Electronic Supplementary Material (ESI) for Chemical Communications

Amplified electrochemiluminescent immunosensing using apoferritin-templated poly(ethylenimine) nanoparticles as co-reactant

Ni Liao, Ying Zhuo*, Yaqin Chai, Yun Xiang, Yaling Cao, Ruo Yuan*, Jing Han

Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China.

(Supplementary Material)

EXPERIMENTAL SECTION

Reagents and Materials. Apoferritin, $Ru(bpy)_3Cl_2 \cdot 6H_2O$, Nafion (5 wt%), Gold chloride ($HAuCl_4$), sodium citrate and BSA (96–99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). N-hydroxy succinimide (NHS) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimidehydrochloride (EDC) were received from Shanghai Medpep Co. Ltd. (Shanghai, China). Poly (ethylenimine) (PEI, 50%) was purchased from Fluka (Switzerland). HCG and goat-anti-HCG were obtained from Biocell Company (Zhengzhou, China). PTCA was gotten from Lian Gang Dyestuff Chemical Industry Co. Ltd. (Liaoning, China). Nafion was diluted to 1 wt% with ethanol solution. Au nanoparticles with mean size of 16 nm were produced by reducing gold chloride tetrahydrate with citric acid at 100 ˚C for half an hour. Phosphate buffer solutions (PBS) with various pH values and concentrations were prepared by mixing standard stock solutions of 0.1 M K_2HPO_4, 0.1 M NaH_2PO_4, 0.1 M KCl and adjusting the pH with 0.1 M H_3PO_4 or NaOH, then diluting with doubly distilled water. All chemicals were analytical grade and used without further purification. All solutions were prepared with doubly distilled water and stored in the refrigerator (4 ºC).

Apparatus. The ECL emission was monitored by a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China). The voltage of the photomultiplier tube (PMT) was set at 800V and scan rate was 100 mVs^{-1} in ECL detection. A conventional three-electrode system was used with Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as auxiliary electrode and a modified glass carbon electrode (GCE, Φ= 4 mm) as the working electrode in the experiment. Cyclic voltammetric (CV) measurements were carried out with a CHI 610A electrochemistry workstation (Shanghai CH Instruments, China). A three-electrode electrochemical cell was composed of a modified glass carbon electrode (GCE, Φ= 4 mm) as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. The morphologies of different nanocomposites were characterized by scanning electronmicroscopy (SEM, S-4800, Hitachi, Tokyo, Japan) at an acceleration voltage of 20 kV.

Preparation of PEI-templated apoferritin nanoparticles (Apo-PEI): Firstly, 50 μL apoferritin solution (48 mg/mL) was dispersed in 1 mL 0.1 M PBS (pH 7.4) and gradually adjusted to pH 2.0 with HCl solution (0.1 M) under continuously stirring for 20 min to make the apoferritin disassociate into its subunits. Then 50 μL 0.1 M PEI solution was added into apoferritin solution (pH 2.0) drop by drop with stirring to allow the PEI to diffuse around the subunits of the apoferritin. Subsequently, the pH was adjusted to 8.5 with 0.1 M NaOH added dropwise. The mixture was under continuously stirring for 2 h to allow the PEI to form a PEI core inside the apoferritin. The obtained

target was centrifugally washed extensively with doubly distilled water for three times to remove the surplus PEI. And then it was dispersed in 1 mL 0.1 M PBS (pH 7.4).

Preparation of the biotin-modified apoferritin-templated PEI nanoparticles (bio-Apo-PEI): Briefly, EDC and NHS were used as coupling agents which catalyze the formation of amide bond between the carboxyl of biotin and the amino of apoferritin. 40 μL 0.5 mg/mL biotin was dissolved in 1 mL doubly distilled water under continual stirring. And then 0.5 mL EDC and NHS (4:1) mixture solution was added to the above solution and stirred for overnight to activate the carboxyl of biotin. Subsequently, 500 μL Apo-PEI was added and the reaction was allowed to stir for overnight at room temperature to produce bio-Apo-PEI. The resulting conjugates were washed for several times to remove free biotin, redispersed in 1 mL 0.1 M PBS (pH 7.4) and stored at 4 ˚C.

