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Amperometric immunosensor for simultaneous detection of three analytes in one interface using dual functionalized graphene sheets integrated with redox-probes as tracer matrixes

Qiang Zhu [a,b], Yaqin Chai [a,*], Ruo Yuan [a,*], Ying Zhuo [a], Jing Han [a], Ya Li [a], Ni Liao [a]

[a] Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, College of Chemistry and Chemical Engineering, The Key Laboratory of Eco-environments in Three Gorges Reservoir Region, Southwest University, Chongqing 400715, China
[b] College of Chemistry, Chongqing Normal University, Chongqing 400047, China

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ABSTRACT

A novel immunoassay protocol for simultaneous electrochemical determination of alpha-fetoprotein (AFP), carcinoembryonic (CEA) and streptococcus suis serotype 2 (SS2) was designed. As standard sandwich-type immunoassay format, three primary antibodies (Ab1), anti-CEA and anti-AFP and anti-SS2, were immobilized via protein A (PA) adsorbed by Nafion modified electrodes, and functionalized graphene sheets (GS), containing abundant gold nanoparticles (AuNPs) and carboxyl group, were used to immobilize secondary antibody@redox-probe so as to act as tracer. Concentration of each analyte was quantitatively related to the reduction peak current of corresponding redox-probe in differential pulse voltammetry (DPV) scan. The resulting immunosensor exhibited high selectivity and sensitivity in simultaneous determination of three analytes. The linear range was from 0.016 to 50 ng/mL for AFP with a detection limit of 5.4 pg/mL, 0.010 to 50 ng/mL for CEA with a detection limit of 2.8 pg/mL and 0.012 to 50 ng/mL for SS2 with a detection limit of 4.2 pg/mL ($S/N=3$).

1. Introduction

Simultaneous multianalytes immunoassays (SMIAs), compared to conventional single-analyte immunoassay, were more attractive analytical methods, which offered significant advantages of requiring less sample and lower cost per test, also increasing throughput efficiency (Wu et al., 2007). As to the SMIAs based on electrochemical techniques, great efforts were paid to develop various spatially-separated arrays or chip (Meyerhoff et al., 1995; Song et al., 2004; Petrou et al., 2002; Malhotra et al., 2012; Kojima et al., 2003), for example, Wilson et al. reported several array-based SMIAs immunosensors using quantum-dot (QD) as signal tags (Wilson, 2005; Wilson and Nie, 2006a, 2006b); Tang et al. (2010) developed a potential immunosensor array for simultaneous determination of 5-type hepatitis virus antigens.

Meanwhile, some researchers focused on developing sensors for simultaneous multianalytes on identical interface, for example, Wang et al. developed QD-tagged immunosensor and aptasensor by detecting released metal ions to perform multianalytes assay (Hansen et al., 2006; Liu et al., 2006). Recently, some researchers paid attention to redox-probe tagged sensors for simultaneous detection of two antigens or aptamers (Song et al., 2010; Xiang et al., 2011; Tang et al., 2011; Bai et al., 2012; Han et al., 2012a). In our previous work, we made use of the three redox-probes, thionine (Thi), ferrocene (Fc) and 2, 2′-bipyridine-4, 4′-dicarboxylic acid ($Co(bpy)_3^{3+}$), as signal labels and fabricated an immunosensor for simultaneous determination of three liver cancer biomarkers (Li et al., 2012). Redox-probe tagged SMIAs method may avoid the complicated and time-consuming QD or metal tag synthesis process, and, on the other way, using distinguishable potential position of redox-probe to indicate corresponding analyte can simplify detection procedures. Even so, further study for determination of three or more analytes on identical surface was extremely rare.

Recently, using the carboxyl groups on graphene edges to prepare PTCA/GS nano-composite attracted a lot of attention (Yuan et al., 2011; Gan et al., 2012). And at the same time, some researches showed that the conjugated ring of PTCA can directly interact with GS through the π–π stacking and hydrophobic forces (Li et al., 2009; Hu et al., 2011), in this way could decorate GS with abundant carboxyl groups. Han et al. (2012b) utilized (L-ascorbic acid) AA to reduce graphene oxide (GO) and $HAuCl_4$ to prepare Au/GS nanohybrid that exhibited excellent conductivity, biocompatibility and dispersibility. Herein, we made use of the above methods and prepared a novel nano-composite described as PTCA/Au/GS, which contained abundant AuNPs and carboxyl group.

* Corresponding authors. Tel.: +86 23 68252277; fax: +86 23 68253172.
E-mail addresses: 21447388@qq.com (Q. Zhu), yqchai@swu.edu.cn (Y. Chai), yuanruo@swu.edu.cn (R. Yuan).

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In this work, we attempt to build a simple and sensitive immunosensor for simultaneous determination of three antigens on identical interface. Based on sandwich-type amperometric assay, three kinds of Ab1 were immobilized onto PA/Nafion modified GCE, and three kinds of Ab2, primarily crosslinked with three redox-probes, were integrated with PTCA/Au/GS nano-composites through AuNPs and carboxyl group and adopted as tracer. Compared with the previously reported approaches, our protocol presents several advantages. First, by using PA/Nafion as base layer, we can immobilize a great deal of Ab1 on the electrode surface, because each molecule of PA contains five homologous immunoglobulins-binding domains and each domain is able to bind protein molecule. Second, functionalized GS with AuNPs and carboxyl group can fully integrate redox-probe@Ab2 composites, thus greatly improved the signal intensity of tracer. To test the feasibility, we randomly selected three biomarkers of AFP, CEA and SS2 as model analytes. The resulting immunosensors exhibited low background current and excellent sensitivity as expected, also wide detection range and low detection limit. Various characters of the proposed immunosensor were studied in detailed.

