Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Redox cycling-amplified enzymatic Ag deposition and its application in the highly sensitive detection of creatine kinase-MB

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Chemicals and Materials. Avidin, biotinylated polyclonal goat antimouse IgG, mouse IgG, and ALP-conjugated polyclonal goat antimouse IgG were obtained from Sigma-Aldrich. Mouse monoclonal anti-CK-MB antibody (10-1363), anti-CK-MB antibody (10-1364), CK-MB protein (30-1082), troponin I protein (30R-AT034), and troponin I-free human serum (90R-106) were purchased from Fitzerald, Inc. (Acton, MA, U.S.A.). CK-MB concentration (0.6 ng/mL) in troponin I-free serum was measured with UniCell DxI 800 Immunoassay analyzer (Beckman Coulter). The ALP labeling kit-NH_2 (LK12) was purchased from Dojindo Laboratories (Tabaru, Kumamoto, Japan). ALP-conjugated anti-CK-MB antibody was prepared using anti-CK-MB antibody (10-1363) and the ALP labeling kit according to the manufacturer's procedure. EZ-link sulfo-NHS-LC-LC-biotin was obtained from Thermo Fisher Scientific Inc. (Meridian, Rockford, USA). 4-Aminophenyl phosphate (APP) monosodium salt hydrate was obtained from Biosynth (Staad, Switzerland). 4-Aminophenol (AP), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), $AgNO_3$, and KNO_3 were obtained from Sigma-Aldrich. All reagents for buffer solutions were received from Sigma-Aldrich.

The phosphate-buffered saline (PBS buffer, pH 7.4) contained 10 mM phosphate, 0.138 M NaCl, and 2.7 mM KCl. The PBSB buffer contained all of the ingredients of the PBS buffer plus 1% (w/v) BSA. The rinsing buffer (pH 7.6) contained 50 mM tris(hydroxymethyl)aminomethane (Tris), 40 mM HCl, 0.05% (w/v) BSA, 0.05 % Tween 20, and 0.5 M NaCl. The Tris-HNO_3 buffer (pH 9.0) for enzymatic reaction contained 50 mM Tris and 10 mM $Mg(NO_3)_2$, and the pH was adjusted by adding concentrated HNO_3. All buffer and other aqueous solutions were

prepared using double distilled water. ITO electrodes were obtained from Samsung Corning (Daegu, Korea).

Preparation of Biotinylated Anti-CK-MB Antibody. Anti-CK-MB antibody (10-1364) was biotinylated by EZ-link sulfo-NHS-LC-LC-biotin. 1.35 μL of sulfo-NHS-LC-LC-biotin (10 mM in water) solution was added to 1 mL of anti-CK-MB antibody (100 μg/mL in PBS) solution and incubated for 30 min at 25 ^{o}C. The mixture solution was then filtered by centrifugation at 12,000 rpm for 20 min. The filtrate was dissolved and collected with 1 mL of PBSB.

Preparation of CK-MB-Spiked Serum. At first a small aliquot of an original solution of CK-MB protein (11.8 mg/mL) was diluted with troponin I-free human serum to prepare a stock solution of CK-MB with a concentration of 100 μg/mL. Then serial dilutions of the stock solution were carried out with the human serum to prepare the samples for various concentrations.

Preparation of Immunosensing Layers and the Immunosensing Procedure. ITO electrodes (1 cm × 2 cm each) were pretreated in a mixture containing H_2O, H_2O_2 (30%) and NH_4OH (30%) in a ratio of 5:1:1 at 70 °C for 1 h. The electrodes were then washed with copious amounts of water and dried under a stream of nitrogen gas.

To obtain avidin-modified ITO electrodes, 70 μL of a carbonate buffer solution (pH 9.6) or a PBS buffer solution (pH 7.4) containing 10 μg/mL avidin was dropped onto the pretreated ITO electrodes, the dropped state was maintained for 2 h at 20 °C, and the electrodes were washed twice with rinsing buffer. Subsequently, 70 μL of a PBSB buffer solution was dropped onto the avidin-modified ITO electrodes, the dropped state was maintained for 30 min at 4 °C, and the electrodes were washed twice with rinsing buffer. To immobilize biotinylated IgG on avidin, 70 μL of a PBSB buffer solution containing 10 μg/mL biotinylated anti-CK-MB antibody (or a PBSB buffer solution containing 10 μg/mL biotinylated antimouse IgG) was dropped onto the avidin- and BSA-modified ITO electrodes, the dropped state was maintained for 30 min at 4 °C, and the electrodes were washed twice with rinsing buffer. The resulting electrodes were stored at 4 °C before their use. For the binding of target protein to the immunosensing electrodes, 70 μL of PBSB solutions (or human serum) containing different concentrations of CK-MB protein (or PBSB buffer solutions containing different concentrations of mouse IgG) were dropped onto the

immunosensing electrodes, the dropped state was maintained for 30 min at 4 °C, and the electrodes were washed twice with rinsing buffer. Afterward, 70 μL of a PBSB buffer solution containing 10 μg/mL ALP-conjugated anti-CK-MB antibody (or a PBSB buffer solution containing 10 μg/mL ALP-conjugated antimouse IgG) were dropped on the target-treated electrodes, and the dropped state was maintained for 30 min at 4 °C, followed by washing twice with rinsing buffer and water.

