetical unstructured Gly-X-Gly tripeptide.

[b] N.D. indicates that modified peptide is not detected in any experiment under these conditions.

[c] This modified peptide is not detected in every experiment.

Figure S1: Tandem mass spectrum of the ANP modified species with a retention time of 12.5 min (see Figure 3b). Unmodified z_2 and z_4 ions, modified z_6 to z_9 ions along with unmodified c_3 to c_6 ions and modified c_7 to c_{10} ions confirm that Cys7 is the site of modification in the peptide ANP.

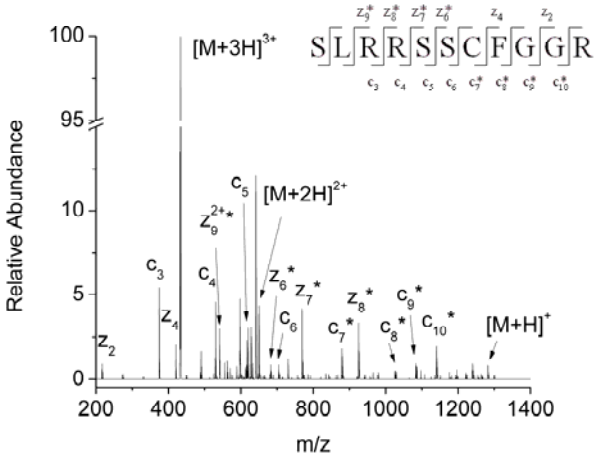
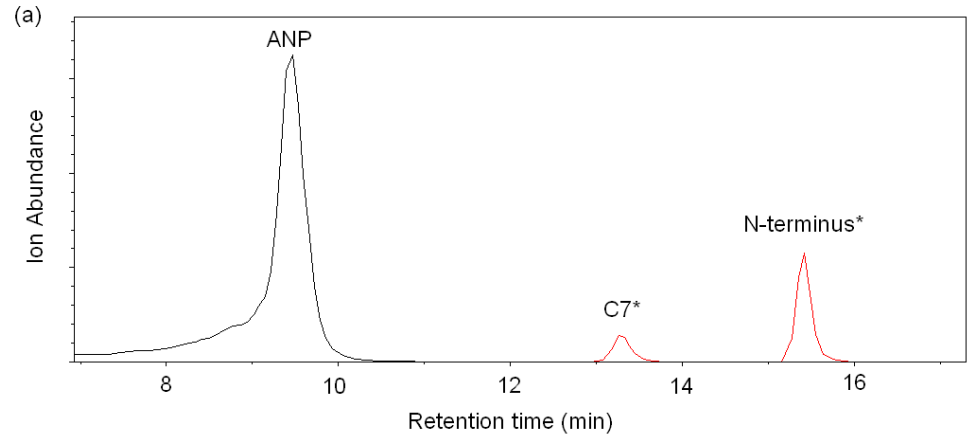
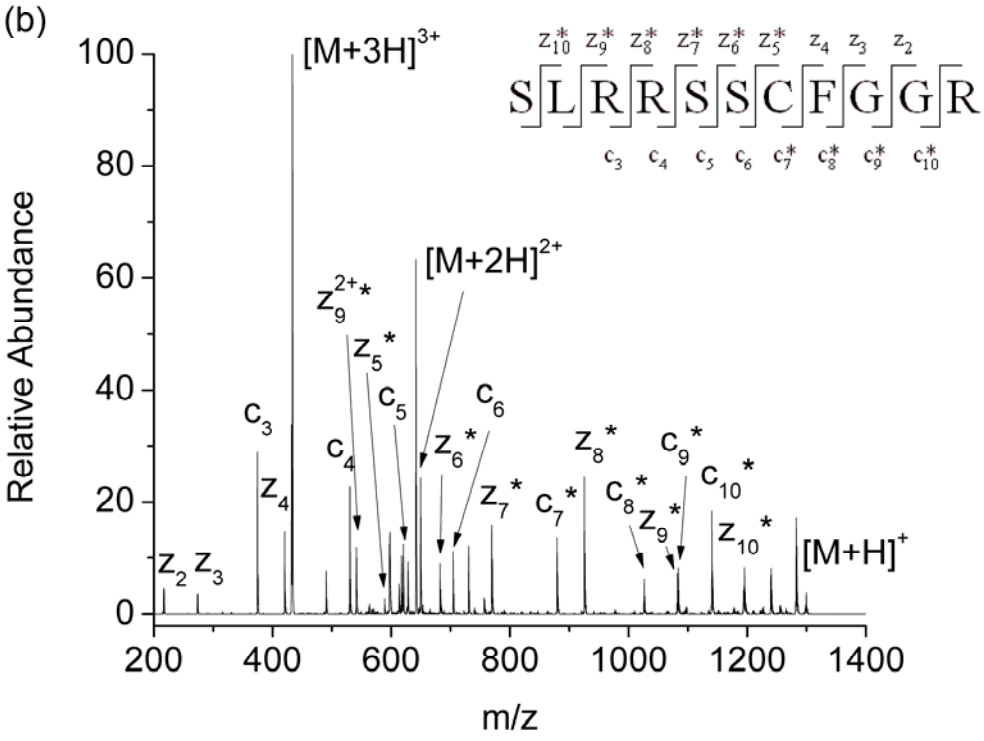


Figure S2: (a) Extracted ion chromatograms (EIC) of ANP (black) and DEPC-modified ANP (red). Chromatographic peaks notated with a residue having an asterisk indicate that the corresponding chromatographic peak is the peptide labeled at only the indicated residue. (b) Tandem mass spectrum of modified ANP with a retention time of 13.2 min. Unmodified z_2 to z_4 ions and modified z_5 to z_{10} ions along with unmodified c_3 to c_6 ions and modified c_7 to c_{10} ions confirm that C7 is the site of modification. (c) Tandem mass spectrum of modified ANP with retention time of 15.5 min. A series of unmodified z_2 through z_{10} ions and modified c_3 to c_9 ions confirm that the N-terminus is the site of modification.


