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A multiple signal amplified colorimetric aptasensor for antibiotics measurement using DNAzyme labeled Fe-MIL-88-Pt as novel peroxidase mimic tags and CSDP target-triggered cycles

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ABSTRACT

An ultrasensitive colorimetric aptasensor was developed for antibiotics detection, with chloramphenicol (CAP) as model target, using DNAzyme labeled Fe-MIL-88-Pt as novel peroxidase mimic signal tags and target-triggered circular strand-displacement polymerization (CSDP) for signal amplification. The system consists of two components which can partially hybridize with each other: one is capture probe which was formed through immobilizing hairpin DNA containing aptamer sequence on magnetic beads (MB-cDNA), another is signal tag which was constructed through labeling single strand DNAzyme (G-quadruplex/Hemin) which can partially hybrid with cDNA on platinum nanoparticles functionalized Fe-MIL-88 (MIL-88-Pt-DNAzyme). All components of MIL-88, Pt and DNAzyme in the tag can act as peroxidase mimic to triply catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 into a blue-colored oxidized TMB (oxTMB) for the colorimetric readout. Thus distinctive signal can be observed by naked eye even in presence of 0.02 nM tags. In the presence of target and primer, cDNA loop can open to form cDNA/CAP intermediates, enabling primer to hybridize with the exposed sequences of the cDNA, which initiated target assisted CSDP recycles. Then numerous signal tags were released into supernatant to catalyze TMB for color development. There was a liner relationship between the absorbance and the concentration of CAP in the range of 0.1 pM (0.0323 pg/mL) to 1000 pM (323 pg/mL) with the detection limit of 0.03 pM (0.0097 pg/mL). The ultra-high sensitivity was ascribed to the multiplex catalytic activities from the tags and CSDP based signal amplification. Furthermore, this method can produce signals being observed by naked eye to facilitate in-situ detection and be further extended to detect other antibiotics in food just by simply replacing cDNA on the sensing system.

1. Introduction

Recently, colorimetric aptasensor has undergone rapid development as a valuable tool in food safety and environmental protection owing to the unique superiority such as high sensitivity and selectivity, rapid feedback and visible inspection [1,2]. During recent years, our group has successfully developed a number of colorimetric aptasensors based on target substitution reaction to detect antibiotics in foods with high sensitivity [3–5]. Normally, in these works, the colorimetric assays usually employed horseradish peroxidase or enzyme linked polymer to catalyze substrates 3,3′,5,5′-tetramethylbenzidine (TMB) for color development. However these natural enzymes can inevitably suffer from some intrinsic defects, such as high price in extraction, demanding long reaction condition, and susceptible stability due to protein denaturation [6]. Thus in the work, we hope to employ some more stable and cheaper substances as mimic enzyme for signal amplification. As alternative to the natural enzyme biocatalyst, a serious of artificial enzyme with surprising potential has gained tremendous attention including Au nanoclusters [7], Fe_3O_4 nanoparticles [8], grapheme oxide [9], Pt nanoparticles (Pt NPs) [10], and metal organic frame (MOF) [11,12]. Among them, MOF has received intensive attention, which mainly because the large specific area and high porosity are beneficial for accomplishing effective catalytic activity. However, the catalytic activity of MOF has rarely been explored.

Composite nanostructures with two or several components have caused serious concern owing to their remarkably optical and catalytic

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performance compared to their individual components [13]. Various composite nanostructures based on MOF have been developed to improve the catalytic activity [14,15]. For example, Liu's group has shown the Au nanoparticles decorated on Fe-MIL-88 possess a much higher catalytic performance than do the single Fe-MIL-88. The well synergetic effect causes the lower detection limit with 11.4 nM for DNA detection [14]. Therefore, the incorporation of platinum nanoparticles (Pt NPs) and Fe-MIL-88 may be an outstanding catalyst for practical applications. Currently, G-quadruplex/Hemin, an artificial nucleic acid enzyme with superior peroxidase-mimicking activity, has attracted special efforts in colorimetric biosensors [5,16]. Moreover, the G-quadruplex/Hemin (DNAzyme) with thiol group can be easily labeled on MIL-88-Pt through Pt-S self-assembly. Thus the resulted MIL-88-Pt-DNAzyme composite as signal tag not only possesses triple catalytic activity to TMB for sensitive color development but also can be easily connected to magnetic capture probes through hybridization reactions to establish the detection probes. As far as we know, there were few reports for development of signal tags consisting of noble metal, MOFs and DNAzyme.

In order to further improve the sensitivity, circular strand-displacement polymerization (CSDP) based target recycling was introduced. CSDP, as a strategy for signal amplification, has been specifically focused in developing high sensitive aptasensor and has recently attracted considerable attention in target recycling amplification [17–19]. This technique uses a new lengthening strand to displace the target into supernatant to initiate a new polymerization cycle under the aid of Bst polymerase. CSDP can be conveniently applied for designing aptamer sensors because it does not require any specific recognition site. Wang and her co-workers developed a sensitive fluorescence method based on isothermal strand-displacement polymerization reaction induced by polymerase for single-strand DNA detection with a detection limit of 6.4×10^{-15} M [20]. Such lower target detection limit maybe ascribe to the CSDP based target recycling. Therefore, CSDP was an outstanding signal amplified method in terms of designing biosensors.

