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A novel colorimetric sandwich aptasensor based on an indirect competitive enzyme-free method for ultrasensitive detection of chloramphenicol

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ABSTRACT

Analytical methods for detection and quantitation of chloramphenicol in blood serum and foodstuffs arse highly in demand. In this study, a colorimetric sandwich aptamer-based sensor (aptasensor) was fabricated for sensitive and selective detection of chloramphenicol, based on an indirect competitive enzyme-free assay using gold nanoparticles (AuNPs), biotin and streptavidin. The designed aptasensor acquires characteristics of AuNPs, including large surface area and unique optical properties, and strong interaction of biotin with streptavidin. In the absence of chloramphenicol, the sandwich structure of aptasensor forms, leading to the observation of sharp red color. In the presence of target, functionalized AuNPs could not bind to 96-well plates, resulting in a faint red color. The fabricated colorimetric aptasensor exhibited high selectivity toward chloramphenicol with a limit of detection as low as 451 pM. Moreover, the developed colorimetric aptasensor was successfully used to detect chloramphenicol in milk and serum with LODs of 697 and 601 pM, respectively.

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1. Introduction

Chloramphenicol (Chl) is a broad-spectrum antibiotic which is widely used in human, animals and fish farming industry for treatment of Gram-negative and Gram-positive infectious diseases and also as the growth promoters in animals (Yadav et al., 2014; Yan et al., 2015). This drug is the first choice for treatment of patients having severe cephalosporin or penicillin allergy, tetracycline-resistant Vibrio cholera and vancomycin-resistant Enterococcus (Yadav et al., 2014). The excessive levels of Chl in animal-derived foods and human blood has adverse side effects on human such as aplastic anemia, leukemia and gray baby syndrome (BagheriHashkavayi et al., 2015; Pilehvar et al., 2014; Wu et al., 2015). Consequently, simple and accurate methods for detection of chloramphenicol in food stuffs and body fluid are required.

Various techniques have been used for detection of chloramphenicol, including high performance liquid chromatography (HPLC), capillary electrophoresis, gas chromatography–mass spectrometry (GC–MS) and LC–MS (BagheriHashkavayi et al., 2015; Pilehvar et al., 2014; Yadav et al., 2014; Yan et al., 2015). Generally, these analytical methods are expensive, time-consuming and need specialized operator and lengthy turn-around time.

Aptamer-based sensors, aptasensors, have been broadly used in analytical methods. Aptamers are short nucleic acid sequences, obtained by an in vitro technique called SELEX (systematic evolution of ligands by exponential enrichment) (Du et al., 2015; Xiang et al., 2015). They bind to a vast range of targets, such as small molecules, proteins and even cells with high selectivity and affinity (Dong et al., 2014; Zhou et al., 2014). Aptamers could replace antibodies for development of biosensors because of their unique advantages, including ease of synthesis and modifications, lack of immunogenicity and toxicity, low cost and small size (Eissa et al., 2015; Lian et al., 2015; Zhao et al., 2015; Zhou et al., 2014). Due to these features, aptamers have potential application in development of different sensors (Ramezani et al., 2015; Taghdisi et al., 2015).

Recently, gold nanoparticles (AuNPs) have been broadly used in construction of fluorescent, colorimetric and electrochemical aptasensors, owing to their unique advantages, including good

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biocompatibility and conductivity, extremely high extinction coefficient, ease of synthesis and functionalization, chemical stability, large surface area and unique optical and electrical properties (Benson et al., 2015; Ma and Chen, 2015; Wei et al., 2015).

Among the different sensing platforms, colorimetric aptasensors have attracted great attention because of their characteristics, including low cost, simplicity and convenient readout without the need for any precise instrument (Emrani et al., 2016; Miao et al., 2015; Wang et al., 2015).

In this study, a novel colorimetric sandwich aptasensor was designed for detection of chloramphenicol, based on an indirect competitive enzyme-free method using AuNPs, biotin and streptavidin. In this work, a 40 mer ssDNA aptamer, which binds to chloramphenicol with high affinity (Feng et al., 2015), was utilized as targeting agent.

2. Materials and methods

2.1. Materials

The chloramphenicol aptamer (Apt), 5′-ACTTCAGTGAGTTGTCCCACGGTCGGCGAGTCGGTGGTAG-Biotin-3′ and AuNPs binding DNA (ABD), 5′-Biotin-ACTTTCCATTCCTTTTAC-Thiol-3′, were obtained from Bioneer (South Korea). Plasma from rat, cefixime, chloramphenicol, amoxicillin, tetracycline, ciprofloxacin, sodium citrate, sodium tetrachloroaurate(III) ($HAuCl_4$), casein from bovine milk, streptavidin and salmon sperm DNA were purchased from Sigma-Aldrich (USA). Chloramphenicol (Chl)–bovine serum albumin (BSA) conjugate was provided by Nankai Biotech (China). Milk was purchased from Razavi (Iran).