Ag Deposition and Electrochemical Measurements. Bare or avidin- and BSA-modified ITO electrodes were incubated with 70 μL of Tris-HNO_3 buffer (pH 9.0) containing 1.0 mM $AgNO_3$, 0.1 M AP or 1.0 mM APP, and 1.0 mM NADH for 10 min at 25 °C. The electrodes were then washed with Tris-HNO_3 buffer and water. The deposited silver was then measured by anodic stripping analysis using cyclic voltammetry or chronocoulometry. In the case of electrochemical immunosensors, after forming the sandwich-type immunocomplex, the sensing electrodes were incubated with 70 μL of Tris-HNO_3 buffer (pH 9.0) containing 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH for 10 min at 30 °C. The electrodes were then washed with Tris-HNO_3 buffer and water. The deposited Ag was then measured by anodic stripping analysis using cyclic voltammetry or chronocoulometry in a three electrode system. The Ag-deposited ITO electrodes were used as working electrodes. A Pt wire and an Ag/AgCl electrode were used as a counter and a reference electrode, respectively. Teflon electrochemical cells were assembled with the resulting sensing electrodes and 1.0 mL of an aqueous solution containing 0.1 M HNO_3 and 0.6 M KNO_3 was injected into the cell and the electrochemical measurement was then carried out using a CHI 708C (CH Instruments, Austin, TX, U.S.A.). The exposed area of the sensing electrodes was 0.28 cm^2.

Scanning Electron Microscope (SEM) Images. The SEM images were obtained at avidin- and BSA-modified ITO electrodes using a VEGA3 TESCAN microscope at 20 kV.

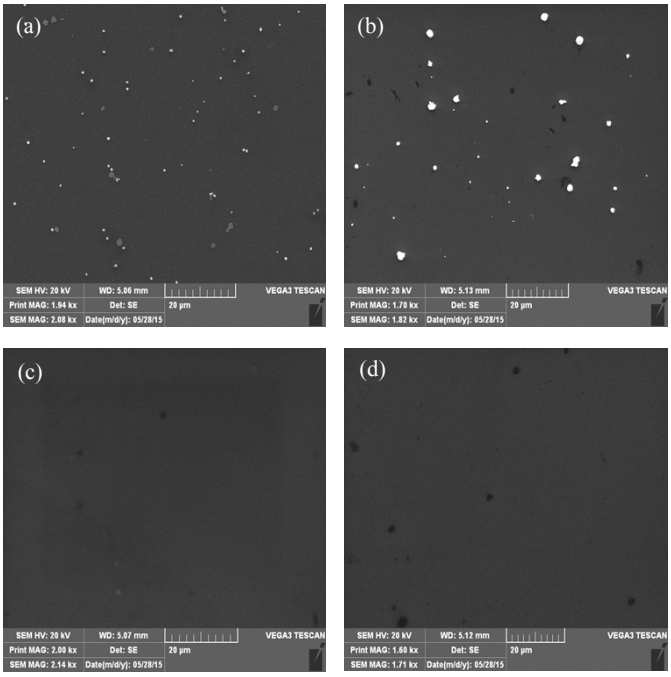

Figure S1. SEM images obtained at avidin- and BSA-modified ITO electrodes after incubation for 10 min at 25 °C in Tris-HNO_3 buffer (pH 9.0) containing (a) 1.0 mM $AgNO_3$ and 0.1 mM AP; (b) 1.0 mM $AgNO_3$, 0.1 mM AP, and 1.0 mM NADH; (c) 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH, and (d) without incubation in Tris-HNO_3 buffer.

