Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Sensitive and Selective Trypsin Detection Using Redox Cycling in the Presence of L-Ascorbic Acid

Seonhwa Park and Haesik Yang*

Department of Chemistry and Chemistry Institute of Functional Materials, Pusan National University, Busan 609-735, Korea

Reason for Wider Dynamic Range in Trypsin Detection Using EC Redox Cycling. When high concentrations of trypsin were used, the chronocoulometric charges were saturated. To investigate the saturation behavior, the charges obtained in a solution containing trypsin and Gly-Pro-Arg-AN were compared to those obtained in a solution containing AN (Fig. S7 and S8). The charges obtained using EC redox cycling after an incubation period of 2 h in a solution containing 1000 ng/mL trypsin and 0.1 mM Gly-Pro-Arg-AN (bars iii in Fig. S7) and after an incubation period of 30 min in a solution containing 2000 ng/mL trypsin and 0.1 mM Gly-Pro-Arg-AN (bar iv in Fig. S7) were much smaller than that obtained in a solution containing 0.1 mM AN (bar ii in Fig. S8). The result indicates that the peptide bond of 0.1 mM Gly-Pro-Arg-AN was not fully cleaved after 2 h (or 30 min).

However, the charges obtained using ECC redox cycling after an incubation period of 2 h in a solution containing 1000 ng/mL trypsin and 0.1 mM Gly-Pro-Arg-AN (bar iii in Fig. S8) and after an incubation period of 30 min in a solution containing 2000 ng/mL trypsin and 0.1 mM Gly-Pro-Arg-AN (bars iv in Fig. S8) were similar to that obtained in a solution containing 0.1 mM AN (bar ii in Fig. S8). It indicates that the saturation behavior occurred although the peptide bond of 0.1 mM Gly-Pro-Arg-AN was not fully cleaved after 2 h (or 30 min). Therefore, when EC redox cycling was employed, higher trypsin concentrations were detected because the saturation behavior was observed at much higher concentrations. As a result, the dynamic range of EC redox cycling was wider than that of ECC redox cycling.

References

S1 M. Choi, K. Jo, H. Yang, *Bull. Korean Chem. Soc.*, 2013, **34**, 421–425.

S2 J. Adjemian, A. Anne, G. Cauet, C. Demaille, *Langmuir*, 2010, **26**, 10347−10356.

S3 L. Hu, S. Han, S. Parveen, Y. Yuan, L. Zhang, G. Xu, *Biosen. Bioelectron.*, 2012, **32**, 297−299.

S4 M. Stoytcheva, R. Zlatev, S..Cosnier, M. Arredondo, *Electrochim. Acta*, 2012, **76**, 43−47.

S5 X. Gao, G. Tang, Y. Li, X. Su, *Anal. Chim. Acta*, 2012, **743**, 131−136.

S6 R. E. Ionescu, S. Cosnier, R. S. Marks, *Anal. Chem*., 2006, **78**, 6327−6331.

Table S1. Comparison of techniques used for trypsin detection

Ref.	Method	System	Preparation time (min, except for electrode pretreatment)	Incubation period (min)	Detection limit (ng/mL)	Dynamic range (ng/mL)
S2	Cyclic voltammetry	Ferrocene-labeled oligopeptide and flexible polyethylene glycol (PEG) monolayer at Au electrodes	>120	11	25	25–100000
S3	Fluorescence	BSA-stabilized Au nanoparticles	720, preparation of BSA-stabilized Au nanoparticles	120	2.0	10–100000
S4	Square wave voltammetry	1,2-Benzoquinone electrochemical reduction at gelatin- coated screen printed electrodes	30, modification of carbon electrode with gelatin	140	10	100–1000000 (10 min reaction)
S5	Fluorescence quenching	Mn:ZnSe d-dots/Arg_6	80	30	100	100–12000
S6	Amperometry	H_2O_2 electro-oxidation at gelatin- and pyrrole-alkylammonium coated Pt electrodes	215	10	1	1–300
The present study	Chronocoulometry	ECC redox cycling at bare ITO electrodes	0	120	1	1–1000
				30	100	100–2000

