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Short communication

Sandwich-type electrochemical biosensor for glycoproteins detection based on dual-amplification of boronic acid-gold nanoparticles and dopamine-gold nanoparticles

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ABSTRACT

Glycoproteins play important roles in a wide variety of biological processes. The change in the concentration levels has been associated with many cancers, as well as other diseases. Thus, rapid, sensitive and selective determination of glycoproteins is much preferred. In this work, we reported a sandwich-type electrochemical biosensor based on dual-amplification of 4-mercaptophenylboronic acid (MBA)-capped gold nanoparticles (MBA-AuNPs) and dopamine (DA)-capped AuNPs (DA-AuNPs). Biological recognition elements such as synthetic receptor and aptamer immobilized onto gold electrodes were used to capture glycoproteins. The captured glycoproteins were then derivatized with MBA-AuNPs through the formation of tight covalent bonds between the boronic acids of MBA-AuNPs and diols of glycoproteins. Electroactive DA-AuNPs were attached by the anchored MBA-AuNPs via the interaction of boronic acids with DA tags, which facilities the amplified voltammetric detection of glycoproteins. With avidin and prostate specific antigen (PSA) as model analytes, we demonstrated the feasibility and sensitivity of the proposed method. The results indicated that sub-picomolar avidin/PSA can be readily measured. We believe that this strategy will be valuable for the electrochemical detection of other glycoproteins.

1. Introduction

Glycoproteins play important roles in a wide variety of biological processes, including protein folding, fertilization, cell proliferation, cell–cell interaction, and tumor cell metastasis (Li, Y., et al., 2011). Changes in protein glycosylation have been observed in many cancers, as well as other diseases (Grubisha et al., 2003; Kannagi et al., 2004). Currently used methods for the routine detection of glycoproteins, such as mass spectrometry, nuclear magnetic resonance, chromatography, polymerase chain reaction (PCR), lectin affinity assay and enzyme-linked immunosorbent assay (ELISA) are usually time-consuming, need complicated instruments and/or lack sensitivity (Bones et al., 2010; Chan Kim et al., 2009; Chen et al., 2007; Dixit et al., 2010; Feng et al., 2009; Liu et al., 2006; Peracaula et al., 2003; Zeng et al., 2011). Therefore, cost-effective and rapid detection assays for glycoproteins are in demand. In recent years, there have been some attempts for glycoproteins detection using electrochemical biosensor in view of its high sensitivity, simplicity, rapid response, and compatibility with miniaturization (Cheng et al., 2008; Dai et al., 2006; Escamilla-Gómez et al., 2009; Yan et al., 2012; Zhang, J.-J., et al., 2010; Zhang, X., et al., 2010).

Sandwich-type electrochemical affinity biosensor is one of the major analytical techniques for sensitive and selective detection of proteins as well as small biomolecules and has found wide applications in clinical diagnosis, biomedical research, food quality control and environmental monitoring (Li et al., 2011a, 2011b; Yang et al., 2010). In this format, the crucial step is the capture (selectivity) and identification (sensitivity) of analytes. Regarding selectivity, the specific binding between the target analytes and the immobilized biomolecules on electrode, such as antigen–antibody, DNA–DNA and protein–nucleic acid, has been extensively used to capture analytes. The increasing demand for detection of ultralow amount of analytes is pushing the enhancement of detection sensitivity by selecting different signal amplification strategy. At present, gold nanoparticles (AuNPs) coated with biological recognition elements (e.g. antibody, natural or synthetic receptors and aptamer) and redox tags (e.g. ferrocene (Fc) and thionine (Th)) have been widely applied for the molecular recognition and signal

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amplification (Dykmana and Khlebtsov, 2012; Perfézou, et al., 2012; Wang et al., 2008; X. Zhang et al., 2010). In particular, the antibody-coated AuNPs, relying on the specific antigen–antibody, have been extensively applied in the recognition of antigen (Huang et al., 2005; Kim et al., 2010). However, the utilization of the antibody-based biosensors might be hindered, especially in resource-poor setting areas such as undeveloped countries, by their high cost and relatively poor stability. Hydrophilic boronic acids are known to form tight covalent bonds with diols (Dowlut and Hall, 2006; Han et al., 2011; James et al., 2006; Matsumoto et al., 2010, 2009; Otsuka et al., 2003). Recent studies have described the use of boronic acids-capped nanoparticles in the enrichment of glycoproteins and indicated such nanoparticles as valuable tools for the analysis of glycoproteins due to their unique merits such as ease of production and labeling as well as highly specific binding affinity for glycoproteins (Qi et al., 2010; Tang et al., 2009; Yao et al., 2009; Zhou et al., 2008).