Preparation of bio-apo-PEI/SA/Ab2/CNTs (Ab2 bioconjugates): To obtain more active -COOH groups of CNTs, 2.0 mg CNTs and 0.5 mg PTCA were dissolved in 5.0 mL doubly distilled water by continuous ultrasonication and then stirred at room temperature for overnight to decorate the surface of CNTs with PTCA via π-π stacking. And then, 2.5 mL EDC and NHS (4:1) mixture solution was added to the above solution and the resulting mixture was kept vigorous agitation for overnight at room temperature. Next, 100 μL 100 mIU/mL anti-HCG solution and 15 μL 1 mg/mL streptavidin (SA) were added simultaneously and stirred at 4 ˚C for 8 h, followed by centrifuged at 10000 rpm for 15 min and dispersed in 2 mL 0.1 M PBS (pH 7.4). Finally, 1 mL bio-Apo-PEI was added to the above solution and stirred continuously

for 4 h to obtain bio-apo-PEI/SA/Ab2/CNTs (Ab2 bioconjugates).

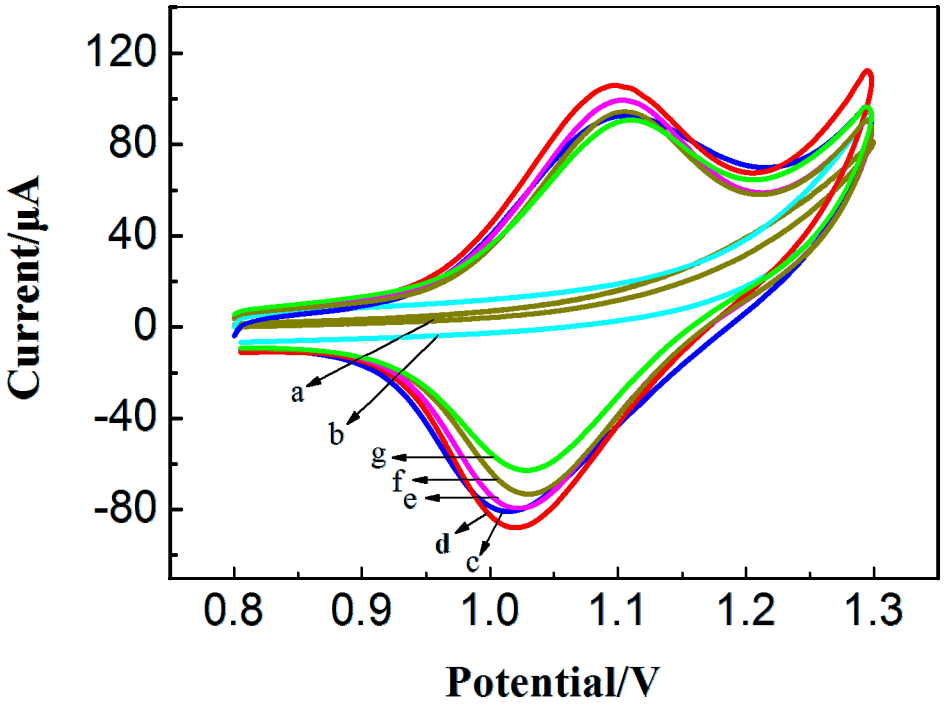
Fabrication of the electrochemiluminescence immunosensor: To obtain a mirror like surface, the glass carbon electrode (GCE, Φ= 4 mm) was polished with 0.3 and 0.05 μm alumina respectively. Then it was cleaned chemically by immersing it into freshly prepared 2:1 mixture of H_2SO_4 and H_2O_2 for 30 s. After a short rinse and followed by successive sonication in doubly distilled water and ethanol, the GCE was allowed to dry at room temperature.