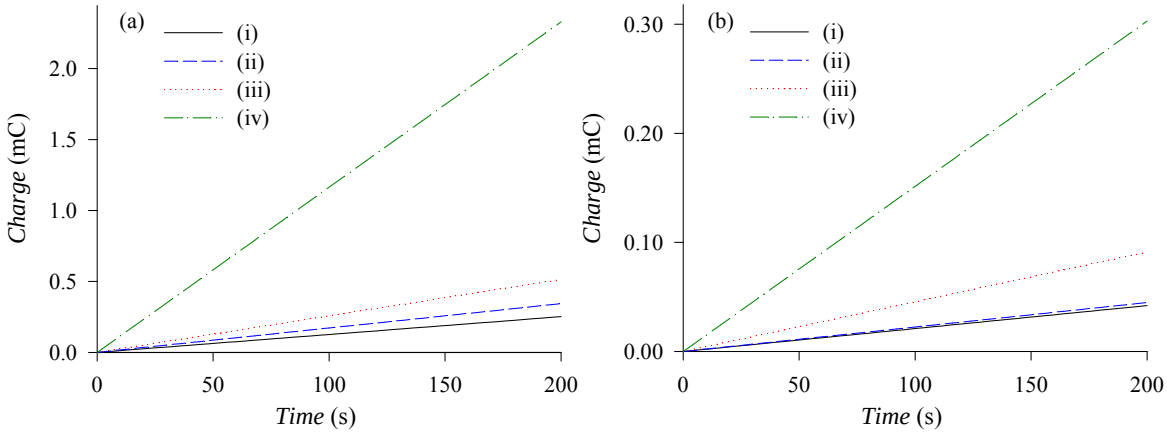

Fig. S1 Chronocoulograms obtained at 0.05 V in Tris buffer (50 mM, pH 8.0) containing 5.0 mM $CaCl_2$, 1.0 mM $Ru(NH_3)_6^{3+}$, 2.0 mM TCEP, and 0.1 mM a) P or b) Gly-Pro-Arg-P. P is (i) 4-aminophenol, (ii) 4-amino-2-chlorophenol (iii) 4-amino-2,6-dichlorophenol, or (iv) 4-amino-1-naphthol (AN).