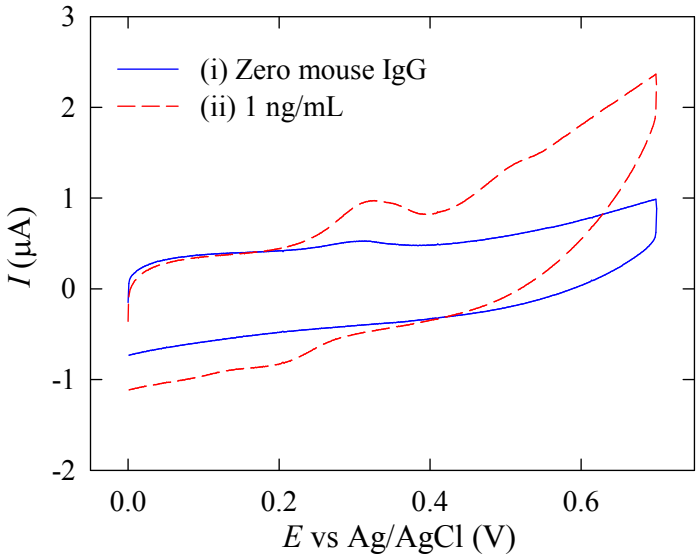

Figure S2. Cyclic voltammograms obtained (at a scan rate of 50 mV/s) at the sensing electrodes in an aqueous solution containing 0.1 M HNO_3 and 0.6 M KNO_3, after the detection of mouse IgG at concentrations of zero and 1 ng/mL and incubation for 10 min at 25 °C in Tris-HNO_3 buffer (pH 9.0) containing 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH.

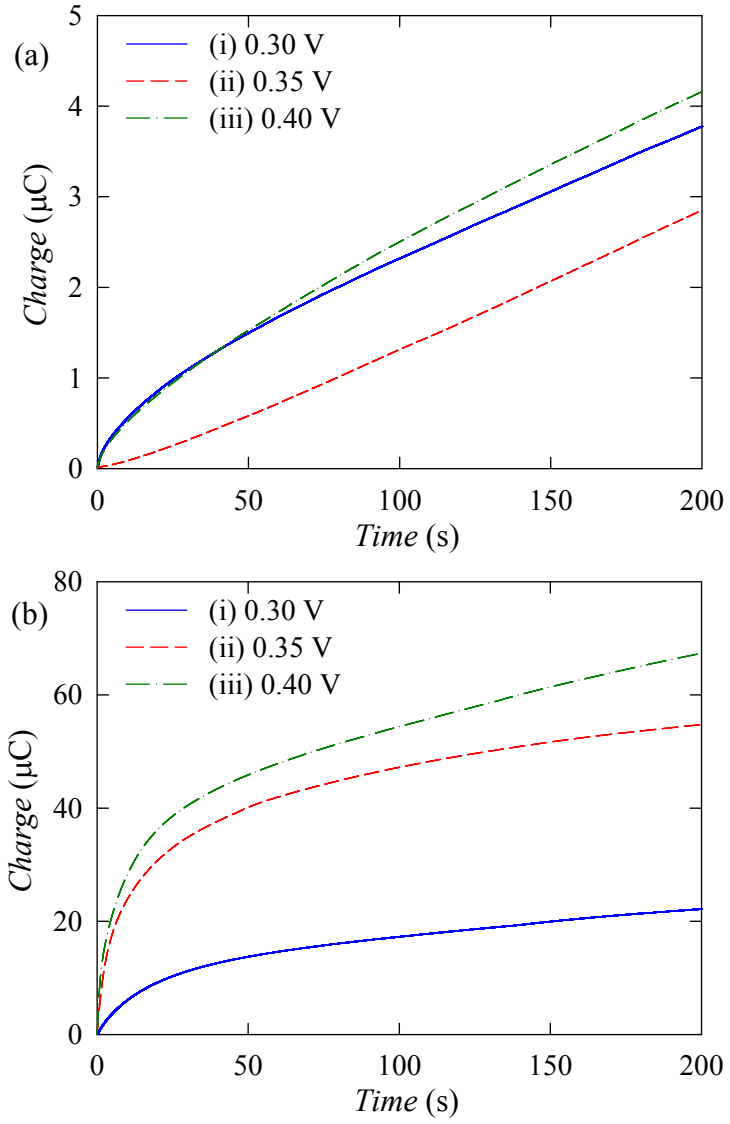

Figure S3. Chronocoulograms obtained at different potentials at bare ITO electrodes in an aqueous solution containing 0.1 M HNO_3 and 0.6 M KNO_3, after incubation for 10 min at 25 °C in Tris-HNO_3 buffer (pH 9.0) containing (a) 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH and (b) 1.0 mM $AgNO_3$, 0.1 mM AP, and 1.0 mM NADH.

