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Analytical Methods

Sensitive and rapid aptasensing of chloramphenicol by colorimetric signal transduction with a DNAzyme-functionalized gold nanoprobe

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ABSTRACT

By combination of the aptamer biorecognition with the colorimetric signal transduction of a DNAzyme-functionalized nanoprobe, a new biosensing method was developed for the rapid and sensitive detection of chloramphenicol (CAP). The nanoprobe was prepared through the functionalization of gold nanoparticles with the complementary oligonucleotide against aptamer and high-content hemin/G-quadruplex DNAzyme. When one-step incubating the nanoprobe and CAP at a constructed aptamer-magnetic bead (MB) biosensing platform, due to the competitive biorecognition reaction, the nanoprobes related with CAP amounts were quantitative captured onto the MB surface. Based on the catalytic reaction of the peroxidase-mimicking DNAzyme, a colored substance was produced for the colorimetric signal transduction of the method. Due to the great signal amplification of the nanoprobe, a very low detection limit down to 0.13 pg/mL was obtained. Considering the excellent performance of the aptasensing method and satisfactory results for milk sample experiments, it indicates good reliability for practical applications.

1. Introduction

Recently, the abuse of antibiotics has caused serious antibiotic residue pollution in environment and food chain (Gaudin, 2017; Lee, Chung, Chung, & Lee, 2007). So the development of analytical methods that are able to sensitively and selectively determine antibiotics is in high demand. In this regard, the aptasensor, which is based on the combination of the specific biorecognition of aptamer with the conventional biosensing technique, has shown powerful capabilities due to its unique characters such as high selectivity, low cost, convenient operation and small sample-consumption (Kim, Raston, & Gu, 2016; Lan, Yao, Ping, & Ying, 2017; Taghdisi, Danesh, Nameghi, Ramezani, & Abnous, 2016; Yang et al., 2017).

Commonly, various analytical techniques including spectrometric (Emrani et al., 2016; Kim & Lee, 2017; Taghdisi et al., 2016), electrochemical (Liu, Lai, Zhang, & Yu, 2017; Yin et al., 2017), photoelectrochemical (Li, Tian, Yuan, Wang, & Lu, 2017) and electrochemiluminescence (Feng et al., 2016) methods can be employed for the signal transduction of biosensors. Among them, colorimetric methods possess attractive advantages like cheap-instrument requirement and direct signal readout even with naked eyes (Bai et al., 2015; Kim, Lee, Min, Lim, & Jeong, 2014; Luan, Gan, Cao, & Li, 2017; Miao, Gan, Ren et al., 2015; Ramezani, Danesh, Lavaee, Abnous, & Taghdisi, 2015). Currently, there are two main categories for the colorimetric signal transduction of biosensors: (1) one is based on the aggregation of noble-metal nanoparticles which induces the change of their plasma absorption spectra (Bai et al., 2015; Ramezani et al., 2015); (2) the other is based on the catalytically chromogenic reaction of various enzymes or mimic enzymes (Kim et al., 2014; Luan et al., 2017; Miao, Gan, Ren et al., 2015). Since the enzymatically catalytic signal amplification can lead to higher sensitivity, the latter strategy is more popularly adopted in applications.

As an important peroxidase-mimicking enzyme, the hemin/G-quadruplex DNAzyme has gained considerable interest in recent years (Deng, Zhang, Zhou, & Zhou, 2008; Teller, Shimron, & Willner, 2009). In the presence of hydrogen peroxide, such an artificial nucleic acid enzyme can effectively catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) to generate a colored product. In comparison with protein peroxidases, it possesses advantages including better stability, easier synthesis and more convenient in manipulation, modification and synthesis. To date, this DNAzyme has been frequently employed as an outstanding catalytic component in a large variety of biosensing systems (Fu, Li, & Park, 2009; Gong et al., 2015; Luo et al., 2014; Wang, Zhao, Bao, & Dai, 2016; Xu, Shen, Li, Zhu, & Zhou, 2017;

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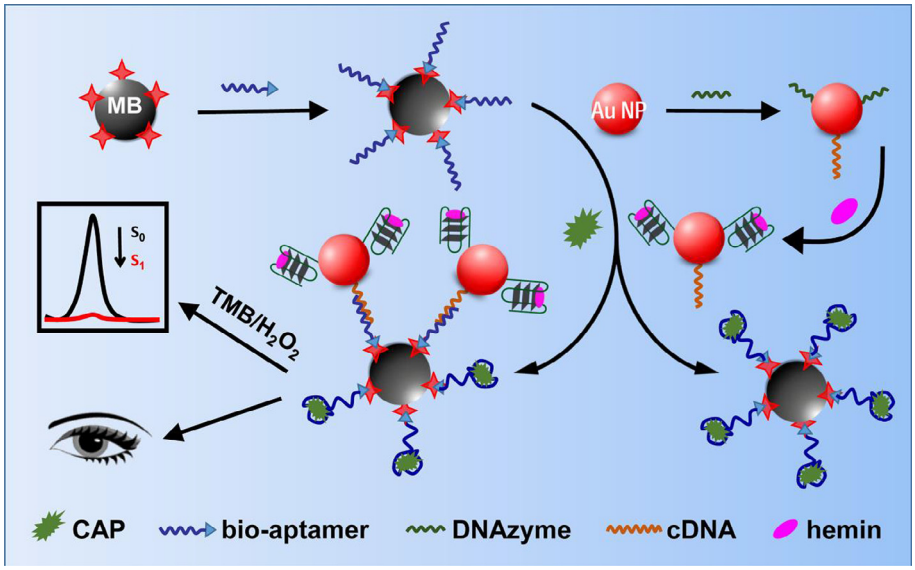

Fig. 1. Schematic representation of the competitive aptasensing of CAP based on the colorimetric signal transduction with the hemin/G-quadruplex DNAzyme functionalized Au NP nanoprobe.