2. Experiment

2.1. Reagents

Anti-AFP, AFP, anti-CEA, CEA were purchased from Biocell Co. (Zhengzhou, China), Streptococcus agalactiae (1.B.501) sc-73072 (Ab1), Streptococcus Group B (072) sc-58045 (Ab2, anti-SS2) and Streptococcus suis 2 (SS2) were achieved from Santa Cruz Biotechnology Inc. (USA). Graphene oxide (GO) was obtained from Nanjing Xianfeng Nano Co. (Nanjing, China). 3, 4, 9, 10-perylenete-tracarboxylic dianhydride (PTCDA) was from Lian Gang Dyestuff Chemical Industry Co. Ltd. (Liaoning, China). Nafion, Protein A, Gold chloride tetrahydrate ($HAuCl_4$), L-ascorbic acid (AA), Carboxyl ferrocene (Fc), 2, 2′-bipyridine-4,4′-dicarboxylic acid ($Co(bpy)_3^{3+}$), thionine (Thi) N-(3-dimethylamino-propyl)-N′-ethylcarbodiimidehydrochloride (EDC) and N-hydroxy succinimide (NHS) were obtained from Sigma Chemical Co. (St. Louis., MO., USA). Serum specimens were provided by Daping Hospital of Third Military Medical University (Chongqing, China). All of the chemicals used were of analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 M Ω cm).

2.2. Apparatus

Cyclic voltammetric (CV) and differential pulse voltammetry (DPV) measurements were carried out with a CHI 660C electrochemical workstation (Shanghai Chenhua Instrument, China), and the parameters of DPV were optimized (Range: −0.7 to 0.7 V, Incre: 0.004 V, Amplitude: 0.05 V, Pluse width: 0.05 s, Sampling width: 0.0167 s, Pluse period: 0.2 s). A three-electrode electrochemical system comprised a modified glass carbon electrode (GCE, Φ=4 mm) served as work electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscope (SEM, S-4800, Hitachi, Japan) and transmission electron microscopy (TEM, H600, HitachiInstrument, Japan) was employed to estimate the morphology of prepared nano-composite.

2.3. Preparation of Au/GS nanohybrid

Preparation of Au/GS nanohybrid was according to the literature (Han et al., 2012b) with slight modification. First, 5 mg graphene oxide was dissolved in 5 mL water by ultrasonic dissolving method, then 50 mg AA was added, and stirred at room temperature until the brown solution changed to pure black. Subsequently, 1 mL 1% (w/w) $HAuCl_4$ was dropped and stirred for another 12 h. Finally, after three cycles of centrifugation and washing, the sediment was dispersed in 5 mL of water for use. The morphology of Au/GS nanohybrid was characterized by SEM (Fig. 1B), and displayed that AuNPs were well-distributed in graphene sheets. For comparative study, the SEM morphology of graphene oxide is also provided in Fig. 1A.

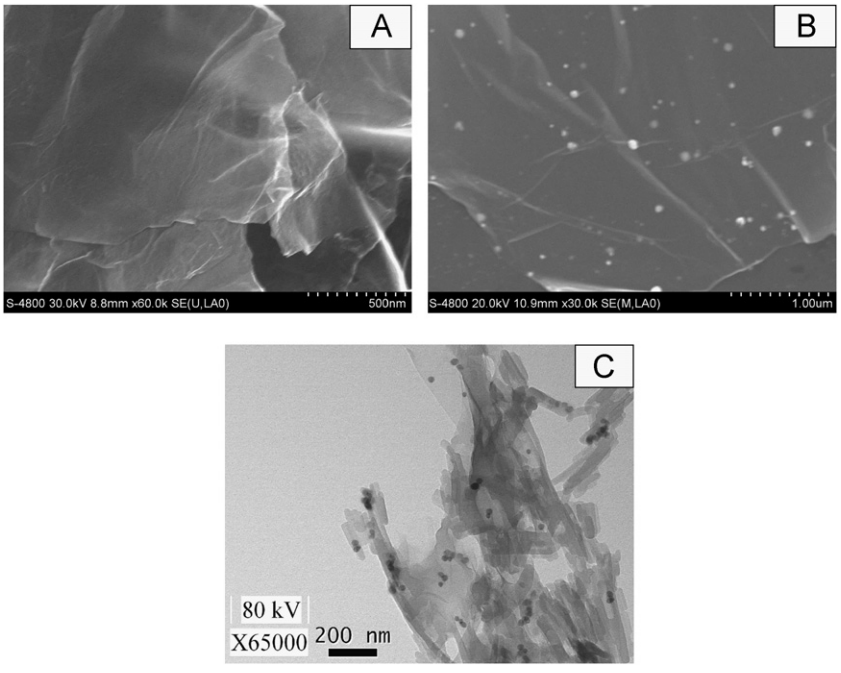

Fig. 1. SEM images of GO (A), Au/GS nanohybrid (B) and TEM image of PTCA/Au/GS nano-composites (C).