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Displacement-type amperometric immunosensing platform for sensitive determination of tumour markers

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ABSTRACT

The sensitive determination of carcino embryonie antigen (CEA)-a set of highly related glycoproteins involved in cell adhesion-is beneficial to the early diagnosis of colorectal cancer. In this study, a novel displacement-type amperometric immunosensing platform, based on the fact that glucose and Alizarin Red S (ARS) compete for phenylboronic acid bindng sites, was projected for sensitive detection of tumour marker (CEA, used as a model) on a PAMAM dendrimer-encapsulated nanogold (PAMAM-Au)-functionlized sensing interface. Firstly, $_4$⁻mercaptophenylboronic acid (S-PBA) was assembled onto the PAMAM-Au via the S-Au interaction, and then ARS was immobilized by S-PBA binding of cis-diol moieties, which was dropped on the surface of glassy carbon electrode as sensing platform to combine antibody. Meanwhile, amylase modified gold nanoparticles were employed as labels. Accompanying the sandwich immunoassay, the carried amylase could hydrolyze amylose into glucose, and the displacement-type format was triggered due to the stronger adhesion between glucose and PBA, which resulted in the change of electrochemical signals due to the decrease of ARS (as an electron mediator). Under optimal conditions, the SWV signals were related to the concentration of CEA, indicating a certain proportional relation in a range of 0.01～50 ng mL^{-1} with a detection limit (LOD) of 0.003 ng mL^{-1}. Intra- and inter-assay coefficients of variation were below 10%, respectively. In addition, the methodology showed good accordance with a commercialized enzyme-linked immunosorbent assay (ELISA) method.

1. Introduction

Cancers, known as malignant tumours, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (Yarden and Caldes, 2013; Lin et al., 2015; Yonezawa et al., 2015). Tens of thousands of people died every year from cancer, which has become the leading killer threating the human health around the world. Tumour marker found in the blood, urine, or body tissues is an important index reflecting the change course of the tumour. Therefore, sensitive and accurate determination of tumour markers is of great importance for screening, diagnosis and monitoring of cancers. Immunoassay, based on the specific interactions of antigen and antibody, is widely utilized for detecting of tumour markers on clinic due to its high sensitivity and selectivity. In recent years, many immunoassay techniques have been exploited, for example, enzyme-linked immunosorbent assay (ELISA), fluorescence, surface-enhanced raman scattering spectroscopy (SERS), electrochemiluminescence, and chemiluminescence, as well as electrochemical (Alonso et al., 2016; Patris et al., 2016; Barroso et al., 2016; Kamińska et al., 2015; Xiao et al., 2016). Although each of technology has certain advantages, electrochemical immunosensors still obtain more and more attention because of the inherent merits of electroanalytical method such as good portability, low cost, low power requirements, high sensitivity, and high compatibility with advanced micromachining technologies.

Sensitivity is one of the most key factors for characterizing the ability of electrochemical immunosensors. With the development of nanotechnology and noanoscience, nanomaterials have open up a door for improving the sensitivity of immunosensors and have obtained more and more application in the immunosensors. A wide range of nanomaterials, including noble metal nanoparticls, metallic oxide, polymer nanospheres, metal sulfide, composite materials and so on (Wang et al., 2015; Bui et al., 2015; Hu et al., 2015; Zhao et al., 2015), have been employed, effectively improving the performance of immunosensors. Among them, poly(amidoamine) dendrimers (PAMAM), a kind of ideal vector for passive targeting, has been extensively employed in clinical practice due to their low cytotoxicity and well biocompatibility (Wang et al., 2013; Sun et al., 2015; Li et al., 2015a, 2015b, 2015c, 2015d; Han et al., 2015). Meanwhile, PAMAM prove to be promising nanocarriers

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with controllable dimensions and good structure homogeneity, which could prevent the aggregation of nanoparticles. As a representative, PAMAM-Au has been not only applied in electrochemical sensing, but also used as nanocatalyst in biosensors. For example, Kavosi et al. prepared PAMAM-Au functionalized wired ethyleneamine-viologen as highly efficient interface in electrochemical immunosensor for detection of AFP (Kavosi et al., 2014). Shen et al. synthesized Fc-Fc/β-CD/PAMAM-Au nanocomplex as label and catalyst to develop immunosensor for procalcitonin detection (Shen et al., 2015).

In general, the nanomaterials with electroactivity were used as labels for constructing sandwich-type immunoassay to enhance sensitivity. Despite the relatively large number of immunosensors were reported to date (Ma et al., 2015; Lai et al., 2015; Fang et al., 2015), there still is a drawback that the activity of biomolecule decreased to some extent due to the modifications of nanomaterials (Nel et al., 2009). To reduce the influence as soon as possible, in this study, PAMAM-Au-PBA-ARS owning electroactivity was fixed at the surface of electrode to guide electrochemical signals. However, this method had larger background signals and lower sensitivity. Fortunately, displacement-type assay (Alizarin Red S-phenylboronic acid-glucose) opens up a door to overcome this problem, which plays an important role in enhancing sensitivity because the electrochemical signal is sensitive to the detachment of electron mediator (ARS). Phenylboronic acid (PBA) is a boronic acid containing a phenyl substituent and two hydroxyl groups attached to boron, which owns high affinity with cis-diol moieties. Based on this principle, phenylboronic acid is usually utilized in biological systems as receptors for carbohydrates, therefore, several of nanomaterials or platforms could be modified with PBA to recognize cell for cancer therapy (Cao et al., 2015; Matuszewska et al., 2015; Brooks and Sumerlin, 2016; Brooks et al., 2015). Moreover, phenylboronic acid has stronger bonding with sugar than other diol moieties. As a result, the displacement assay could be constructed by taking advantage of the competitive binding between glucose and another molecule (such as Alizarin Red S) for the phenylboronic acid-binding site. For example, Chen et al. reported a glucose sensing based on glucose compete with ARS-PBA complex (Chen et al., 2011). Bi et al. proposed to establish a virtual lectin array based on the displacement of each monosaccharide with molecules of a dye called Alizarin Red S (ARS) (Bi et al., 2015). To the best of our knowledge, there is no report focusing on the Alizarin Red S-phenylboronic acid-glucose displacement assay for the development of advanced electrochemical immunosensors.

