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Multiplexed aptasensor for simultaneous detection of carcinoembryonic antigen and mucin-1 based on metal ion electrochemical labels and $Ru(NH_3)_6^{3+}$ electronic wires

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ABSTRACT

In this paper, a dual-target electrochemical aptasensor has been developed for simultaneous detection of carcinoembryonic antigen and mucin-1 based on metal ion electrochemical labels and $Ru(NH_3)_6^{3+}$ electronic wires. When targets are present, the interaction between targets and their respective aptamers leads to the dissociation of double-strand DNA because the targets have higher affinity to its aptamer than the complementary strand. And the qualitative and quantitative analyses of the two targets are realized by the differential pulse voltammetry (DPV) peaks generated by metal ion electrochemical labels. For the effective loading of a large number of metal ions, Au/bovine serum albumin (Au/BSA) nanospheres are employed as carriers to develop Au/BSA–metal ions. After $Ru(NH_3)_6^{3+}$ complexes are embedded into double-strand DNA to form the electronic wires, the electrical conductivity and the electron transfer of the detection system are greatly improved. The detection limit of the proposed assay was calculated as 3.33 fM ranging from 0.01 pM to 100 nM. Therefore, this novel sensing assay provides a new and sensitive platform for detecting several targets simultaneously in biochemical research and clinical diagnosis.

1. Introduction

The portable and rapid analysis of clinical samples is rather significant in early screening and therapy of diseases (Ferguson et al., 2011; Kang et al., 2011), especially the simultaneous detection of multiple targets (Li et al., 2016a; Shin and Park, 2016). As a low-cost and portable platform, microfluidic paper-based analytical devices (μ-PADs) are widely applied in the field of bio-analysis and detection (Martinez et al., 2007; Zhang et al., 2017, 2014; Li et al., 2016b; Liang et al., 2017). Aptamers are short oligonucleotide sequences that could specifically bind with a wide range of targets (Iliuk et al., 2011; Jhaveri et al., 2000), including ions (Kim et al., 2012), peptides (Rhinehardt et al., 2015), purified proteins (Chen et al., 2013), and even living cells (Wan et al., 2012). In addition, aptamers provide novel and efficient platforms for the specific recognition of targets in the detecting system (Bamrungsap et al., 2011; Ma et al., 2015, 2016). And the introduction of excellent aptamers into portable μ-PADs may make great sense to realize the specific recognition and low-cost analysis of multiple targets (Cate et al., 2015; Ge et al., 2017; Zhu et al., 2014).

For signal generation and amplification, metal ions may be a rational choice to realize a simple and fast detection on μ-PADs. In fact, metal ions are readily applicable as the electrochemical labels via their redox reaction on the working electrode (Feng et al., 2012; Yuan et al., 2015). By introducing multiple metal ions into the detection system, the simultaneous detection of multiple targets becomes easier to be implemented, due to the electrochemical peak separation of metal ions for qualitative analysis and the peak height for quantitative analysis (Li et al., 2015; Lu et al., 2005), so that a pathway between aptamer-based target recognition and metal ion electrochemical labelling can be realized. Interestingly, the cationic $Ru(NH_3)_6^{3+}$ redox species can electrostatically interact with the polyanionic sugar–phosphate backbone of DNA to form the electronic wire to reduce the resistance of the electronegative DNA backbone to the electron transport (Cheng et al., 2015; Abi and Ferapontova, 2012).

Given all these considerations, a dual-target electrochemical aptasensor has been constructed for simultaneous detection of carcinoembryonic antigen (CEA) and mucin-1 (MUC1) based on metal ion electrochemical labels and $Ru(NH_3)_6^{3+}$ electronic wires. As shown in Scheme 1, two aptamers (AP) bind with their respective thiolated capture probes (CP) through the complementary base pairing principle, after the self-assembly immobilization of the two CPs on the prepared

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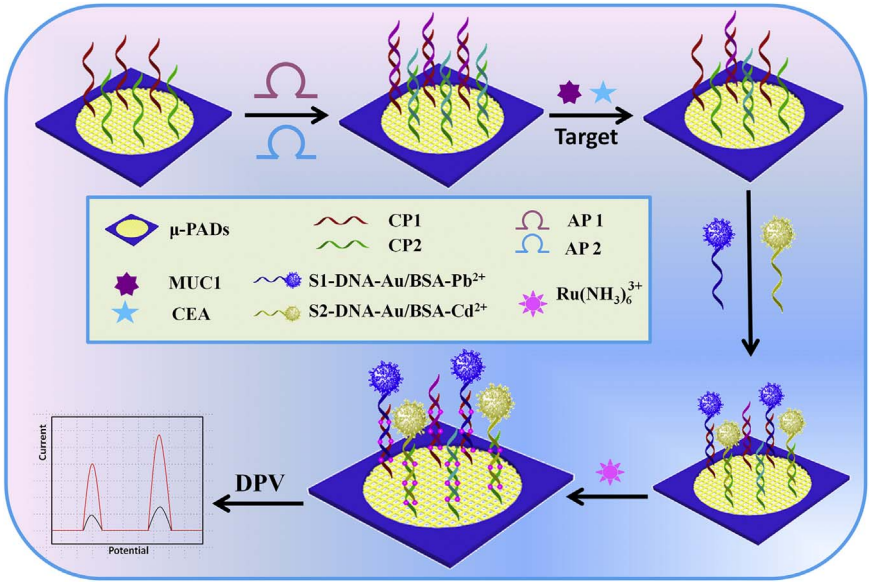

Scheme 1. Schematic representation of the dual-target electrochemical aptasensor for the detection of carcinoembryonic antigen and mucin-1 based on metal ion electrochemical labels and $Ru(NH_3)_6^{3+}$ electronic wires.