Subsequently, 1 mg GR and 1 mg CNTs was dissolved in 1mL 1 % Nf solution by ultrasonic dispersion. Then 5 μL GR-CNTs-Nf composite was coated onto a pretreated GCE surface and dried in the air. Then the GR-CNTs-Nf modified GCE was soaked in 10 mM $Ru(bpy)_3^{2+}$ for 30 min and then immersed in nano-Au solution for 2 h at 25 ˚C. Subsequently, 15 μL anti-HCG (abbreviated as Ab1) was attached by incubating at 4 ˚C for 12 h. The immunosensor was then washed with doubly distilled water to remove the physical adsorbed Ab1 and then incubated for 1 h with 15 μL of 1 % BSA, followed by washing with doubly distilled water. Ultimately, the obtained immunosensor was stored at 4 ˚C when not in use.

Experimental measurements: The measurement was based on a sandwich immunoassay method. Before measurement, the immunosensor was incubated in HCG solution with various concentrations at 37 ˚C for 40 minutes. Next, the modified electrode was incubated in Ab2 bioconjugates for 40 minutes at 37 ˚C. 5 μL 0.1 M PBS (pH 2.0) was added to the immunosensor surface at room temperature for 2 h to release the PEI. Finally, the resultant immunosensor was investigated with a MPI-A

electrochemiluminescence analyzer in 3 mL 0.1 M PBS (pH 7.4) at room temperature.

Electrochemical behaviors of the electrochemiluminescence immunosensor: To gain a better understanding of the fabrication process of the electrochemiluminescence immunosensor, we also performed the cyclic voltammograms (CVs) experiments at different modified electrodes in 0.1M PBS solution (pH 7.4). No obvious redox peaks were observed at bare GCE (FigureS1, curve a) and GR-CNTs-Nf coated GCE (FigureS1, curve b) due to the lack of redox active molecule. Then $Ru(bpy)_3^{2+}$ was coated on the modified electrode surface, the resulting electrode exhibited a stable and well-defined redox peaks between 1.02 and 1.15 V (FigureS1, curve c), indicating the good electrochemical redox activity of $Ru(bpy)_3^{2+}$. The redox peak currents increased significantly when nano-Au was assembled by electronic adsorption, since the excellent conductivity of nano-Au could promote the electron transfer (FigureS1, curve d). When the electrode was modified with anti-HCG, the peak currents were decreased (FigureS1, curve e). Subsequently, the redox peak currents further decreased after the modified electrode was blocked with 0.25% BSA solution (FigureS1, curve f). Finally, when HCG antigen molecules were coupled covalently onto the antibody molecules, the redox peak currents decreased apparently (FigureS1, curve g). The reason for this was that the BSA and HCG protein layers on the electrode would retards the electron transfer.



FigureS1. Cyclic voltammograms performed in 0.1 M PBS (pH 7.4, 3 mL) (a) bare GCE; (b) GR-CNTs-Nf; (c) $Ru(bpy)_3^{2+}$/GR-CNTs-Nf; (d) nano-Au/$Ru(bpy)_3^{2+}$/GR-CNTs-Nf; (e) anti-HCG/nano-Au/$Ru(bpy)_3^{2+}$/GR-CNTs-Nf; (f) BSA/anti-HCG/nano-Au/$Ru(bpy)_3^{2+}$/GR-CNTs-Nf modified GCE; (g) the proposed immunosensor after reacted with 100 mIU/mL HCG. The scan was from 0.8 to 1.3V with the rate of 100 mV/s and all potentials are given versus SCE.

Application: The feasibility of the immunoassay for clinical applications was investigated by analyzing several real samples in comparison with the ELISA method. Five clinical serum samples were from the Ninth People's Hospital of Chongqing, China, were used. Serum samples were diluted to different concentrations with a PBS solution of pH 7.0. The results are shown in Table 1 and the recovery was in the range of 96.0%-104.0%, which confirmed that the proposed immunosensor could be reasonably applied in the clinical determination of HCG.

Table1

Experimental results of different methods obtained in serum samples.

Sample number	Added (mIU/mL)	Found (mIU/mL)[a]	Recovery (%)
1	0.01	0.0104 ± 0.006	104.0
2	0.10	0.096 ± 0.02	96.0
3	1.00	0.98 ± 0.03	98.0
4	10.00	10.24 ± 0.01	102.4
5	20.00	20.36 ± 0.14	101.8

[a] Mean value ± SD of three measurements.