In this study, the displacement-type of Alizarin Red S-phenylboronic acid-glucose was employed to enhance the sensitivity of immunosensors via the change of electrochemical signals. Au nanoparticles were first loaded in the PAMAM (designed as PAMAM-Au), and then 4-mercaptophenylboronic acid bind with PAMAM-Au by Au-S to further form PBA/ARS complexe. PAMAM-Au-PBA-ARS composite was used as sensing probe to modify the electrode and combine with antibody. Based on the sandwich-type immunoassay format, glucose could be produced by enzyme-catalysis which displaced the ARS together with the formation of PAMAM-Au-PBA-glucose at the surface of electrode and then lead to a large change in the electrochemical signals (SWV) due to the drop of ARS. By monitoring the shift in SWV, we could quantitatively determine the concentration of target protein in the sample.

2. Experimental

2.1. Materials and reagents

CEA standards with various concentrations were obtained from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). Monoclonal CEA antibody (Ab_1, 0.1 mg mL^{-1}) and polyclone CEA antibody (Ab_2, 0.1 mg mL^{-1}) were purchased from Sangon Biotech. Co., Ltd (Shanghai, China). Alizarin Red S (ARS), 4-mercaptophenylboronic acid (S-PBA), polyamidoamine dendrimers (PAMAM), chloroauric acid ($HAuCl_4 \cdot 3H_2O$), amylase, amylose, sodium borohydride ($NaBH_4$) were purchased from Aladdin®. Ultrapure water (≥ 18 MΩ) was used through the experiments. Acetic acid buffered saline solution (ABS, 0.1 M) was prepared by mixing 0.1 M NaAc and 0.1 M HAc and 0.1 M KCl was added as the supporting electrolyte. Phosphate buffer solution (PBS, 0.1 M) was prepared by mixing 0.1 Na_2HPO_4 and 0.1 M NaH_2PO_4 and 0.1 M KCl was added as the supporting electrolyte. Clinical serum samples were made available by Fujian Provincial Hospital, China.

2.2. Preparation of PAMAM dendrimer-encapsulated nanogold (PAMAM-Au)

According to the previous literatures (Wang et al. 2013; Qiu et al. 2016), PAMAM dendrimer-encapsulated nanogold (designated as PAMAM-Au) was synthesized by employing the method of *in situ* reduction. Firstly, $HAuCl_4$ aqueous solution (1.0 wt%, 10 mL) was added into PAMAM dendrimer solution (1.0 wt%, 15 mL), then, $AuCl_4^-$ion absorbed into the PAMAM through the electrostatic interactions by vigorously stirring for 60 min at room temperature (RT). Next, $NaBH_4$ (0.5 M) was added into the above solution dropwise under the stirring. In this process, $AuCl_4^-$was reduced to zero-valent Au nanoparticles accompanied with the color variation of the solution from pale yellow to red-brown. Following that, the resulting solutions were centrifuged and washed to remove impurities and gold nanoparticles alone. Finally, the as-prepared PAMAM-Au nanocomposites were dispersed into 5 mL double-distilled water and stored at 4 °C.

2.3. Preparation of ARS-decorated PAMAM-Au by S-PBA (PAMAM-Au-PBA-ARS)

To construct the displacement-type, ARS was decorated into the PAMAM-Au composite by the binding connection of 4-mercaptophenylboronic acid (S-PBA), and the preparation referring to the previous document with some mini modification (Chen et al. 2011). Initially, S-PBA (2 mM, 2 mL) was added into the above prepared PAMAM-Au solution (1 mL), and the mixed solution was stirred for 5 h at RT in order to insure S-PBA bind with PAMAM-Au by Au-*S* bonding action (designed as PAMAM-Au-PBA). Then, the solutions were centrifuged and washed to remove redundant S-PBA. Following that, ARS (2 mM, 2 mL) was added into the resulted solution and slightly shaked at RT for 30 min to form PAMAM-Au-PBA-ARS complex, and therein S-PBA as molecular receptor binds with cis-diol moieties. Then, the solution was centrifuged and washed with bi-distilled water, collected and redispersed in PBS buffer solution (pH 7.4) and stored at 4 °C when not in use.

2.4. Preparation of amylase/anti-CEA-conjugated AuNP (Am-Au-Ab_2)

Prior to experiment, Au nanoparticles (16 nm) were synthesized according to our previous report (Zhang et al. 2012a, 2012b) and the pH of the solution was adjusted to 9.0–9.5 by Na_2CO_3. The Am-Au-Ab_2 conjugates were synthesized according to previous report (Tang et al. 2013). Briefly, amylase (140,000 units mL^{-1}, 150 μL) and Ab_2 (1 mg mL^{-1}, 50 μL) were added into gold colloids, and the mixed solutions were gently shaked for 1 h at RT and then transferred into the refrigerator for overnight to guarantee enough integrated between protein and AuNP. Finally, the resulted conjugates were centrifuged, washed and suspended in PBS buffer