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Ultrasensitive electrochemical detection of protein tyrosine kinase-7 by gold nanoparticles and methylene blue assisted signal amplification

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ABSTRACT

We present here an ultrasensitive and simple strategy for protein tyrosine kinase-7 (PTK7) detection based on the recognition-induced structure change of sgc8 aptamer, and the signal change of methylene blue (MB) that interacted with sandwiched DNA complex. To construct such a sensor, an homogeneous nano-surface was formed firstly on the glass carbon electrode (GCE) by using negatively charged Nafion (Nf) as the inner layer and positively charged gold nanoparticles ((+)AuNPs) as the outer layer, followed by the immobilization of sgc8 aptamer based on Au–S bond. In the presence of helper probe (HP), sandwiched DNA complex was formed between the sgc8 aptamer and the DNA modified gold nanoparticle probe (DNA-AuNPs). Then, a strong current signal was produced due to the capture of abundant MB molecules by both the sandwiched DNA complex and the multiple DNAs that modified on AuNPs surface. However, the specific binding of sgc8 aptamer with PNK7 would trigger a structure transition of it, and directly prevented the following formation of sandwiched structure and the capture of MB. Thus, PTK7 detection could be realized based on monitoring the signal reduction of MB upon incubation of sgc8 aptamer with PTK7. Under optimal conditions, a low detection limit of 372 fM was obtained for PNK7 detection. Due to the employment of sgc8 aptamer, the proposed biosensor exhibited high selectivity to PNK7. Moreover, satisfactory results were obtained when the proposed method was applied for PNK7 detection in cellular debris.

1. Introduction

Protein tyrosine kinase-7 (PKT7), as one type of cell membrane protein, is directly associated with human diseases such as acute myeloid leukemia (AML), colon cancer, gastric cancer, lung cancer and so on (Endoh et al., 2004; Gorringe et al., 2005; Kampen et al., 2011; Shangguan et al., 2008). PTK7 also plays vital roles in numerous physiological functions including molecular recognition, energy transduction and ion regulation (Arinaminpathy et al., 2009; Grimm et al., 2011). Thus, developing novel, sensitive and effective strategies for PKT7 detection is of great importance in clinical diagnosis.

Aptamers are oligonucleotide (DNA or RNA) receptors that obtained in vitro by an evolution process named systematic evolution of ligands by exponential enrichment (SE-LEX) from larger and om-sequence nucleic acid libraries (Ellington et al., 1990; Tuerk et al., 1990). Such aptamers are highly specific to the corresponding targets, and they possess the merits such as easy preparation, thermal stability, reusability and so on. Due to the properties of these aptamers, a number of aptamer-based biosensors have been developed for the quantification of proteins coupling with colorimetric (Su et al., 2013; Wang et al., 2015; Zheng et al., 2014), fluorescent (Feng et al., 2013; He et al., 2012; Wei et al., 2015) and electrochemical (Gui et al., 2014; Jiang et al., 2015; Yu et al., 2015; Zhuang et al., 2014) strategies. Among these biosensors, electrochemical aptasensors have attracted increased research interest because of the merits of them including low cost, easy operation, high sensitivity and good selectivity.

Due to the large surface area and excellent biocompatibility, gold nanoparticles (AuNPs) have been shown to be an excellent candidate for the preparation of biosensors. Thereinto, in electrochemical strategies, AuNPs are mainly utilized for the modification of DNA (Cui et al., 2015; Dong et al., 2012; Wang et al., 2014) and the immobilization of protein (Liu et al., 2014 and Xu et al., 2012), or used to improve the sensitivity of the biosensors due to the high conductivity of them (Li et al., 2015a, 2015a, 2015b; Wang et al., 2013). Methylene blue (MB), as one type of the redox indicators, can interacts with proteins and lipids, and also has high interact capability with single-stranded DNA (ssDNA) that contained high GC contents or double-stranded DNA (dsDNA) based on electrostatic adsorption, such properties of MB allow the great

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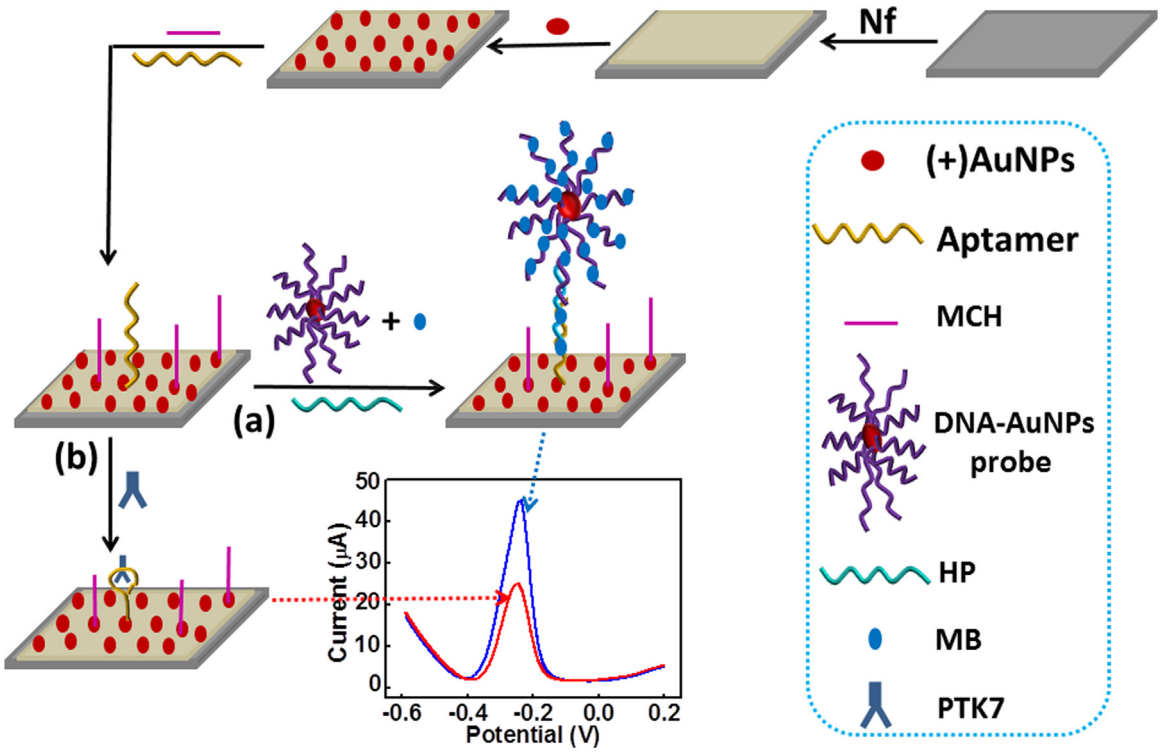

