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DNAzyme-functionalized Pt nanoparticles/carbon nanotubes for amplified sandwich electrochemical DNA analysis

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ABSTRACT

A novel DNAzyme-functionalized Pt nanoparticles/carbon nanotubes (DNAzyme/Pt NPs/CNTs) bioconjugate was fabricated as trace tag for ultrasensitive sandwich DNA detection. The Pt NPs/CNTs were prepared *via* layer-by-layer (LBL) assembly of the Pt NPs and polyelectrolyte on the carboxylated CNTs, followed by the functionalization with the DNAzyme and reporter probe DNA through the platinum—sulfur bonding. The subsequent sandwich-type DNA specific reaction would confine numerous DNAzyme/Pt NPs/CNTs bioconjugate onto the gold electrode surface for amplifying the signal. In the presence of 3,3′,5,5′ tetramethylbenzidine (TMB) which could be oxidized by the DNAzyme, electrochemical signals could be generated by chronoamperometry *via* the interrogation of reduction electrochemical signal of oxidized TMB. The constructed DNA sensor exhibited a wide linear response to target DNA ranging from 1.0 fM to 10 pM with the detection limit down to 0.6 fM and exhibited excellent selectivity against even a single base mismatch. In addition, this novel DNA sensor showed fairly good reproducibility, stability, and reusability.

1. Introduction

Among the various DNA assay techniques including fluorescence, chemiluminescence, surface plasmon resonance and quartz crystal microbalance, and electrochemical assay have attracted considerable interest for its intrinsic advantages such as good portability, low cost, and high sensitivity (Fan et al., 2005; Drummond et al., 2003). A few DNA molecules are sufficient to affect the biological functions of cells and trigger pathophysiological processes. The conventional electrochemical methods can hardly detect low abundance DNA. So, it is urgent to develope sensitive detection strategies.

In the previously reported electrochemical DNA assays (Liu et al., 2008; Dong et al., 2011a; Zhang et al., 2008; Loaiza et al., 2008; Wan et al., 2009), numerous signal amplification strategies have been developed with the aim to achieve a highly sensitive signal. For example, a large number of oligonucleotides were assembled on one nanoparticle (NP) probe for a biobarcode assay of DNA (Garcia et al., 2008); polyelectrolyte-coated quantum dots on carbon particles have been used to label oligonucleotides for highly sensitive sandwich DNA detection (Dong et al., 2011b). In these reports, the NPs have been demonstrated as excellent carriers for the analytical signal amplification (Wang et al., 2003; Zhang et al., 2006; Xiang et al., 2011; Xu et al., 2009; Huang et al., 2009; Sanchez-Pomales et al., 2009). Pt nanoparticles(Pt NPs)were proved to have high electrocatalytic activity (Bahshi et al., 2008). On the other hand, as one of the most popular tracer labels, enzymes, such as horseradish peroxidase(HRP) (Mao et al., 2008; Fu, 2008; Gao et al., 2011) and alkaline phosphatase(AP) (Tu et al., 2004; Lucarelli et al., 2006; Liao et al., 2007; Miranda-Castro et al., 2007) have been immobilized onto Au NPs (Lai et al., 2009), CNTs (Gao et al., 2011; Munge et al., 2005), bio-nanospheres (Cui et al., 2008; Tang and Ren, 2008) and magnetic beads (Piao et al., 2011) for enhancing the enzyme-catalyzed signal. Another kind of enzyme label is DNAzyme with catalytic capability (Li et al., 2009a,b; Yuan et al., 2011; Nakayama et al., 2011; Freeman et al., 2010) that could be generated *via* in vitro selection process (Travascio et al., 2001). An interesting example of a catalytic DNA possessing peroxidase-like activity includes a guanine-rich nucleic acid (aptamer) that binds hemin in a supramolecular G-quadruplex configuration (Travascio et al., 2001; Willner et al., 2008). For practical applications, DNAzymes are cost-effective to produce, much more stable than proteins or RNA and can be denatured and renatured many times without losing activity or binding ability (Ito and Hasuda, 2004). These potential advantages of DNAzymes offer a feasible analytical basis for developing highly selective sensors.

Benefitting from the peroxidase-like activity of both DNAzymes and Pt NPs (Polsky et al., 2006; Ma et al., 2011), DNAzyme-functionalized Pt nanoparticles/carbon nanotubes (DNAzyme/Pt NPs/CNTs) bioconjugate was fabricated as trace label to doubly

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catalyze the oxidation of their substrate TMB (reduced form) with H_2O_2 and enhance the electrochemical signal for ultrasensitive DNA analysis. Experimentally, highly dispersive CNTs were prepared by acid treatment for the subsequent employment as matrix for the homogeneous loading of Pt NPs. Through the Pt—S bonding, the DNAzyme and reporter probe was anchored onto the Pt NPs/CNTs composites, whereas the capture probe DNA was self-assembled onto the gold electrodes *via* Au—S bonding. A three-component sandwich assay was then employed, in which the target DNA would specifically bind to both of the capture DNA and the fabricated trace label. This dual-hybridization would render numerous DNAzyme proximal to the electrode surface, thus offering a significant amplification for the target DNA detection. In the presence of DNAzyme/Pt NPs/CNTs, TMB (reduced form) would react with to form it oxidation state. The oxidized TMB could then be electro-reduced and determined amperometrically. Since the amount of target DNA is directly related with the reduction charge of the TMB (oxidized form), by tracking the transduction signal that monitors the extent of hybridization, and a new DNA biosensor can be tailored. Since both the high-content DNAzyme and Pt NPs in the tracer amplified the detectable signal for the sandwich-type DNA biosensor, the approach proposed here showed high sensitivity for target detection. The experimental results further demonstrate that the constructed DNA sensor is reproducible, stable, reusable, and can sensitively detect target DNA with excellent differentiation ability for single-base mismatched DNA.