Recently, we have achieved the amplified voltammetric detection of dopamine using ferrocene (Fc)-capped gold nanoparticle/streptavidin (SA-AuNPs) conjugates and amplified surface plasmon resonance detection of amyloid-β peptides with streptavidin conjugated to a specific antibody in sandwich format (Liu et al., 2013; Xia et al., 2010). In the present work, we reported a dual-amplified sandwich-type electrochemical biosensor for the detection of glycoproteins at low levels using 4-mercaptophenylboronic acid (MBA)-capped AuNPs (MBA-AuNPs) and dopamine (DA)-capped AuNPs (DA-AuNPs). The sandwich-type system was formed by specific recognition of the biosensor surface-confined elements to glycoproteins, followed by the successive attachment of MBA-AuNPs and DA-AuNPs. The analytical method based on this concept is very sensitive because a MBA-AuNP can capture more than one DA-AuNP (Kong et al., 2011) and each DA-AuNP contains a large number of electroactive DA molecules. Moreover, this biosensor obviates the need of expensive and less stable antibody conjugates for the recognition of captured glycoproteins.

2. Experimental

2.1. Chemicals and reagents

Dithiobis(succinimidyl propionate) (DSP), 4-mercaptophenylboronic acid (MBA), biotin, 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC), avidin, trisodium citrate, 1-hexanethiol (HT), $HAuCl_4$ and cysteamine hydrochloride were obtained from Sigma-Aldrich. Prostate specific antigen (PSA) was obtained from Linc-Bio Science Co. Ltd. (Shanghai, China). Dopamine hydrochloride, single strand DNA aptamer (ssDNA1, 5′-HS-$(CH_2)_6$-ATTAAAGCTCGCCATCAAATAGC-3′) and random single strand DNA (ssDNA2, 5′-HS-$(CH_2)_6$-TTTTGCCATCGGGGC-CATGTTCCA-3′) were purchased from Sangon Biotech. Co., Ltd. (Shanghai). The supporting electrolyte was 10 mM PBS solution containing 0.05 M Na_2SO_4. All aqueous solutions were prepared with a Millipore system (Simplicity Plus, Millipore Corp.) and saturated with N_2. Unless otherwise noted, the reactions were conducted at room temperature.

2.2. Preparation of MBA-AuNPs and DA-AuNPs

AuNPs of 13 nm were prepared using a trisodium citrate reduction method as reported previously (Grabar et al., 1995). The MBA-AuNPs were prepared by ligand-exchange reaction between MBA and citrate-stabilized AuNPs, as reported previously (Liu, S., et al., 2012; Yao et al., 2009). It was performed at room temperature by mixing a 2 mL of diluted AuNPs suspension with a 20 μL of ethonal solution of MBA under stirring for 2 h. The particle concentration of the diluted AuNPs solution was determined to be 3.2 nM by using a molar absorptivity of $2.7\times10^8\ M^{-1}\ cm^{-1}$ at 520 nm (S. Liu et al., 2012). High concentration of MBA could cause the aggregation and color change of AuNPs due the formation of a planar 6-membered boroxine ring with the elimination of three water molecules from three MBA molecules (Liu, S., et al., 2012). The optimized concentration ratio of MBA to AuNPs was found to be about 156 (500 nM MBA/3.2 nM AuNPs) in this work.

The DA-AuNPs were prepared by ligand-exchange reaction between citrate-stabilized AuNPs and DSP, and by the standard amine coupling reaction between the amino groups of DA and activated-carboxyl groups of DSP (Cooper et al., 2009; Kong et al., 2011; Liu et al., 2012). Briefly, two-equivalent DA (20 mM) was allowed to react with one-equivalent DSP (10 mM) in DMF containing 20 mM triethylamine for 12 h to produce the DA–DSP complex. The result of mass spectrometry indicated that DA reacted completely with DSP (Fig. S1 in Supplementary material). The mixed solution of synthesized DA–DSP and citrated-stabilized AuNPs was then stirred at room temperature for 2 h to produce the DA-AuNPs. The unmodified and modified AuNPs were characterized by UV/vis spectrometry and transmission electron microscopy (TEM) (Fig. S2 in Supplementary material). The immobilization ability of AuNPs for DA–DSP was studied by centrifuging the nanoparticles suspension at 13,000 rpm for 15 min and then measuring the oxidation current of DA–DSP in the supernatant solution with differential pulse voltammetry (Fig. S3 in Supplementary material). The average number of DA molecules per gold nanoparticle was determined to be 857 ± 60, implying a high loading amount of DA groups.

