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Analytical Methods

Colorimetric aptasensors for determination of tobramycin in milk and chicken eggs based on DNA and gold nanoparticles

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L-Arginine (PubChem CID: 6322)
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ABSTRACT

Colorimetric aptasensors were designed for detection of tobramycin (TOB) based on unmodified gold nanoparticles (AuNPs) and single-strand DNA (ssDNA). In the absence of TOB, the DNA aptamer was coated on the surface of AuNPs to keep it against salt-induced aggregation. In the presence of TOB, aptamer will bind with TOB and detach from the surface of AuNPs because of higher affinities between aptamer and TOB. Then less protection of DNA may result in the aggregation of AuNPs by salt and an apparent color change from red to purple-blue. The developed aptasensors showed a high selectivity and sensitivity for TOB detection. The linearity range and the detection limit were 40–200 nM and 23.3 nM respectively. The validity of the procedure and applicability of aptasensors were successfully used to detect TOB in milk and chicken eggs, and the results were excellent in accord with the values obtained by spectrofluorimetric detection.

1. Introduction

Tobramycin (TOB) is an antibiotic of the aminoglycoside (AG) class, which was primarily used for the treatment of bacterial infections of the human and veterinary. However, incorrect and uncontrolled application of TOB could cause serious irreversible side effects on human, including nephrotoxicity, neuromuscular blocking and hypersensitivity (Santos et al., 2014). Since the price of TOB is low that it is still often widely used in animal husbandry leading to potential residues in the food chain, such as milk, eggs and meat (Derbyshire et al., 2012; Tao et al., 2012). The European Commission has established maximum residue limits (MRLs) of some antibiotics about 200 μg/kg in milk (Arsand et al., 2016). To keep the harmful residues out of the human food chain, it is necessary to develop simple and convenient determination methods of the TOB residues in milk, egg, and meat etc. (McKeating, Couture, Dinel, Garneau-Tsodikova, & Masson, 2016; Yu, He, Fu, Xie, & Gan, 2009).

A variety of literatures reported several traditional and reliable methods for the determination of AGs antibiotic including TOB, for instance spectrofluorimetry (Omar, Ahmed, Hammad, & Derayea, 2015), thin-layer chromatography (Hubicka, Krzek, Woltynska, & Stachacz, 2009), gas chromatography (Preu, Guyot, & Petz, 1998), high performance liquid chromatography (HPLC) (Elzaher & Mahrouse, 2012), liquid chromatography-mass spectrometry (LC-MS) (Arsand et al., 2016), and capillary zone electrophoresis (Ahmed & Ebeid, 2015). But these methods have some disadvantages, such as high detection limit, pre-column derivative, expensive equipment, long testing cycle, trained personnel skill and complicated sample preparation.

Aminoglycoside antibiotics detection is a considerable sample preparation and analytical instrumental analysis challenge because they lack UV chromophores and fluorescence group (Kaufmann, Butcher, & Maden, 2012) and their amino and hydroxyl group tend to establish strong links with matrix protein. In order to accomplish optical detection and improve the lower detection limit, the derivative procedure is

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indispensable (Omar et al., 2015). Although LC-MS by using ion pair reagents was successfully to quantify polar AG in biological samples (Arsand et al., 2016), it is difficult to remove the ion pair reagent and cause the fouling of MS instrument (Zhiyong et al., 2010). Hence, simple and inexpensive alternative methods are needed to detect aminoglycoside antibiotic molecules in animal original foods.

Presently, most methods used to detect molecules require molecular recognition events (Pagba, Lane, Cho, & Wachsmann-Hogiu, 2010). Aptamers are versatile used to molecule recognition because of their high affinity and specificity with target molecule (Sun, Tan, & Zu, 2015). Aptamers are mainly oligonucleotides (single stranded DNA or RNA), which exhibit a number of advantages such as simple synthesis, easy labeling, good stability, and wide applicability (Mairal et al., 2008; Song, Wang, Li, Fan, & Zhao, 2008). Thus, aptamers are becoming promising recognition probes for protein analysis, disease diagnosis, new drug selection, aptasensor and molecular switch development, etc. (Piro, Shi, Reisberg, Noël, & Anquetin, 2016; Ruscito & DeRosa, 2016; Wu et al., 2007).

Small molecules are important targets of aptamers since they possess many biological functions, and their detection is needed for the protection and wellbeing of humans and animals (Pfeiffer & Mayer, 2016). However, aptamers for small molecule targets detection, such as toxins, antibiotics, drug, and heavy metals, are relative difficult because of their low molecular weight and small molecule size (Ruscito & DeRosa, 2016). During the past years, novel colorimetric aptasensor based on aptamer and gold nanoparticles (AuNPs) is becoming a promising alternative to conventional methods for small molecule detection (Soh, Lin, Rana, Ying, & Stevens, 2015).

