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A homogeneous and "off–on" fluorescence aptamer-based assay for chloramphenicol using vesicle quantum dot-gold colloid composite probes

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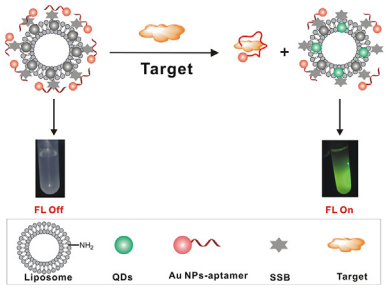
HIGHLIGHTS

- Homogeneous and "off–on" fluorescence aptamer-based assay was developed to detect chloramphenicol (CAP) residues in food.
- This probe was fabricated based on a vesicle QDs signal tracer (SSB/L-QD) combining with Au-Aptamer.
- The detection mechanism was based on FRET with high specificity.
- The results for CAP detection in the milk samples agreed well with those from ELISA, while detection limit down to 0.3 pM.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a novel homogeneous and signal "off–on" aptamer based fluorescence assay was successfully developed to detect chloramphenicol (CAP) residues in food based on the fluorescence resonance energy transfer (FRET). The vesicle nanotracer was prepared through labeling single stranded DNA binding protein (SSB) on limposome-CdSe/ZnS quantum dot (SSB/L-QD) complexes. It was worth mentioning that the signal tracer (SSB/L-QD) with vesicle shape, which was fabricated being encapsulated with a number of quantum dots and SSB. The nanotracer has excellent signal amplification effects. The vesicle composite probe was formed by combining aptamer labeled nano-gold (Au-Apt) and SSB/L-QD. Which based on SSB's specific affinity towards aptamer. This probe can't emit fluoresce which is in "off" state because the signal from SSB/L-QD as donor can be quenched by the Au-aptas acceptor. When CAP was added in the composite probe solution, the aptamer on the Au-Apt can be preferentially bounded with CAP then release from the composite probe, which can turn the "off" signal of SSB/L-QD tracer into "on" state. The assay indicates excellent linear response to CAP from 0.001 nM to 10 nM and detection limit down to 0.3 pM. The vesicle probes with size of 88 nm have strong signal amplification. Because a larger number of QDs can be labeled inside the double phosphorus lipid membrane. Besides, it

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was employed to detect CAP residues in the milk samples with results being agreed well with those from ELISA, verifying its accuracy and reliability.

1. Introduction

Antibiotic residues in foodstuff have been attached widespread attention due to their health hazard [1–3]. For example, chloramphenicol is not approved for use in food-producing animals in the United States. A number of methods have been developed for the detection of Antibiotic residues, including fluorescence biosensor [4], colorimetric aptasensor [5], and electrochemical analysis [6]. The detection limit of some of the methods can reach to ng/Kg [6]. However, in many cases, the above assays are based on heterogeneous detection strategy which means some necessary separation procedures are needed for pretreatment after target was added into the reaction system. Moreover, some immobilization, and washing steps are needed which made some of the assays time-consuming and tedious [7–9]. On the contrary, homogeneous assays are normally performed in a single reaction system, which means that the signal can be triggered and detected without separation after the analyte was added. This can greatly simplify the detection and pretreatment procedures and thus are more attractive [10]. With the use of antibodies or aptamers as affinity ligands, a variety of homogeneous assays have been fundamentally developed [11,12]. Compared with antibody, the aptamer possesses many merits, such as in vitro synthesis, high specificity, and non-immunogenic usage. Above all, the fluorescent aptamer assays in homogenous detection strategy have attracted great attention because of their facile operation, high sensitivity, and high specificity for a variety of targets, which result from higher affinity towards targets than antibody.