2.3. Procedure

The gold disk electrode with a diameter of 2 mm was polished with diamond pastes down to 3 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The cysteamine self-assembled monolayers (SAMs) were formed by immersing the cleaned gold electrode in a solution of 10 mM cysteamine in the darkness for 18 h. This step was followed by washing the electrode thoroughly with water and soaking the cysteamine-covered electrode in an aqueous solution containing 1 mM biotin and 4 mM EDC for 2 h. After the biotin film had been formed, 10 μL of PBS comprising a given concentration of avidin was cast onto the electrode surface for 90 min (Kerman and Kraatz, 2009). Again, the electrode was rinsed with water to rid any non-specifically adsorbed substance. Throughout this work, the avidin concentration was reported based on the molecular weight of the avidin monomer. For the capture of PSA, immobilization of the thiolated ssDNA1 was first carried out by casting onto the bare gold electrode 10 μL of PBS containing 1.0 μM ssDNA1 for 12 h. This step was followed by washing the electrode thoroughly with water and soaking the electrode in an aqueous solution containing 0.1 mM HT for 5 min. After the surface had been carefully rinsed with water, 5 μL of PBS containing a given concentration of PSA was cast onto the electrode surface for 20 min. Then, the electrode was rinsed with water to remove the unattached PSA molecules. To attach DA-AuNPs, the electrode was first allowed to react with 10 μL of MBA-AuNPs suspension for 30 min. Upon rinsing with a copious amount of water, the electrode was exposed to 10 μL of DA-AuNPs suspension for 30 min. After the electrode had been rinsed with water, voltammetric determination in PBS solution containing Na_2SO_4 was performed on a DY2013 electrochemical workstation (Digi-Ivy, Inc., Austin, TX) using a homemade plastic electrochemical cell. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively.

3. Results and discussion

3.1. Amplified voltammetric determination of avidin

To demonstrate the feasibility of our strategy, we first realized the detection of less expensive avidin, a biotin-binding glycoprotein. The schematic representation of the amplified voltammetric detection of avidin via oxidation of catechol tags on the AuNPs is illustrated in Fig. 1. Biotin was immobilized onto the cysteamine self-assembled monolayers (SAMs) through the standard amine coupling reaction. The strong biotin–avidin non-covalent interaction ($K_d = 10^{-15}$ M) (Samanta and Sarkar, 2011) allows avidin molecules to be anchored onto the electrode surface. Then, MBA-AuNPs were captured by avidin through the interaction between boronic acids on AuNPs and carbohydrate moieties of avidin, which was followed by the attachment of DA-AuNPs via the formation of diol-boronate ester. Since each gold nanoparticle was capped with 857 ± 60 DA molecules (Fig. S3 in Supplementary material), the electrochemical signals were greatly amplified.

Curve a in Fig. 2A is a representative cyclic voltammogram (CV) collected at an electrode modified with biotin for avidin capture and the subsequent attachment of MBA-AuNPs and DA-AuNPs. The redox peaks with $E_{pa} = 0.175$ V and $E_{pc} = 0.138$ V were attributed to the oxidation/reduction of the catechol moieties of DA-AuNPs (Ji et al., 2012; Medintz et al., 2010). The control CV (curve b) was acquired at an electrode modified with biotin but without having exposed the electrode to the avidin solution. In the absence of MBA-AuNPs (curve c), the voltammetric peak currents dropped to a background level, indicating that the attachment of DA-AuNPs is dependent on the anchored MBA-AuNPs. For comparison, the same procedure was implemented with electrodes covered with streptavidin (curve d), a biotin-binding protein lacking any carbohydrate modification (Kerman and Kraatz, 2009). The absence of any discernible peaks in curve d indicates that binding of MBA-AuNPs is greatly dependent on the carbohydrate moieties of captured avidin. As the previously reported data for DA/quantum-dot conjugates (Ji et al., 2012; Medintz et al., 2010), we also found that the redox potential of DA-AuNPs depended on the pH values of electrolyte solution (Fig. S4 in Supplementary material).

Another electrochemical technique, differential pulse voltammetry (DPV), can decrease the background charging currents and in turn increase the detection sensitivity. Therefore, we evaluated the sensitivity and dynamic ranges of the proposed method using DPV (Fig. 2B) instead of CV (Bard and Faulkner, 2001). The dependences of the oxidation current on the avidin concentrations are presented in Fig. 2C. The relative standard deviations (RSDs), shown as the error bars in Fig. 2C, are all less than 5%. The oxidation peak current (i_{pa}) increases linearly with the concentrations of avidin ranging from 157 fM to 4.40 pM. The linear regression equation is expressed as i_{pa} (μA) = 0.018 + 0.081[avidin] (pM) ($R^2 = 0.999$). The detection limit (3σ) of the method was determined to be 75 fM ($n = 11$). Notice that the calibration curve reveals a slight response of the electrode at [avidin] = 0. This is probably attributed to a residual non-specific adsorption of the DA-AuNPs to the electrode surface.

