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A signal amplification strategy using the cascade catalysis of gold nanoclusters and glucose dehydrogenase for ultrasensitive detection of thrombin

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ABSTRACT

This work reports a novel signal amplification strategy for ultrasensitive detection of thrombin by cascade catalysis of gold nanoclusters (AuNCs) and glucose dehydrogenase (GDH). Herein, the AuNCs prepared by using polyamidoamine dendrimer as template were constructed not only as nanocarriers for anchoring the large amounts of secondary thrombin aptamers but also as nanocatalysts to catalyze the oxidation of NADH efficiently. Moreover, a large amount of GDH was loaded through the immobilization technology of DNA hybridization and a large amount of toluidine blue (Tb) was intercalated into the DNA grooves *via* electrostatic interaction. Significantly, the electrochemical signal was greatly enhanced based on cascade catalysis: firstly, GDH catalyzed the oxidation of glucose to gluconolactone with the concomitant generation of NADH in the presence of NAD^+. Then, AuNCs as nanocatalysts could effectively catalyze NADH to produce NAD^+ with the help of Tb as redox probe. Under the optimal conditions, the proposed aptasensor exhibits a linear range of 1.0×10^{-14}–5×10^{-9} M with a low detection limit of 3.3×10^{-15} M for thrombin detection and shows high sensitivity and good specificity.

1. Introduction

For the construction of ultrasensitive electrochemical biosensor, the signal amplification strategies employing enzyme (Su, et al., 2012; Zhang et al., 2011; Zhao et al., 2012a; Zhuo et al., 2009), nanoparticles (Lin et al., 2012; Sun et al., 2013; Wang et al., 2004; Zhang et al., 2010) or redox probe (Xu et al., 2010) as labels are the most popular. Usually, biomolecules immobilized methods are resort to sorption, entrapment, covalent bonding, as well as layer-by-layer self-assembly. Lately, great attention has been focused on the amplification of detectable signals by employing a new immobilization technology according to DNA hybridization, which could increase the amount of immobilized biocatalysts or signal molecules under mild conditions and maintain their bioactivity. For example, Lee et al. (2013) developed an ultrasensitive electrochemical biosensor by binding methylene blue (MB) to the complementary DNA to greatly amplify the detection signals. Wu et al. (2010) reported polymerase chain reaction (PCR) as carriers to immobilize MB, a kind of signal molecule, making an extraordinarily low detection limit possible. Yu's group (Zhao et al., 2012b) reported utilizing hybridization chain reaction (HCR) as carriers to immobilize the biocatalyst of streptavidin–alkaline phosphatase for signal enhancement. In our previous work (Gui et al., 2013), we have employed long-range self-assembled DNA nanostructures as carriers to immobilize a large amount of $Ru(phen)_3^{2+}$, a well-known luminophore, as a signal molecule for biosensors construction. The above signal amplification strategy depends on the binding of signal molecules (*e.g.* redox probe) or biocatalysts (*e.g.* enzyme) according to DNA hybridization interaction which also reveals the important advantages of DNA immobilization technology: (1) to simplify bioconjugate preparation; (2) to avoid denaturation and leakage of enzyme; and (3) to have better control over the loading under mild conditions. Motivated by the above observations, we propose here a new signal amplification strategy based on DNA immobilization technology to simultaneously load enzyme and redox probe for the development of ultrasensitive electrochemical biosensors.

As is well known Au is a poor catalyst in bulk form, but recent studies showed that the highly dispersed, nanometer-sized Au particles exhibit excellent catalytic activity (Jena and Raj, 2006), since the large surface-to-volume ratio and the presence of active sites on the fine particle are the driving forces in nanoparticle-assisted catalysis (Maye et al., 2000). For example, Raj and Jena (2005) exploited nanosized Au particles as an efficient electrocatalyst that could efficiently catalyze the oxidation of NADH. Wang et al. (2009) reported, by utilizing gold nanoparticles-modified Pb nanowires, the construction of a biosensor which exhibited excellent electrocatalytic activity and good response performance to glucose. In our previous work (Yi et al., 2013), we demonstrated the AuNPs as an

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electro-catalyst that could catalyze the oxidation of NADH efficiently with the help of electroactive material MB as an electron mediator. On the other hand, polyamidoamine (PAMAM) dendrimer with a hyperbranched and three-dimensional structure has been widely used in biosensor designing owing to its high density of surface active groups, good structural homogeneity, and intense internal porosity (Tully and Frechet, 2001; Zhang and Hu, 2007). Interestingly, the unique structure of PAMAM includes the incorporation of metal nanoparticle with precise control over the nanoparticle size and prevents nanoparticle aggregation (Frasconi et al., 2010). Inspired by this property, gold nanoclusters (AuNCs), prepared by using PAMAM as template, showed smaller particle size and better dispersive stability. More importantly, the AuNCs were chosen in our work as they combined the following advantages: (1) to increase the amount of immobilized secondary thrombin aptamer (TBA II) and initiator strands (S1) as nanocarriers; and (2) to catalyze the oxidation of NADH efficiently as nanocatalysts. Indeed, the unique properties make AuNCs a promising candidate for biosensor designing with increased sensitivity.