Recently, owing to their unique optical properties of high florescence efficiency and photochemical stability, semiconductor quantum dots (QDs) as a kind of excellent fluorescent probe have been broadly used in biological labeling, biomedical detection research, and vitro/vivo imaging [13]. Small-molecule pollutants which combines CAP detection with the QDs has shown innateness advantages of high signal-to-noise ratio, low sample consumption, improved sensitivity, and near-zero background signal in comparison with the conventional ensemble fluorescence measurements [14–16]. In addition, the detection system based on signal “off–on” transition in homogenous phase reaction system is more attractive because the signal can turn on after adding the targets. Nowadays, most of the signal “off–on” detection system in homogenous phase are based on fluorescence resonance energy transfer (FRET). For example, Qian and co-workers utilized QDs and AuNPs composite to develop homogeneous immune assay for the analysis of carcinoembryonic antigen based on FRET [17]. This assay was consisted of polyclonal goat anti-CEA antibody labeled luminescent CdTe quantum dots (QDs) as donor and monoclonal goat anti-CEA antibody labeled gold nanoparticles (AuNPs) as acceptor [17]. In FRET processes, a donor fluorophore is excited by incident light, and if an acceptor is in close proximity, the excited state energy from the donor can be transferred [18]. The transfer efficiency was predicted by Förster to decrease with R, the distance between the two dyes, as $1/(1+(R/R_0)^6$. R_0, the Förster radius, is the distance corresponding to 50% energy transfer and depends on the photo physical properties of the dyes and their relative orientations [19]. For the conventional QDs florescence sensors based on FRET, signal enhancement is usually achieved by the assembly of a number of target molecules onto probes labeled with a single QD, thus the sensitivity is limited [20]. In order to amplify the signal, it's a good choice to fabricate a probe encapsulated with larger numbers of QDs. In the study, we developed a novel kind of vesicle probe which was fabricated by liposome–QD (L-QD) complex. Moreover, with the probe was employed for establishing an assay to detect CAP in homogeneous phase. The liposomes have attractive vesicle structure with size in magnitude of nanometer (30 nm–100 nm) and a double phosphate lipid membrane structure. Thus it can encapsulate a number of QDs (2–10 nm) in the vesicle membrane and emit strong florescence signal [21]. After then, the liposome-QD composite can be labeled with aptamer to fabricate the signal tracer. Moreover, it's a good candidate to fabricate a sensitive homogenous signal tracer.

As shown in Scheme 1, firstly, the vesicle nanotracer was prepared through labeling single stranded DNA binding protein (SSB) on limposome-CdSe/ZnS quantum dot (SSB/L-QD) complexes. It is worth mentioning that SSB has higher specific affinity towards single stranded DNA while not double stranded DNA [22]. Secondly, a novel capture probe was fabricated by labeling CAP aptamer onto Au nanoparticles. Thirdly, a composite probe for CAP was prepared between the vesicle L-QD nanotracer and Au-apt capture probe based on the specific recognition of SSB to aptamer. The composite vesicle probe doesn't emit fluorescence signal which is in “off” state because the fluorescence of the nanotracer as donor can be quenched by the Au NPs-apt as acceptor. Upon mixing the composite probe solution with CAP, the aptamer on the capture probe preferentially bounded with CAP, and then Au-Apt-CAP complex can be released from the composite probe, which can turn the fluorescence signal from “off” to “on”. Moreover, the signal conversion strategy was performed in homogenous phase without separation for pretreatment. In the end, this assay was successfully employed to detect CAP in milk samples with results being comparable with these by commercial ELISA.

2. Experimental

2.1. Reagents and chemicals

The oligonucleotides used in this paper are as the following sequences: ssDNA1,CTA CCA CCG ACT CGC, ssDNA2 (thiolated Apt bound to CAP), 5′$(CH_2)_6$-ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG [23] which were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). Cadmium oxide (CdO, 99.99%), selenium (99.5%), sulfur (S, 99.98%), zinc oxide (ZnO, 99.99%), stearic acid, 1-octadecene (ODE), hexadecylamine (HDA), octadecylamine (ODA), oleic acid (OA) and trioctylphosphine (TOP) and Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4$), CAP, oxytetracycline (OTC), streptomyces erythreus (SE), kanamycin (Kana), chlortetracycline (CTC) and gentamicin sulfate (GS), thiamphenicol (TAP), florfenicol (FF), florfenicol amine (FFa) and single stranded DNA binding protein (SSB) were purchased from Sigma–Aldrich (St. Louis, MO). Hexane, methanol and chloroform were bought from Shanghai Lingfeng Chemical Reagent Co., Ltd. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 4-cholesterol-3-one, 1,2- distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-NH_2) were purchased from Avanti Polar Lipids, Phosphate