2. Experimental

2.1. Materials and reagents

CNTs were purchased from Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). Hexachloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$), dimethyl sulfoxide (DMSO), Triton X-100 were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Tri (2-carboxyethyl) phosphinehydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), poly (dimethyldiallyl ammonium chloride) (PDDA) and hemin were purchased from Sigma-Aldrich (St. Louis, MO). TMB was purchased from Neogen (Lexington, KY) in the format of a ready-to-use reagent (K-blue low-activity substrate, H_2O_2 included). All of the synthetic oligonucleotides were purchased from Shanghai Sangong biotechnology Co. Their base sequences are as follows:

Capture probe sequence:

5′-HS-$(CH_2)_6$CGATTCGGTACTGG-3′.
Target DNA sequence:
5′-TTGAGCATGCGCATTATCTGAGCCAGTACCGAATCG-3′.
DNAzyme with reporter probe sequence: 5′-ATGCGCATGCT-$CAAT_{10}$GGGTAGGGCGGGTTGGGT$_{17}$$(CH_2)_6$SH-3′.
One-base mismatched DNA sequence:
5′-TTGAGCATGCGCATTATCTGAGCCAGTA**G**CGAATCG-3′.
Non-complementary DNA sequence:
5′-TTGAGCATGCGCATTATCTGAGCCAGTACC-3′.

The other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared using ultra-pure water ($\geq$ 18.2 MΩ cm, Milli-Q, Millipore). The buffers involved in this work were as follows: DNA immobilization buffer containing 10 mM Tris–HCl, 1.0 mM EDTA, 1 M NaCl, and 1.0 mM TCEP (pH 7.4); DNA hybridization buffer containing 10 mM Tris–HCl, 1.0 mM EDTA, and 1 M NaCl (pH 7.4); Washing buffer containing 10 mM phosphate buffer (PBS), 0.1% sodium lauryl sulfate (SDS) and 0.1 M NaCl (pH 7.4).

2.2. Apparatus

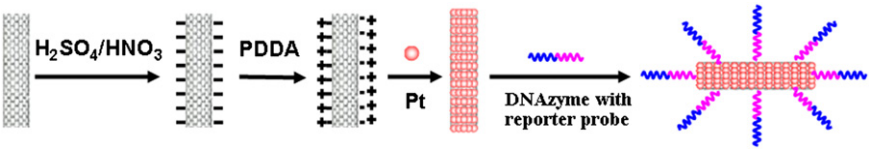
The electrochemical measurements for cyclic voltammetry(CV) and chronoamperometry(i-t) were performed on a CHI 660C electrochemical workstation (Shanghai Chenhua Apparatus Co, Shang, China). Electrochemical impedance spectroscopy (EIS) experiments were carried out on an Autolab PGSTAT-30 potentiostat/galvanostat (Eco Chemie BV, Utrecht, The Netherlands) with FRA software. UV–visible absorption spectra were recorded with a Shimadzu UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co.). Transmission electron microscopy (TEM) images were taken with a JEOL model 2000 instrument operating at 200 kV accelerating voltage.

2.3. Preparation of Pt NPs, Pt NPs/CNTs conjugates, DNAzyme/Pt NPs/CNTs bioconjugates

Pt NPs were prepared according to the literature (Polsky et al., 2006). Briefly, 1 mL of 1 % H_2PtCl_6 solution was added to 100 mL aqueous solution in a three-neck round-bottom flask under stirring. Then the above solution was added to 10 mL of 38.8 mM aqueous sodium citrate, followed by boiling of the mixture for 50 min and the solution turned from clear to black. The heat was then turned off, and the solution was stirred for an additional 10 min. After cooled to room temperature, the solution was kept in a refrigerator at 4 °C for further use.

The preparation procedure of the Pt NPs/CNTs conjugates was showed in Scheme 1. CNTs were dispersed in HNO_3 (30%) and then refluxed for 24 h at 140 °C to shorten the nanotubes and to produce carboxylic acid groups mainly on the open ends as well as the sidewalls, which introduced negative charges on the CNTs and improved their dispersion in water. The resulting suspension was centrifuged, and the sediment was washed with ultrapure water until the pH reached 7.0. Then, the oxidized CNTs were dispersed in ultrapure water to a concentration of 1 mg mL^{-1}. 4 mL of PDDA solution (2 mg mL^{-1}, containing 0.5 M NaCl) was added into 2 mL of CNTs suspension by ultrasonicating for 60 min to obtain a homogeneous brown suspension. The suspension was followed by centrifuging at 11,000 rpm for 30 min. The supernatant was then carefully removed, and the CNTs coated with PDDA (PDDA/CNTs) were washed by three alternate cycles of centrifugation. Then the prepared Pt NPs were added into the PDDA/CNTs suspension by 20 min sonication. Residual Pt NPs were removed by high-speed centrifugation, and the complex was washed with water for four times to obtain Pt NPs/CNTs. Then, Pt NPs/CNTs were redispersed in 10 mM pH 7.4 Tris–HCl solution and kept in a refrigerator at 4 °C.

The process of DNAzyme with reporter probe labeling was performed as follows: 0.5 μM DNAzyme with reporter probe was activated in 10 mM Tris–HCl including 10 mM TCEP for 1 h, then 1 mL Pt NPs/CNTs dispersion was added and shaken gently



Scheme 1. Schematic representation of preparation procedure of DNAzyme /Pt NPs/CNTs bioconjugates.