3.2. PSA detection

To demonstrate the amenability of our method to other glycoproteins analysis, we tested PSA, the most common serum

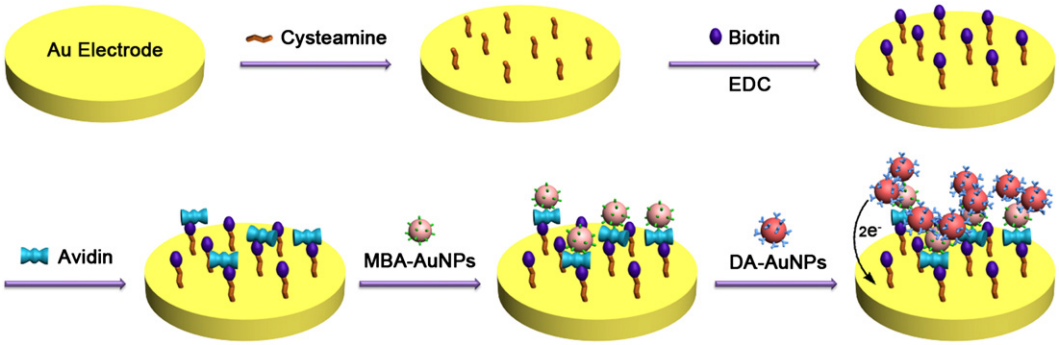

Fig. 1. Schematic illustration of the strategy of avidin detection using MBA-AuNPs and DA-AuNPs.

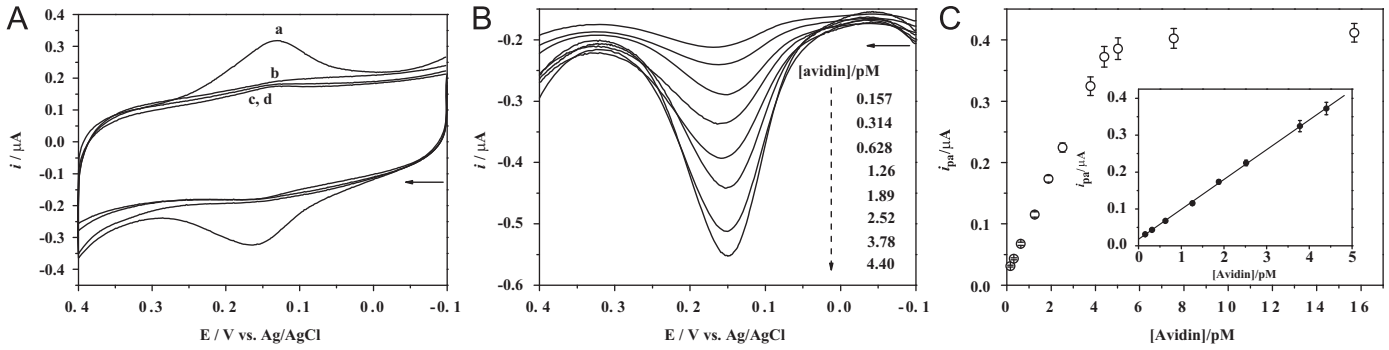

Fig. 2. (A) CVs acquired at the electrodes covered with biotin after avidin binding and attachment of MBA-AuNPs and DA-AuNPs (curve a). The electrochemical responses in the absence of DA (curve b) or MBA-AuNPs (curve c) followed by the attachment of DA-AuNPs were also shown. Curve d corresponds to that after exposure to streptavidin instead of avidin. PBS solution (pH 7.4) containing 0.05 M Na_2SO_4 was used as the electrolyte, and the arrow indicates the scan direction. (B) DPV peak currents for the detection of different concentrations of avidin. (C) Plots of the i_{pa} against the avidin concentration (0.157–15.7 pM). The inset in panel C showed the linear plots at concentrations of 0.157, 0.314, 0.628, 1.26, 1.88, 2.52, 3.78 and 4.40 pM. Each point was averaged from at least three replicates, and the relative standard deviations (RSDs) are shown as the error bars.