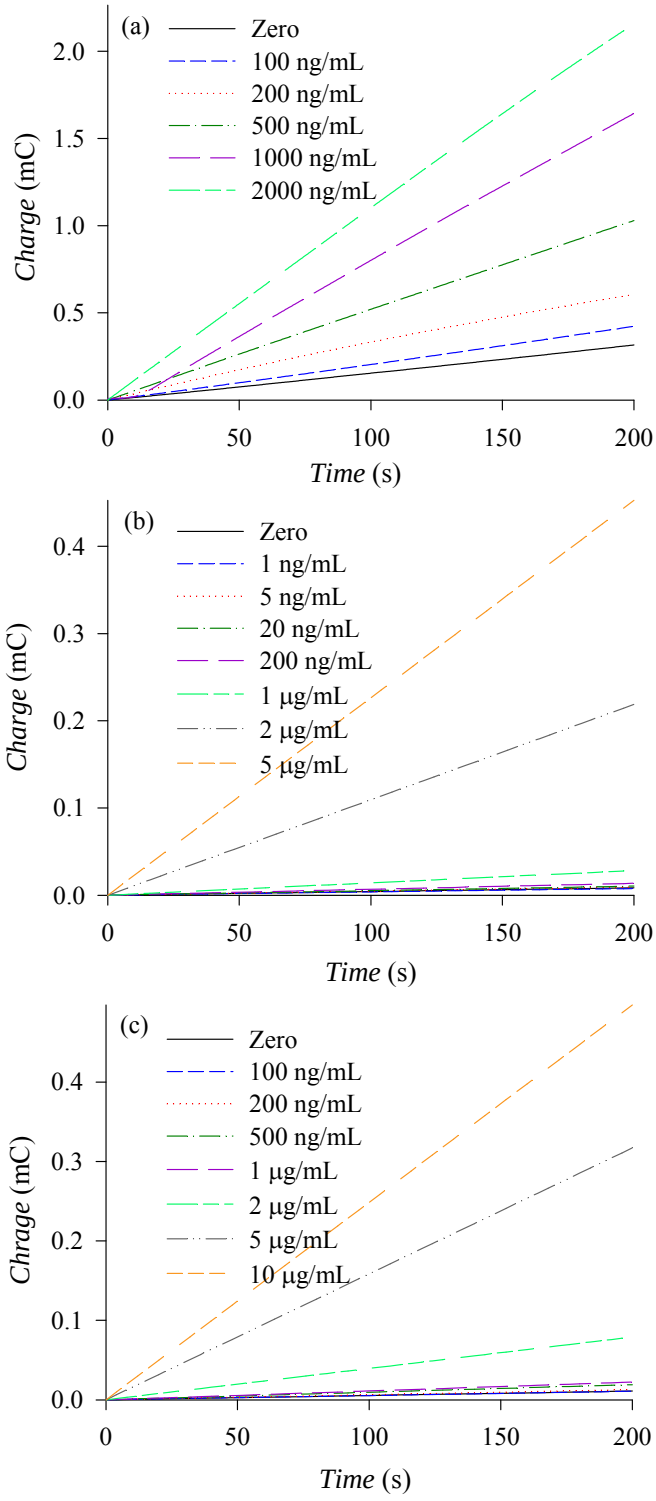

Fig. S2 (a) Chronocoulograms for trypsin detection obtained using ECC redox cycling with an incubation period of 30 min in tris buffer (pH 8.0) at 37 °C. The chronocoulograms were obtained at 0.05 V in tris buffer containing 5.0 mM $CaCl_2$, 1.0 mM $Ru(NH_3)_6^{3+}$, 2.0 mM TCEP, and 0.1 mM Gly-Pro-Arg-AN. Chronocoulograms for trypsin detection obtained using EC redox cycling with an incubation period of (b) 2 h or (c) 30 min in tris buffer at 37 °C. The chronocoulograms were obtained at 0.10 V in tris buffer containing 5.0 mM $CaCl_2$, 2.0 mM TCEP, and 0.1 mM Gly-Pro-Arg-AN.

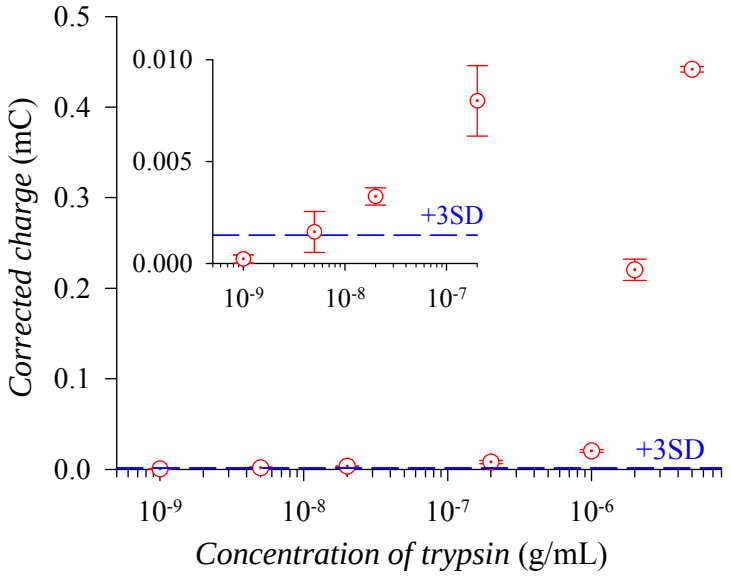

Fig. S3 Calibration plot for the chronocoulograms in Fig. S2b obtained using EC redox cycling with an incubation period of 2 h in tris buffer (pH 8.0) at 37 °C.