Here we designed AuNCs as nanocarriers to immobilize a large amount of TBA II and S1 forming AuNCs–TBA II–S1 bioconjugate. In the simultaneous presence of glucose dehydrogenase labeled S2 (S2-GDH) and S3 (S3 is partially complementary to the S2), initiator S1 on the AuNCs–TBA II–S1 bioconjugate propagates a chain reaction of hybridization events to form long nicked double helices analogous (dsDNA) with the numerous GDH labeled. Subsequently, numerous redox probes (toluidine blue, Tb) could be intercalated into the grooves of dsDNA with high affinity. The cascade catalysis was accomplished in the following way: firstly, GDH catalyzed the oxidation of glucose to gluconolactone with the concomitant generation of NADH in the presence of NAD^+. Then, with the help of Tb as redox probe, the AuNCs as nanocatalysts could catalyze the oxidation of NADH effectively, thus resulting in the amplification of the electrochemical signal. Moreover, bovine serum albumin (BSA) modified Au electrode as carrier platform can be an effective and directional immobilization primary thrombin aptamer (TBA I) with low background. The proof-of-concept of novel signal amplification strategy by using the cascade catalysis of AuNCs and GDH has been shown herein to lead to low femtomolar detection of thrombin. The fabrication process and sandwiched assay principle of the proposed electrochemical aptasensor are presented in Fig. 1.

2. Experimental

2.1. Reagent and materials

Glucose dehydrogenase (GDH), β-nicotinamide adenine dinucleotide hydrate (NAD^+), toluidine blue (Tb), thrombin, hexanethiol (96%, HT), gold chloride ($HAuCl_4$) and bovine serum albumin (BSA, 96–99%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Carboxyl-terminated polyamidoamine (PAMAM, G 4.5) dendrimer was obtained from Aldrich (St. Louis, MO, USA). *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimidehydrochloride (EDC) and *N*-hydroxy succinimide (NHS) were acquired from Shanghai Medpep Co. (Shanghai, China). $NaBH_4$ was obtained from Kelong Chemical Company (Chengdu, China). D-glucose was obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Gold nanoparticles (AuNPs) of 16 nm diameter were prepared by reducing $HAuCl_4$ with sodium citrate at 100 °C for half an hour (Enustun and Turkevich, 1963). All other chemicals were of reagent grade and used as received. Double distilled water was used throughout this study. Thrombin binding aptamer (TBA): 5′-SH–$(CH_2)_6$–GGTTGGTGTGGTTGG-3′ and all the synthetic DNA oligonucleotides were ordered from TaKaRa (Dalian, China), and the sequences are listed below:

S1 (Initiator strands): 5′-NH_2–CGTA AAGA TAGC CACT GCGT TGGA CCGT TTCG *ATAT CCAA CGTG TTAG*-3′

S2: 5′-NH_2–TGAC ATTT GCTC GATT **AGTG GCTA TCTT TACG** *CTAA CACG TTGG ATAT*-3′

S3 (Partially complementary to the S2): 5′-**CGTA AAGA TAGC CACT** GCGT TGGA CCGT TTCG *ATAT CCAA CGTG TTAG*-3′

Phosphate buffered solution (PBS) (pH 7.4) was prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, and 0.1 M NaCl. 20 mM Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$ was used to prepare aptamer solutions and DNA oligonucleotides. The acetate buffer solution (pH 5.5) was prepared with 0.1 M HAc–NaAc containing 0.1 M KCl.

2.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed with a CHI 660D electrochemical workstation (Shanghai Chen Hua Instrument, Co., China). Electrochemical impedance spectroscopy (EIS) measurements were done with a Model IM6e (Zahner Elektrik Co., Germany). A three-electrode electrochemical system was composed of a platinum wire as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference electrode and a modified gold electrode (∅=4 mm) as the working electrode. The scanning electron micrographs were taken with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan).

2.3. Synthesis of AuNCs

The stable AuNCs in aqueous medium have been prepared by chemical reduction of $HAuCl_4$ in the presence of PAMAM as template, according to the literatures with minor revision (Rahman et al., 2008; Zhang and Hu 2007). In brief, 10 mg PAMAM was first dispersed in 2 mL of 2 mM $HAuCl_4$ solution under continual stirring for 1 h at room temperature. By this step, $AuCl_4^-$ was coordinated to the nitrogen ligand in the interior of the PAMAM forming PAMAM–Au (III) complex. Second, 1 mL of 20 mM $NaBH_4$ was quickly added to PAMAM–Au (III) solution with vigorous stirring. The color of the resulting mixture solution immediately turned from light yellow to red-brown that of reducing Au (III) to Au (0) and forming PAMAM–Au (AuNCs). After centrifugation at 15,000 rpm for 30 min to remove the free PAMAM and $NaBH_4$, the sediment of AuNCs was resuspended in double distilled water and stored at 4 °C for further use.

2.4. Synthesis of AuNCs–TBA II–S1 bioconjugate

The synthesis was performed in the following manner. The first step: 100 μL of TBA II (2 μM) was added into 0.5 mL AuNCs solution and the mixture was allowed to react at 4 °C for 12 h under gently stirring to produce TBA II conjugated AuNCs (AuNCs–TBA II) *via* Au–S affinity. The second step: 50 μL S1 (2.5 μM) was added into AuNCs–TBA II solution to block the nonspecific adsorption sites of the AuNCs surface. The product was centrifuged, washed and finally dispersed in 1 mL Tris–HCl buffer.

2.5. Synthesis of S2-GDH

1 mg GDH was firstly suspended in 1 mL Tris–HCl buffer (pH 7.4), and then a proper amount of EDC and NHS was added and stirred for 8 h. Subsequently, the NH_2-modified S2 (100 μL, 2.5 μM) was added and the mixture was allowed to react overnight under gentle stirring to obtain GDH labeled S2 (S2-GDH). After centrifugation at 15,000 rpm for 15 min to remove surplus reagent, the product of S2-GDH was resuspended in 500 μL Tris–HCl and stored at 4 °C for further use.