Fig. 1. The scheme of PTK7 detection based on the signal change of MB.

application of it as an excellent redox indicator in electrochemical biosensors preparation (Chen et al., 2008; Li et al., 2015b; Kong et al., 2014; Rohs et al., 2000; Tuite et al., 1994).

To expand the application of MB for biomolecules detection, we reported here an ultrasensitive and simple electrochemical sensor for PTK7 detection based on monitoring the signal change of MB before and after the incubation of sgc8 aptamer with PTK7. As shown in Fig. 1, an homogeneous nano-surface was formed firstly on the glass carbon electrode (GCE) by using negatively charged Nafion (Nf) as the inner layer and about 4 nm of positively charged gold nanoparticles ((+)AuNPs) as the outer layer to enlarge the electrode surface for more aptamer immobilization. Then, numerous sgc8 aptamer strands were effectively immobilized onto above mentioned nano-surface based on Au–S bond. Subsequently, sandwiched DNA complex was formed between the sgc8 aptamer and DNA modified gold nanoparticle probe (DNA-AuNPs) in the presence of helper probe (HP), and a strong current signal of MB was obtained due to the capture of abundant MB on such sandwiched DNA complex. However, the specific binding of PTK7 with sgc8 aptamer in advance would trigger the structure change of sgc8 aptamer, accordingly prevented the following formation of sandwiched structure and the capture of MB. Thus, PTK7 detection could be realized based on monitoring the current signal change of MB oxidation peak by using differential pulse voltammetry (DPV) experiments. Here, about 4 nm of (+)AuNPs with high conductivity and large surface area, was utilized to immobilize the sgc8 aptamer for the first time, which can greatly enhance the amount of aptamer that immobilized on GCE surface, and accordingly in favor of the formation of more sandwiched DNA complex that used for MB capture. In addition, the introduction of (+)AuNPs made the nano-surface positively charged, which would has an electrostatic repulsion to positively charged MB, and accordingly minimized the undesired adsorption of MB on the GCE surface. Thus, the use of (+)AuNPs could effectively improve the accuracy of the sensor for PTK7 detection. Moreover, the proposed method was highly specific for PTK7 detection because of the application of sgc8 aptamer.

2. Experimental section

2.1. Preparation of (+)AuNPs and DNA-AuNPs probes

(+)AuNPs were prepared according to our previous method based on the reduction of 15 mL of $HAuCl_4$ (1.0 mM) using 2 mL of $NaBH_4$ (100 mM) in the presence of 2 mL of CTAB (10 mM) (Li et al., 2015b). The sizes of such (+)AuNPs characterized by TEM (Fig. 2A) and HRTEM (Fig. 2B) were about 4 nm. Another type of AuNPs (16 nm) that used for the preparation of DNA-AuNPs probes and the DNA-AuNPs probes were prepared according to our previous method (Miao et al., 2011).

2.2. Fabrication of DNA-AuNPs/aptamer/(+)AuNPs/Nf modified electrode

Before modification, the glass carbon electrodes (GCE, φ=3 mm, CHI) were polished with 1.0, 0.3, 0.05 μm alumina slurry continuously, and then rinsed with deionized water and sonicated in an ethanol/water bath for 5 min. Then, 5 μL of 1.0 wt% Nf was dipped onto the GCE surface. Subsequently, the Nf/GCE was immersed into (+)AuNPs solution (10.6 nM) and incubated for 90 min at room temperature (RT). After rinsing with deionized water to remove the excess and unreacted regent, the modified electrodes were incubated in aptamer solution over night at RT. Then, the aptamer/(+)AuNPs/Nf/GCE was immersed in 2.0 mM of MCH for 1.5 h to reduce the nonspecific DNA adsorption and to optimize the orientation of the aptamer. At last, the sandwiched DNA complex was obtained on the GCE surface based on the incubation of aptamer/(+)AuNPs/Nf/GCE with DNA-AuNPs solution for 1 h in the presence of helper probe (HP). Before using, the modified electrodes were kept at 4 °C.