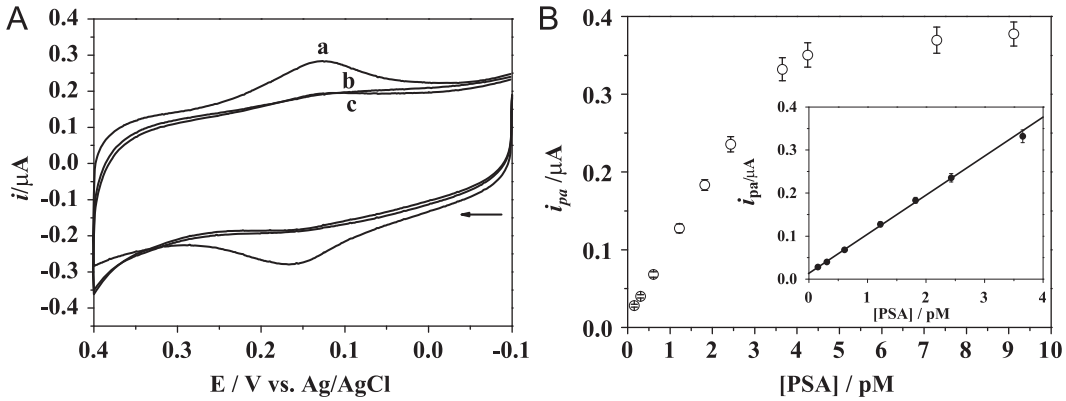

Fig. 3. (A) CVs acquired at electrodes covered with single strand DNA and hexanethiol mixed SAM after PSA binding and attachment of MBA-AuNPs and DA-AuNPs. Curves a and b correspond to ssDNA1 and ssDNA2 after exposure to 4.25 pM PSA, respectively, and curve c was obtained without exposing the electrode to a PSA solution. (B) Plots of the i_{pa} against the PSA concentrations (0.152–9.12 pM). The inset in panel B showed the linear plots at concentrations of 0.152, 0.304, 0.608, 1.22, 1.82, 2.43 and 3.65 pM.

marker for diagnosing prostate cancer (Grubisha et al., 2003; Uludag and Tothill, 2012). Aptamers which are selected from random sequence pools *in vitro* are single strand DNA or RNA that can specially and strongly bind to various molecular targets from small molecules to proteins, even to whole cells. In contrast to antibodies, aptamers are advantageous in their quick and reproducible synthesis, easy and controllable chemical modification, long-term stability, and ability to sustain reversible denaturation (Liu et al., 2011). Therefore, the single strand DNA aptamer (ssDNA1, 5′-HS-$(CH_2)_6$-ATTAAAGCTCGCCATCAAATAGC-3′) which was specific to PSA was used here to capture PSA in this work (Chen et al., 2012; Liu, B., et al., 2012; Savory et al., 2010). As shown in Fig. 3A, a pair of well-defined redox waves was observed at the DNA-modified electrode with PSA capture. For the experiment wherein the DNA-modified electrode was not exposed to the PSA solution (curve c), no peaks were observed. This experiment indicates that the DNA-modified electrode without the PSA capture step does not allow MBA-AuNPs and DA-AuNPs to adsorb onto the electrode. The control CV (curve b) was acquired at an electrode modified with the random single strand DNA (ssDNA2, 5′-HS-$(CH_2)_6$-TTTTGCCATCGGGGCCATGTTCCA-3′). The absence of any discernible peaks in curve c indicates that nonspecific interaction between PSA and ssDNA2 is negligible. The sensitivity of the sensor for PSA was also evaluated by monitoring the oxidation peak current of DPV in the presence of different concentrations of PSA. As shown in Fig. 3B, i_{pa} linearly increased with the increase of the PSA concentration ranging from 152 fM to 3.65 pM. The linear regression equation is expressed as i_{pa} (μA)=0.014+0.091[PSA] (pM) (R^2=0.999). The detection limit was estimated to be 50 fM (1.6 pg/mL), which is comparable to (or even lower than) those achievable using other electrochemical strategy (Table S1 in Supplementary material) (Barton et al., 2008; Fragoso et al., 2008; Liu et al., 2007; Wei et al., 2011; Yu et al., 2006).

4. Conclusion

In summary, we reported a dual-amplified sandwich-type electrochemical affinity biosensor for the detection of glycoproteins. The captured glycoproteins were derivatized with MBA-AuNPs for the attachment of electroactive DA-AuNPs. With avidin and PSA as model analytes, we demonstrated the feasibility and sensitivity of the proposed method. Due to the dual-amplification effect of MBA-AuNPs and DA-AuNPs, relatively low detection limits were obtained. We believe that our method would be valuable in the electrochemical detection of glycoproteins.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.12.020.

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