2.2. Synthesis of water resuspended gold nanoparticles

AuNPs were synthesized using the classical reduction of $HAuCl_4$ by citrate, based on the previously reported protocol (Storhoff et al., 1998). Briefly, a 100 mL solution of 1 mM $HAuCl_4$ was boiled and stirred vigorously. 10 mL of a 38.8 mM sodium citrate solution was added quickly to the solution, which led to an obvious color change from light yellow to wine red. After boiling for 15 min, the reaction flask was allowed to cool to room temperature and stored at 4 °C before use. The prepared nanoparticles solution was centrifuged at 14,000*g* for 20 min at 4 °C. The supernatant was discarded and AuNPs were resuspended in ultrapure water. Concentrations of AuNPs were determined based on extinction coefficient of $2.7 \times 10^{8}\ M^{-1}\ cm^{-1}$ at $\lambda = 520$ nm for 14 nm AuNPs. The size, zeta potential and morphology of synthesized AuNPs were evaluated by a particle size analyzer (Malvern, UK) and transmission electron microscopy (TEM) (CM120, Philips, Holland).

2.3. Preparation of ABD-modifed AuNPs

The concentration of AuNPs was determined to be 40 nM. 1 mL AuNPs (40 nM) was mixed with 30 μL ABD for 16 h at room temperature. After this time, the sample was stored at 4 °C.

2.4. Optimization of concentration of Chl-BSA conjugate

Increasing concentrations of Chl-BSA conjugate (0–20 μg/mL, 100 μl) in coating buffer (10 mM Tris–HCl, pH 8) were added to 96-well plates (Nunc-Immuno™ MicroWell™ 96 well solid plates, Sigma-Aldrich) and incubated overnight at 4 °C. The wells were washed two times with washing buffer (10 mM Tris–HCl, 0.05% tween-20, pH 7.4). The free sites of the wells were blocked using 0.25% (W/V) casein in coating buffer for 4 h. Then, 30 nM aptamer in binding buffer (10 mM Tris–HCl, pH 7.4) was added to each well. After incubating for 30 min at room temperature, the wells were rinsed thoroughly with the washing buffer and 60 nM streptavidin was added to each well and incubated for 1 h. Finally, the wells were washed with washing buffer and 40 nM functionalized AuNPs (100 μL) were added to each well. After incubation for 1 h, the wells were washed and A_{520} was recorded using a Synergy H4 microplate reader (BioTek, USA).

2.5. Effect of aptamer concentration on the absorbance of AuNPs

10 μg/mL (100 μL) Chl-BSA conjugate in the coating buffer was added to 96-well plates. After overnight incubation at 4 °C, the wells were rinsed with the washing buffer, followed by blocking the wells using 0.25% (W/V) casein in coating buffer for 4 h. After that, each well was incubated with different concentrations of aptamer (0–60 nM) in the binding buffer for 30 min. Then, the wells were washed with the washing buffer and each well was incubated with 60 nM streptavidin for 1 h. After rinsing the wells, 40 nM modified AuNPs (100 μL) were added to each well and after 1 h, A_{520} was measured.

2.6. Optimization of streptavidin concentration

Increasing amounts of streptavidin (0–120 nM) were added to 96-well plates containing Apt–Chl–BSA complex. After incubation for 1 h, the wells were rinsed and 40 nM modified AuNPs were added to each well. After incubation for 1 h, A_{520} was determined.

2.7. Effect of the concentration of modified AuNPs on the absorbance

Different concentrations of functionalized AuNPs (0–60 nM) were added to 96-well plates containing streptavidin–Apt–Chl–BSA complex. After incubation for 1 h, the wells were rinsed thoroughly with washing buffer and A_{520} was measured.

2.8. Evaluation of the designed colorimetric aptasensor

The interaction of chloramphenicol and the developed aptasensor was analyzed by colorimetric measurement. 120 nM chloramphenicol in the binding buffer (100 μL) was added to a well containing Chl–BSA conjugate, followed by addition of 30 nM aptamer to the well. After incubation for 30 min at room temperature, the well was rinsed and 60 nM streptavidin was added to the well and incubated for 1 h. Then, the well was incubated with 40 nM functionalized AuNPs for 1 h, followed by rinsing the well with washing buffer and absorbance spectra was recorded.

2.9. Quantitative analysis of chloramphenicol based on colorimetric measurement

Increasing concentrations of chloramphenicol (0–360 nM) in the binding buffer were added to 96-well plates containing Chl–BSA conjugate. Then, the wells were treated with 30 nM aptamer for 30 min, followed by rinsing the wells with the washing buffer. Next, the wells were incubated with 60 nM streptavidin for 1 h. The wells were washed and 40 nM modified AuNPs were added to each well. After 1 h, the wells were rinsed and A_{520} was recorded.

2.10. Specificity of the designed colorimetric aptasensor

The selectivity was assessed in the presence of 120 nM chloramphenicol, cefixime, ciprofloxacin, tetracycline and amoxicillin.

2.11. Serum stability of aptamer

Serum stability of aptamer was analyzed using agarose gel

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