(a)
ANP
C7*
N-terminus*
Ion Abundance
8
10
12
14
16
Retention time (min)


(b)
[M+3H]3+
[M+2H]2+
[M+H]+
Relative Abundance
m/z
100
80
60
40
20
0
200
400
600
800
1000
1200
1400
S L R R S S C F G G R

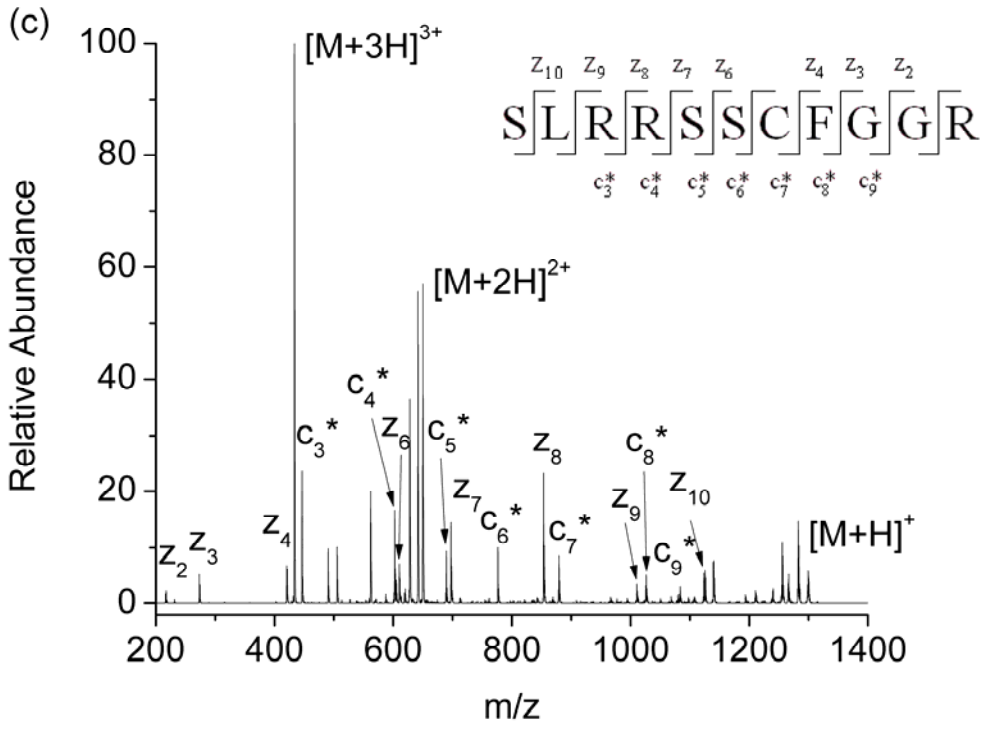
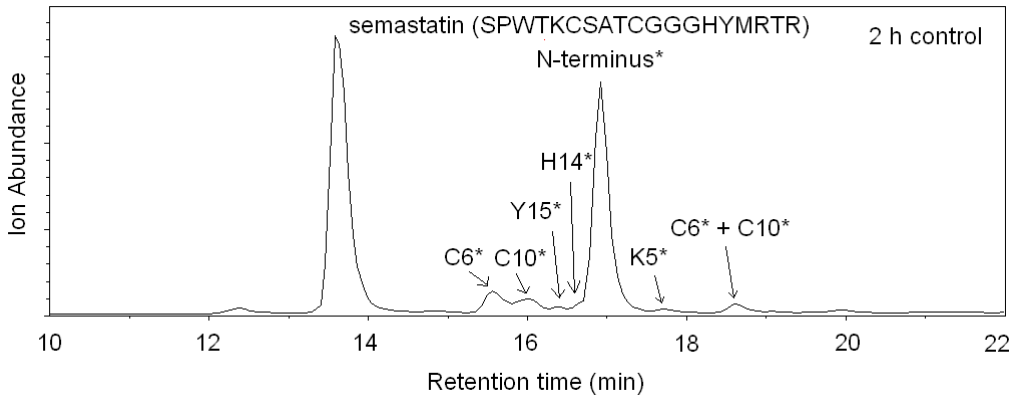


Figure S3: Total ion chromatogram (TIC) of semastatin modified by DEPC at a peptide:DEPC ratio of 1:4 with TCEP added 2 h after the reaction with DEPC. The sample was analyzed immediately. This is a control experiment that demonstrates that the capture of the carbethoxy group by the Cys residues is the primary cause of the decreases in the Tyr15 and His14 modification levels. The figure indicates that modification at Tyr15 and His14 is observed, while no modification of these two residues is observed in the case when the sample was incubated with TCEP for 2 h as indicated in Figure 5(b). This result demonstrates that the decrease of the modification for some of the residues is directly related with the existence of free thiol goup. Chromatographic peaks notated with a residue(s) having an asterisk indicate that the corresponding chromatographic peak is the peptide labeled at only the indicated residue(s).



Reference

1. Fraczkiewicz, R., Braun, W.: Exact and efficient analytical calculation of the accessible surface areas and their gradients for macromolecules. *J. Comput. Chem.* **19,** 319-333 (1998)