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Rapid and sensitive detection of cholera toxin using gold nanoparticle-based simple colorimetric and dynamic light scattering assay

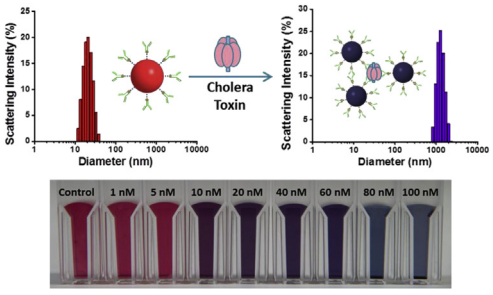
Sadia Afrin Khan*, Jeffrey A. DeGrasse, Betsy Jean Yakes, Timothy R. Croley

Center for Food Safety and Applied Nutrition (CFSAN), U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740, USA

HIGHLIGHTS

- Gold nanoparticle based colorimetric and dynamic light scattering assay was developed for cholera toxin.
- The reported assay is sensitive enough to visually detect as low as 10 nM of cholera toxin.
- This assay is very fast and can specifically recognize cholera toxin over other diarrhetic toxins.

GRAPHICAL ABSTRACT



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ABSTRACT

Herein, a rapid and simple gold nanoparticle based colorimetric and dynamic light scattering (DLS) assay for the sensitive detection of cholera toxin has been developed. The developed assay is based on the distance dependent properties of gold nanoparticles which cause aggregation of antibody-conjugated gold nanoparticles in the presence of cholera toxin resulting discernible color change. This aggregation induced color change caused a red shift in the plasmon band of nanoparticles which was measured by UV–Vis spectroscopy. In addition, we employed DLS assay to monitor the extent of aggregation in the presence of different concentration of cholera toxin. Our assay can visually detect as low as 10 nM of cholera toxin which is lower than the previously reported colorimetric methods. The reported assay is very fast and showed an excellent specificity against other diarrhetic toxins. Moreover, we have demonstrated the feasibility of our method for cholera toxin detection in local lake water.

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1. Introduction

Cholera toxin, secreted by *Vibrio cholerae*, is a protein enterotoxin with a hexametric structure consisting of one enzymatically active A-subunit which is non-covalently linked to a pentameric core of five identical B-subunits [1,2]. Both subunits are required to initiate the severe diarrhea, vomiting, and acidosis associated with the intestinal infection cholera [2,3]. According to World Health Organization (WHO), there are an estimated 3–5 million cholera cases and 100,000–120,000 deaths due to cholera every year [4]. Cholera can become an epidemic if proper treatments are not readily available and the disease can be fatal: an outbreak in Haiti killed almost 5000 people [5,6]. Even though most of the cholera cases are in developing countries, the disease can enter the USA through importation of foods where cholera is endemic and by travelers from the affected regions [7–9]. Because this toxin easily spread by the ingestion of contaminated food and water and can spread within very short time, the use of cholera toxin as a

* Corresponding author. Tel.: +1 240 402 1469.
E-mail address: sadia.khan@fda.hhs.gov (S.A. Khan).

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bioterrorism weapon is also of great concern [6,10].

Traditional bioassays for detecting cholera toxin require the use of animal testing or tissue culture methods, which are time-consuming and labor-intensive [11–13]. The current laboratory method used by the Centers for Disease Control and Prevention (CDC) for cholera toxin includes enzyme-linked immunosorbent assay (ELISA), latex agglutination and polymerase chain reaction (PCR). While effective, these assays take several hours, which makes them inconvenient for rapid detection for field applications [11]. Several groups have used ganglioside GM1, a cell membrane ligand, as a recognition element for cholera toxin [1,14–16]. However, the large molecular size, complex structure and stability of ganglioside GM1 is a matter of concern [3]. A number of sensitive methods also have been reported in the literature for cholera toxin, though some of these require complex and expensive instrumentation or prolonged sample preparation [17–19]. To this end, development of a simple, fast and cost effective approach for the sensitive detection of cholera toxin is an ongoing need for practical field screening, and a colorimetric assay could be an ideal choice [20–23]. Previously, Pan and Charych reported sensing of cholera toxin using a ganglioside GM1-based colorimetric technique without quantitation [24]. Schofield et al. described a colorimetric assay for the detection of only cholera toxin B subunit using a lactose derivative stabilized nanoparticle, which required complex multistep synthesis of the lactose derivative [3]. In recent years, gold nanomaterials have been used in different analytical techniques for sensing a wide range of targets including metal ion, pathogen, small molecule and protein [22,23,25–28]. The reason behind gold nanomaterials having received extensive attention is its unique optical properties resulting in high molar extinction coefficient and scattering intensity [25–28]. In addition, ease of synthesis and functionalization make them a potential colorimetric probe for sensing applications [29–31].