μ-PADs. When targets are present, the interaction between targets and their respective aptamers leads to the dissociation of dsDNA because targets have a higher affinity to its aptamer than the complementary strands (Dong et al., 2016; Wu et al., 2015). By employing Au/bovine serum albumin (Au/BSA) nanospheres as carriers for the loading of numerous metal ions (Pb^{2+} and Cd^{2+}), the labelling Au/BSA–metal ions are prepared for the conjugation with the two auxiliary sequences (S1 and S2). And importantly, when numerous $Ru(NH_3)_6^{3+}$ interacted with DNA base-pairs to form effect electronic wires, the conductivity of the detecting system can be significantly improved. This novel biosensing assay may have potentials in detecting different targets simultaneously in biochemical research and clinical diagnosis.

2. Experimental section

2.1. Reagents and apparatus

All solutions were prepared by using DNase and RNase free water. The ultrapure water used in the assay was obtained from the Millipore water purification system. And bovine serum albumin (BSA), ascorbic acid (AA), 6-mercapto-1-hexanol (MCH) and HAc/NaAc were purchased from Sigma-Aldrich (St. Louis, Missouri). Tetrachloroauric acid ($HAuCl_4$) and hydroxylammonium chloride ($NH_2OH{\cdot}HCl$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). KH_2PO_4 and Na_2HPO_4 were used for the preparation of phosphate buffered solutions (PBS, 10.0 mM) with different pH values.

The oligonucleotide sequences of the two aptamers specific for CEA and MUC1 were selected according to the references (Ferreira et al., 2006; Wen et al., 2016). And all synthetic oligonucleotides were ordered from Takara Bio (Dalian, China), and the sequences were listed below (from 5′ to 3′).

CP1: SH- CCAGGGTATCCAAAGGATCAACTGC;
CP2: SH- CCACGAATTGAATAAGCTGGTATCGTGG;
AP1: GCAGTTGATCCTTTGGATACCCTGG;
AP2: CCACGATACCAGCTTATTCAATTCGTGG;
S1: SH-TTAGCAGTTGATCCTTTGGATACCCTGG;
S2: SH-TACCCACGATACCAGCTTATTCAATTCGTGG.

Scanning electron microscopy (SEM) images were recorded on a QUANTA FEG 250 thermal field emission SEM (FEI Co., USA). And all electrochemical measurements were performed on a CHI 760D workstation (Chenhua, Shanghai, China).

2.2. Preparation of metal ion electrochemical labels

The synthesis of DNA–Au/BSA–metal ions was experimented at room temperature. Firstly, 40 mg BSA was dispersed into ultrapure water (10 mL) with continually magnetic stirring. And then 10 mL of $HAuCl_4$ solution (10 mM) was mixed with the prepared BSA solution under magnetic stirring for 10 min. Finally, 40 mg AA was dispersed into the obtained mixture with which reacting for 10 min. The final gained product was reserved at 4 °C after centrifuged and washed with ethanol and ultrapure water for many times.

The solid product of Au/BSA was formulated as solution (40 mg/mL). Aiming to load numerous metal ions, the aforesaid Au/BSA solution (0.5 mL) was respectively mixed with 10 mL of 10 mM $Pb(NO_3)_2$ and $Cd(NO_3)_2$ solution with magnetic stirring for 24 h. After being centrifuged and washed with ultrapure water many times, the two different compounds were respectively added into 1.0 mL ultrapure water.

Before the following experiments, the two DNA sequences (S1 and S2) were separately heated at 95 °C for 5 min, and slowly cooled down to room temperature for 1 h. For immobilization of DNA, 1.0 mL S1 (1 μM) solution was mixed with the Au/BSA–Pb^{2+} and respectively the same volume of S2 (1 μM) solution into the Au/BSA–Cd^{2+}. Then the two obtained products were shaken for 5 h. After centrifugation and washed with DNase and RNase free water for many times, the obtained products were re-dispersed into DNase and RNase free water (5 mL).

2.3. Sensing procedure

Prior to experiments, the two capture DNAs (CP1 and CP2) and aptamers (AP1 and AP2) were separately heated at 95 °C for 5 min, and slowly cooled down to room temperature for 1 h.

The preparation of the μ-PADs is displayed in Supporting information (Scheme S1 and S2). Firstly, 10 μL CP1 and CP2 (2 μM) were respectively dropped onto the working zone of μ-PADs and incubated for 12 h at room temperature. And the working zone of μ-PADs was washed with PBS and blocked by using 1 mM MCH for 2 h to eliminate the nonspecific binding effect and block the left active groups, and MCH could also stand up the CPs improving their hybridization efficiency with the APs. After being washed with PBS, 10 μL AP1 and