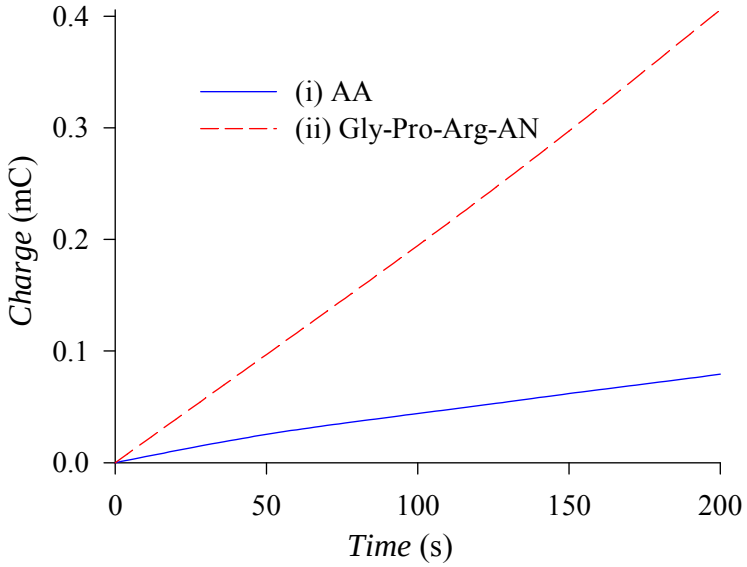

Fig. S4 Chronocoulograms obtained at 0.05 V in PBS buffer containing 2.0 mM TCEP, 1.0 mM $Ru(NH_3)_6^{3+}$, and (i) 0.1 mM AA or (ii) 0.1 mM Gly-Pro-Arg-AN after incubating for 30 min at 37 °C.

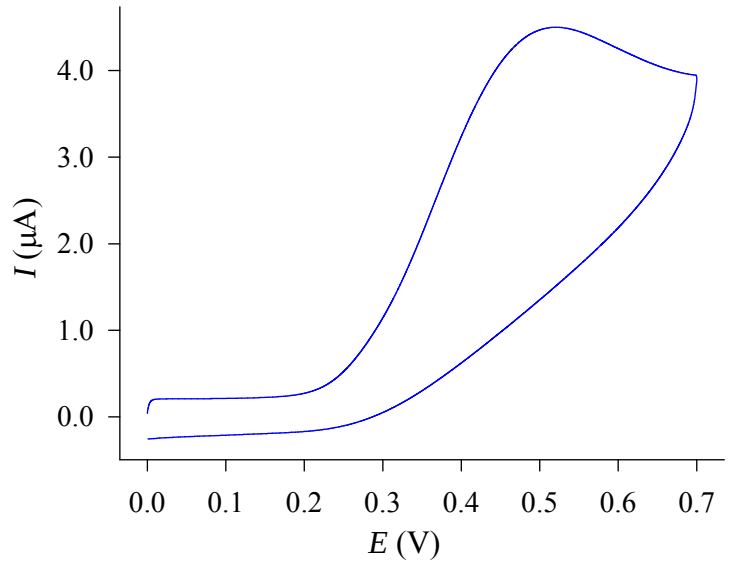

Fig. S5 Cyclic voltammogram of 0.1 mM UA obtained (at a scan rate of 100 mV/s) in PBS buffer (pH 7.4) at a bare ITO electrode.

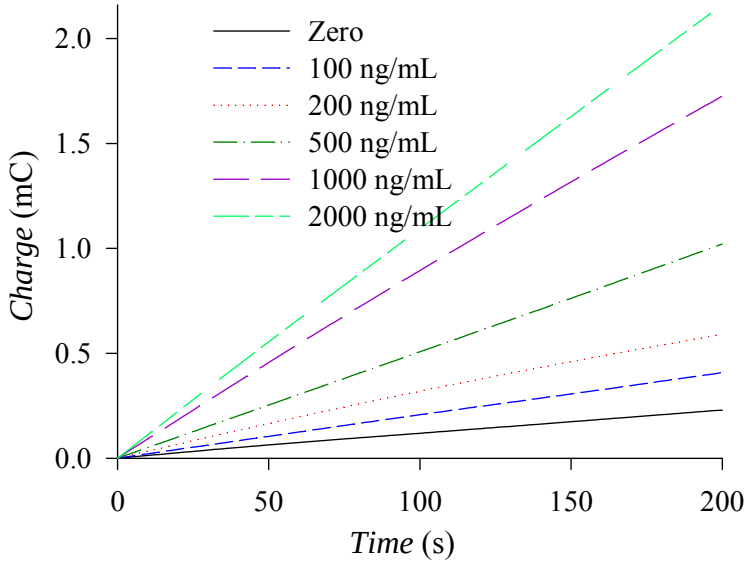

Fig. S6 Chronocoulograms for trypsin detection obtained using ECC redox cycling with an incubation period of 30 min at 37 °C in PBS buffer (pH 7.4). The chronocoulograms were obtained at 0.05 V in PBS buffer containing 5% human serum albumin, 0.1 mM AA, 0.1 mM UA, 1.0 mM $Ru(NH_3)_6^{3+}$, 2.0 mM TCEP, and 0.1 mM Gly-Pro-Arg-AN.