We describe a colorimetric assay, using cholera antibody-conjugated gold nanoparticles, which could serve as a rapid, sensitive, and cost efficient way to screen cholera toxin. Since the color and surface plasmon band of gold nanoparticles strongly depends on its inter-particle distance, we utilized this distance dependent property to develop the gold nanoparticle based colorimetric assay for the detection of cholera toxin [20,21]. The visual limit of detection (LOD) of our assay is 10 nM which is an order of magnitude more sensitive than the previously reported colorimetric methods [3,24]. Furthermore, we report the successful use of gold nanoparticle based Dynamic Light Scattering (DLS) assay for cholera toxin detection, which was used to confirm the results of the colorimetric assay. DLS is a quasi-elastic light scattering technique used for measuring the hydrodynamic size and size distribution of nanoparticle, polymer and protein [32–36]. Since the light scattering cross section of gold nanoparticle is very high, we employed the nanoparticle as a light scattering enhancer in the DLS assay [36,37]. Ideally, our strategy could be applied to the rapid screening of water and food samples, and then subsequently confirmed by other analytical techniques where necessary.

2. Experimental section

2.1. Chemicals and materials

Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate dihydrate, mercaptopropionic acid (MPA), N-(3-dimethylamino propyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfo succinimide (sulfo – NHS), bovine serum albumin (BSA), potassium cyanide and cholera toxin from *V. cholerae* were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). The chemicals used were of analytical grade and used without further purification. Monoclonal anti-cholera toxin antibody was procured from Abcam (Cambridge, MA). Reagent grade water (pH 5–7) from Thermo Fisher Scientific (Waltham, MA) was used throughout the experiment unless otherwise specified. Diarrhetic toxins were purchased from the National Research Council Canada (NRC) Certified Reference Materials Program, Institute for Marine Bioscience (Halifax, Nova Scotia, Canada).

2.2. Synthesis and characterization of gold nanoparticles

Gold nanoparticles were prepared by citrate reduction of $HAuCl_4 \cdot 3H_2O$ according to the literature with a minor modification [38]. Briefly 5 mL of 0.01 M $HAuCl_4 \cdot 3H_2O$ in 50 mL water in a round bottom flask was heated to reflux with stirring for 30 min, then 3 mL solution of 1% sodium citrate was added quickly, which changed the solution color from pale yellow to wine-red. The solution was refluxed for 30 min then cooled to room temperature and stirred continuously for another 1hr. Each batch of the synthesized nanoparticles was characterized by UV–Vis spectroscopy and Transmission Electron Microscopy (TEM). Absorption spectra were collected using a Varian Cary 100 Bio UV–Visible spectrophotometer (Agilent Technologies, Santa Clara, CA). TEM images were acquired using a JEM-1400 transmission electron microscope operating at 120 kV. The average particle size of gold nanoparticle was determined to ~20 nm using TEM images. Nanoparticle concentration was calculated according to the literature by using elemental gold content and the average diameter of gold nanoparticle determined by ICP-MS and TEM respectively [36].

2.3. Preparation and characterization of antibody-conjugated gold nanoparticle

To modify the surface of the gold nanoparticles, 100 μl of 10^{-3} M MPA was added to 1 mL of gold nanoparticle solution (approximately 15.5 nM); the resulting solution was stirred for 2 h. This mixture was allowed to react at 4 °C for overnight. Next, these gold nanoparticles were conjugated with cholera antibody using EDC and sulfo-NHS as linking reagents [39]. Briefly 15 μl of EDC (1 mg/mL) and 15 μl of NHS (1 mg/mL) were added to the MPA modified nanoparticle solution. Then 100 μL of cholera antibody (45 μg/mL) was added to the surface modified nanoparticle solution, stirred for 30 min and reacted for 2 h at room temperature. The final antibody conjugated gold nanoparticle solution was centrifuged to remove any unbound antibodies and resuspended in phosphate-buffered saline (PBS) solution. TEM and DLS were used to characterize the antibody-conjugated nanoparticle. After conjugation, the average number of covalently attached antibodies per nanoparticle was determined according to the literature [40,41]. To separate the gold nanoparticles prior to the protein assay, antibody-conjugated gold nanoparticles were treated with 10 μM potassium cyanide to dissolve the gold nanoparticles [41,42]. Then, the amount of released antibody was measured by light absorption at 280 nm and Qubit protein assay kit separately (Invitrogen) [40]. By dividing the total number of antibodies by the total number of nanoparticles, an average of 10–15 antibodies per particle was estimated.

2.4. Colorimetric detection of cholera toxin

Initially 500 μL of antibody-conjugated gold nanoparticle was diluted with 500 μL water to optimize the absorbance. Then, 100 μL of cholera toxin ranging from 100 nM to 0.01 nM was added to the solution. The color change from red to blue was both instant and visible by the naked eye. Photographs were taken using a Pentax K-30 camera, and absorption spectra were collected from 400 to