During these years, antibiotics residues in animal-derived foods have increasingly threatened public health [21]. The accumulation of chloramphenicol (CAP) in bodies can cause serious side effects, e.g. severe aplastic anaemia, cardiovascular collapse disease and “grey syndrome” [22,23]. Thus it's urgent to develop sensitive and in-situ analytical assays for antibiotics detection in foods. Thus, based on the research background above, we developed a multiplex signal amplified colorimetric aptasensor for CAP detection using Fe-MIL-88-Pt-DNAzyme as novel peroxidase mimic with target-triggered CSDP signal amplification. The scheme was shown in Scheme 1. Firstly, capture probe (MB-cDNA) was synthesized by fixing hairpin DNA (cDNA) containing aptamer sequence on magnetic beads (MB). Furthermore, signal tag (MIL-88-Pt-DNAzymes) was synthesized through DNAzymes containing the partial complementary sequence of cDNA and G-quadruplex/Hemin modifying on MIL-88-Pt (Fe). Finally, the detection system was formed through the hybridization between cDNA and DNAzymes on the capture probe and signal tag. After introducing CAP and primer into the system, the hybrid double stranded DNA would unwind owing to the higher affinity of cDNA towards target than DNAzymes. Subsequently, the primer would hybridize with the sequences on the stem part of the cDNA, triggering a polymerization for forming a new double stranded DNA with replacing the target into supernatant in the assist of the Bst DNA polymerase. Afterwards, numerous signal tags with highly peroxidase-like activity would be obtained after magnetic separation and they can effectively catalyze the classical peroxidase substrate TMB in the presence of H_2O_2, producing an obviously visible blue color. The novelties of the work were as following: we fabricate a triple signal amplification strategy based on MIL-88-Pt-DNAzyme composite as novel peroxidase mimic and CSDP for signal amplification; and all components of MIL-88, Pt and DNAzyme can catalyze TMB-H_2O_2 for color development. The proposed colorimetric aptasensor exhibits excellent analytical performance towards CAP detection, which might provide a promising sensing platform for organic analysis and environmental monitoring.

2. Materials and methods

2.1. Materials and reagents

Chloramphenicol (CAP), Kanamycin (KANA), streptomycin (STR), oxytetracycline (OTC), gentamicin sulfate (GS) and chlortetracycline (CTC) were purchased from sigma (St. Louis, MO, USA). $FeCl_3$ $6H_2O$, H_2O_2 and acetic acid were obtained from Chongqing Pharmaceutical Co., Ltd. Keyi Assay Glass Branch (Chongqing, China). Amino-functionalized magnetic beads (MB) were purchased from Enriching Biotechnology LTD (Shanghai, China). Polyvinylpyrrolidone (PVP, Mw = 55000), terephthalic acid (BDC), Hemin, dimethyl sulfoxide (DMSO) and phosphate buffer saline (PBS, pH 7.4, 0.1 M KH_2PO_4-K_2HPO_4, 0.1 M KCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroplatinic acid (H_2PtCl_6, 37 wt% Pt), hydrogen peroxide (H_2O_2, 30%) and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The blocking buffer solution 6-mercapto-1-hexanol (MCH), glutaraldehyde (25% aqueous solution) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Aladdin Co., Ltd (Shanghai, China). DNA-500 kits, containing DNA-500 marker, DNA-500 separation buffer, 25 bp DNA ladder and TE buffer solution (pH = 8.0) were obtained from Shimadzu Co., Ltd. (Shimadzu, Kyoto, Japan). DNA polymerase (Bst) and the mixture of deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd. (Canada). All other reagents were analytical grade and were used without further purification, and double-distilled water was used throughout the study. All DNA oligonucleotides used in this paper were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China) and purified using high performance liquid chromatography, and the sequences of DNA oligonucleotides are the sequences of the oligonucleotides were given below (the italics in cDNA refer to the aptamer sequence of CAP [24] and the bold letters in cDNA refer to the complementary sequences of primer):

cDNA: 5′-CTA GTA *ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTA G***AC AAC C**-$(CH_2)_6$-NH_2–3′

DNAzyme: 5′-TCA CTG AAG TTA CTA GAG GGA GGG AGG GAG GGA-$(CH_2)_6$-SH-3′

Primer: 5′-GGT TGT-3′

2.2. Apparatus

Scanning electron micrographs (SEM) were obtained with a S3400N scanning electron microscope (Hitachi, Tokyo, Japan). The transmission electron microscopic (TEM) image was obtained with a H600 transmission electron microscope (Hitachi, Tokyo, Japan). Infrared spectra were recorded with a Nicolet 6700 FT-IR spectrophotometer (Madison, Wisconsin, USA). The UV–Vis spectra were recorded on a UV-1800 spectrophotometer (Shimadzu, Japan). The powder X-ray diffraction (XRD) characterization was performed using X-ray diffraction (Bruker, D8 Focus, Karlsruhe, Germany) with Cu Kα radiation at room temperature. DNA analysis was performed by microchip electrophoresis system (MCE-202 MultiNA, Shimazu, Japan). Dynamic Light Scattering (DLS) was carried out by the Malvern zetasizer Nano ZS90, Malvern instruments Ltd., (MalvinCo., UK).

2.3. Synthesis of capture probes (MB-cDNA)

The capture probes (MB-cDNA) were prepared according to literature with further modification [25]. Originally, 200 μL 10 μM cDNA containing aptamer sequences was denatured by heating for 5 min at 95 °C in a water bath and cooled to room temperature, ensuring to