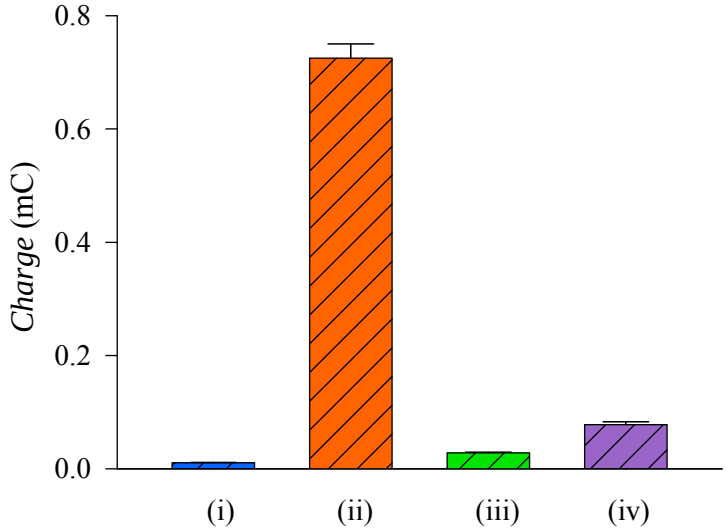

Fig. S7 Charge values at 100 s in chronocoulograms obtained using EC redox cycling in tris buffer (pH 8.0) containing 5 mM $CaCl_2$, 2.0 mM TCEP, and (i) 0.1 mM Gly-Pro-Arg-AN, (ii) 0.1 mM AN, (iii) 0.1 mM Gly-Pro-Arg-AN + 1000 ng/mL trypsin after incubating for 2 h at 37 °C, or (iv) 0.1 mM Gly-Pro-Arg-AN + 2000 ng/mL trypsin reaction for 30 min at 37 °C. All our experiments were carried out with 3 different electrodes for the same sample. The error bars represent the standard deviation of three measurements.

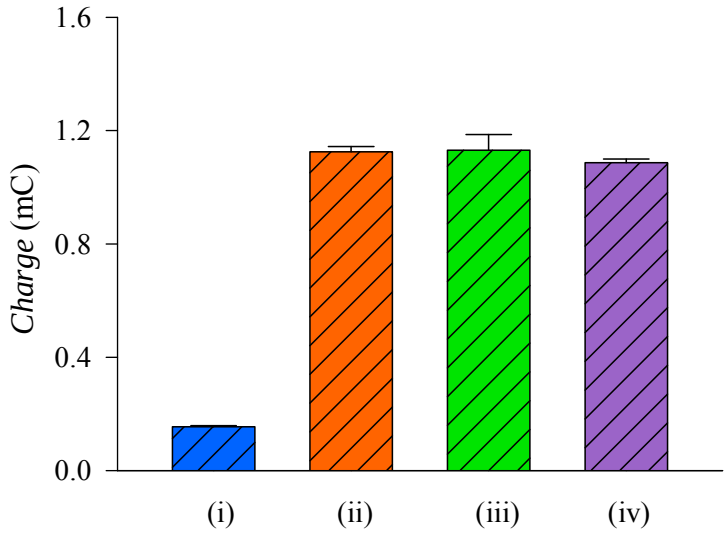

Fig. S8 Charge values at 100 s in chronocoulograms obtained using ECC redox cycling in tris buffer (pH 8.0) containing 5 mM $CaCl_2$, 1.0 mM $Ru(NH_3)_6^{3+}$, 2.0 mM TCEP, and (i) 0.1 mM Gly-Pro-Arg-AN, (ii) 0.1 mM AN, (iii) 0.1 mM Gly-Pro-Arg-AN + 1000 ng/mL trypsin after incubating for 2 h at 37 °C, or (iv) 0.1 mM Gly-Pro-Arg-AN + 2000 ng/mL trypsin reaction for 30 min at 37 °C. All our experiments were carried out with 3 different electrodes for the same sample. The error bars represent the standard deviation of three measurements.