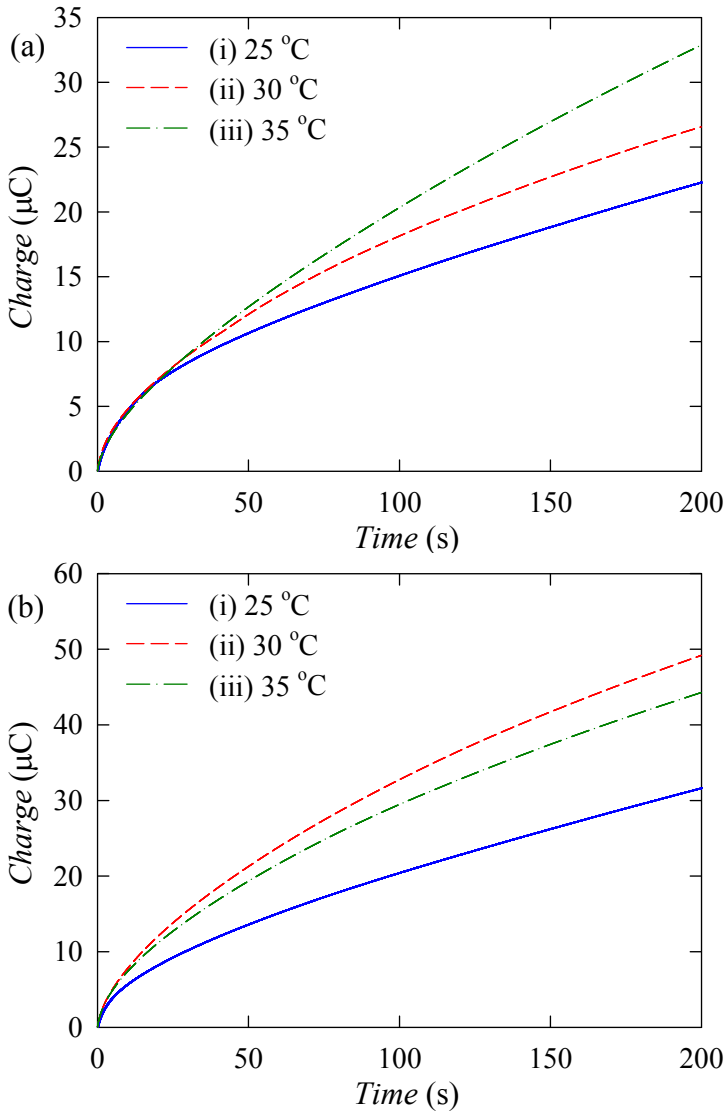

Figure S4. Chronocoulograms obtained at 0.35 V at the sensing electrodes in an aqueous solution containing 0.1 M HNO_3 and 0.6 M KNO_3, after the detection of CK-MB at concentrations of (a) zero and (b) 1 ng/mL and incubation for 10 min at different temperatures in Tris-HNO_3 buffer (pH 9.0) containing 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH.

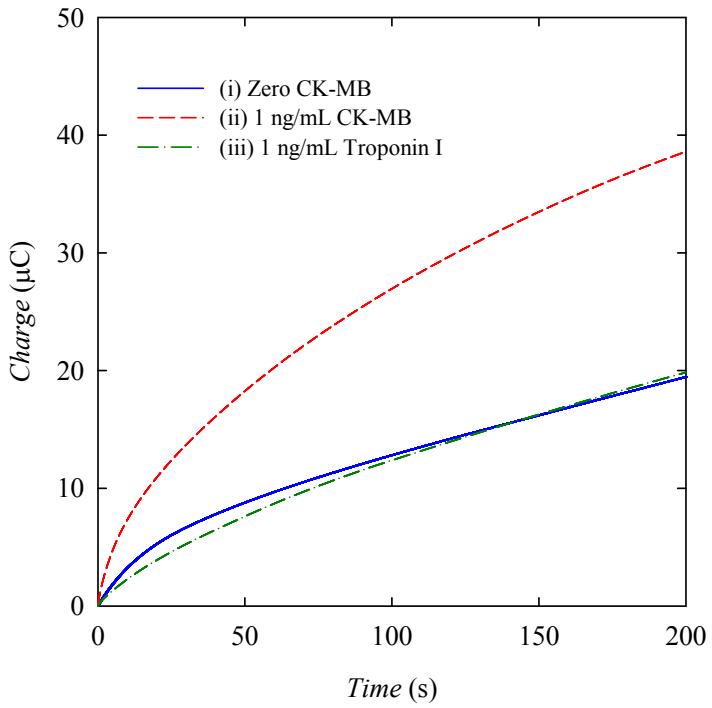

Figure S5. Chronocoulograms obtained at 0.35 V at the sensing electrodes in an aqueous solution containing 0.1 M HNO_3 and 0.6 M KNO_3, after the detection of CK-MB at concentrations of (i) zero and (ii) 1 ng/mL, and troponin I at concentration of (iii) 1 ng/mL and incubation for 10 min at 30 °C in Tris-HNO_3 buffer (pH 9.0) containing 1.0 mM $AgNO_3$, 1.0 mM APP, and 1.0 mM NADH.