Yan et al., 2015; Zhou et al., 2009).

To fulfill the accurate detection of low-level analytes, furthermore, various nanoprobes prepared by loading high-content signal labels on a large variety of nanomaterials have been extensively designed recently. When employed for signal transduction, the multi-label signal amplification of these nanoprobes greatly improved the analytical sensitivity of biosensors (Lei & Ju, 2012; Song et al., 2010). As gold nanoparticle (Au NP) possesses unique properties such as narrow size distribution, excellent biocompatibility and easy surface modification, it often serves as an ideal nanocarrier to load different kinds of signal labels for the nanoprobe preparation (Cao, Ye, & Liu, 2011; Fu et al., 2009; Lai, Zhang, Tamanna, & Yu, 2014; Lin et al., 2015; Luo et al., 2014; Zhou et al., 2009). In contrast to the conventional single-label probes, these nanoprobes with multi-label signal amplification could greatly improve the signal transduction and analytical sensitivity of biosensors.

Herein, this work designs a high-DNAzyme labeled Au NP probe. By combining its colorimetric signal transduction with a competitive biorecognition reaction at an aptamer-conjugated magnetic bead (MB) platform, a new biosensing method for the selective and rapid detection of the antibiotic of chloramphenicol (CAP) is developed (Fig. 1). The nanoprobe is prepared through the sulfhydrylation assembly of the complementary oligonucleotide against CAP-aptamer (cDNA) and high-content hemin/G-quadruplex DNAzyme on the surface of Au NPs. Based on the one-step competitive reaction between the aptamer-CAP target biorecognition and the aptamer-cDNA hybridization at the MB-assay platform, the gold nanoprobes can be quantitatively captured onto the MB surface, thus producing corresponding colorimetric signal transduction through the enzymatically catalytic reaction of the DNAzyme labels. Owing to the high-efficient signal amplification of the nanoprobe, this method shows a very high sensitivity even rivaling with many electrochemical biosensing methods.

2. Materials and methods

2.1. Reagents and materials

MBs (4.0–4.5 μm in diameter) modified with streptavidin were purchased from Charm Biotech Corporation (USA). Bovine serum albumin (BSA) and TMB were purchased from Sigma-Aldrich Co., Ltd. CAP, thiamphenicol (TAP), florfenicol (FF), kanamycin (Kana), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and hemin were purchased from Aladdin Co. Ltd (Shanghai, China). All other reagents were of analytical grade and directly used without further purification. The oligonucleotides of CAP-aptamer (1), G-quadruplex DNAzyme (2) and cDNA strand (3) with the following sequences were synthesized by Sangon Biotechnology Co. Ltd (Shanghai, China): (1) 5′-biotin-ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTA G-3′; (2) 5′-(SH)-$(CH_2)_6$-AAAAAA GGG TAG GGC GGG TTG GG-3′; (3) 5′-(SH)-$(CH_2)_6$-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA CTA CCA CCG ACT CGC C-3′.

A 10 mM pH 7.0 Tris-HCl buffer containing 100 mM NaCl, 5 mM $MgCl_2$ and 5 mM KCl was prepared as the working solution. Hemin was dissolved in 1% DMSO containing 0.2 M KCl, which was used as the stock solution and then diluted to the required concentration with the Tris-HCl buffer. A pH 5.0 citrate buffer containing 26.6 mM citric acid, 51.4 mM Na_2HPO_4 and 40 mM KCl was used for the preparation of the chromogenic solution.

2.2. Construction of the MB-assay platform

First, 20 μL of the streptavidin-coated MBs at the concentration of 10 mg/mL were transferred into a 2-mL centrifuge tube. These beads were washed twice with 100 μL of pH 7.0 Tris-HCl and then re-suspended in 100 μL of the Tris-HCl buffer. Subsequently, 10 μL of the biotinylated CAP aptamer (10 μM) were added. After 60 min mixing at room temperature, the product was collected by magnetic separation and then suspended in 2% BSA for 30 min incubation. After washing with Tris-HCl buffer for three times, the resulting aptamer-conjugated MBs were finally obtained as the assay platform and suspended in 300 μL of pH 7.0 Tris-HCl.

2.3. Preparation of the gold nanoprobe

First, Au NPs with an average diameter of 13 nm were prepared with the conventional citrate-reduction method (Lai, Yan, & Ju, 2009). Then, 1.0 mL of the colloidal Au NPs were adjusted to pH 8.5 with 0.1 M K_2CO_3, and 5 μL of the sulfhydrylated cDNA (10 μM) containing 5 mM TCEP and 40 μL of the sulfhydrylated DNAzyme (20 μM) containing 5 mM TCEP were successively added into the above colloidal solution. This mixture was gently stirred at room temperature for 16 h. After that, a 1.0 M NaCl solution was dropwisely added into the solution until its final concentration became to 0.1 M. After ageing in this condition overnight, the product was collected by 15 min centrifugation at 10,000 rpm and then mixed with 800 μL of 10 μM hemin to conduct a 2-