Gold nanoparticles play a key role in biochemical analysis of low molecular weight chemicals because of its signal amplification (Kim & Gu, 2014; Contreras et al., 2014). In their analysis, the aptamers can adsorb on the gold nanoparticle surface and stabilize the AuNPs, which can control salt-induced aggregation due to surface plasmon resonance coupling among neighboring particles. AuNPs novel optical properties based on distance-dependent lead the color change from red to purple-blue, and which are easily observed by naked eye and quantified by UV–Vis spectrophotometer. Some literatures about antibiotic detection were reported with aptamer and gold nanoparticles (Emrani et al., 2016; Soh et al., 2015; Taghdisi, Danesh, Nameghi, Ramezani, & Abnous, 2016). Some else literatures about electrochemical aptamer-based (E-AB) sensors on a gold microelectrode platform (Schoukroun-Barnes, Wagan, & White, 2014) and Quartz crystal microbalance (QCM) nanosensor about antibiotic determination were published (Yola, Uzun, Özaltın, & Denizli, 2014).

In this article, a highly sensitive and selective colorimetric aptasensor for the detection of tobramycin based on DNA and nanogold was developed. The amount of aptamer adsorbed on the AuNPs surface was changed depended on the small molecule target concentration. Different amount of aptamer, served as the stabilizer of AuNPs, has different preventing aggregation ability in NaCl solution and resulted in different colors, which can be observed by naked eye. UV–Vis absorption spectroscopy was used to quantitative detection. The proposed colorimetric aptasensor was applied to real samples detection of TOB in milk and chicken eggs. The spectrofluorimetric method was employed for TOB detection to evaluate the accuracy of the developed method.

2. Materials and methods

2.1. Materials

The tobramycin aptamer, 5′-GGG ACT TGG TTT AGG TAA TGA GTC CC-3′ (Liu, Wagan, Dávila, Taylor, & White, 2014), was synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Tobramycin (TOB), streptomycin (STR), and kanamycin (KAN) was purchased from Aladdin Industrial Corporation (Shanghai, China). L-glycine (Gly), L-histidine (His), L-serine (Ser), L-arginine (Arg), L-proline (Pro), L-tyrosine (Tyr), L-lysine (Lys), L-tryptophan (Try), L-alanine (Ala), and bovine albumin (BSA) were purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China). Sulfamethoxazole (SMZ), sulfadimethoxine (SDM), sulphachlorpyridazine (SCD), and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroauric acid ($HAuCl_4$), sodium chloride (NaCl), ferric chloride ($FeCl_3$), magnesium chloride ($MgCl_2$), potassium chloride (KCl), and calcium chloride ($CaCl_2$) were purchased from Sinopharm Chemical Reagent Company (Beijing, China). Sodium citrate was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Synthesis of gold nanoparticles and characterization

All glassware for preparation of AuNPs were first dipped thoroughly in aqua regia (3:1 (v/v) HNO_3-HCl), then washed with double distilled water and dried in an oven. AuNPs with a diameter of 13.0 nm were prepared via the reduction reaction of $HAuCl_4$ and sodium citrate (Storhoff, Elghanian, Mucic, Mirkin, & Letsinger, 1998). Briefly, $HAuCl_4$ (1 mM, 200 mL) was heated to reflux under vigorous stirring. Subsequently, sodium citrate (38.8 mM, 20 mL) was added rapidly to the boiling solution, with a color change from pale yellow to deep red within 5 min. The mixture was allowed to reflux for another 15 min and then cooled down slowly to room temperature while stirring continued. The resulting wine-red solution was filtered through a 0.22 μm ultrafiltration membrane (PVDF), which was stored in the 4 °C refrigerator before use (Turkevich, Stevenson, & Hillier, 1951). Concentrations of AuNPs were calculated based on the obtained absorbance at 520 nm and the extinction coefficients (ε) of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ for 13 nm AuNPs (New et al., 2014).

In order to characterize the dispersion of AuNPs aqueous, transmission electron microscopy (TEM) images were acquired on an H-7650 microscope (Hitachi, Japan) operating at 80 kV. The zeta potentials of AuNPs aqueous were also measured with before and after coat by TOB aptamer at 25 °C using a Zetasizer Nano ZS90 (Malvern instruments Ltd., England) equipped with a red (633 nm) laser and an Avalanche photodiode detector (APD).

2.3. Pretreatment of the milk and chicken egg sample

All of milk and the egg samples were purchased from a local supermarket. The milk and the whole egg samples were pretreated with trichloroacetic acid to remove the proteins in terms of the procedure described by Tan and Chen, with a slight modification (Tan & Chen, 2012). A 2% trichloroacetic acid solution and samples were mixed well in a centrifuge tube and ultrasonicated for 30 min. The mixture was centrifuged at 12,000 rpm for 10 min, and the supernatant was filtered through a 0.22 μm ultrafiltration membrane (PVDF) to remove lipids. The filtrate was stored at 4 °C for further detection (Luo et al., 2014). The samples were diluted for 100-fold and spiked with the standard solutions of TOB for validating the effectiveness detection of TOB in real samples.

2.4. Tobramycin detection based on colorimetric technique

In a typical experiment, 50 μL of 10 nM AuNPs and 25 μL of 1 μM aptamer solution were added into a centrifuge tube, mixed well, and incubated for 30 min at room temperature. Then, 25 μL of different concentrations of TOB were added into the assay solutions, mixed thoroughly, and incubated for another 15 min under the same conditions. Subsequently, 10 μL of 1 M NaCl solution was added to centrifuge tube and diluted to 250 μL with ultrapure water. After the solution was equilibrated for 5 min, the resulting solution was transferred to a quartz cell. The absorbance of the above solutions at 520 nm and 620 nm were recorded using a Scinco S3100 UV–Vis Spectrophotometer (Scientific