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Horseradish peroxidase and antibody labeled gold nanoparticle probe for amplified immunoassay of ciguatoxin in fish samples based on capillary electrophoresis with electrochemical detection

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ABSTRACT

This paper describes a new amplified immunoassay with horseradish peroxidase (HRP) and antibody (Ab) labeled gold nanoparticles (AuNPs) probe hyphenated to capillary electrophoresis (CE) with electrochemical (EC) detection for ultrasensitive determination of ciguatoxin CTX1B. AuNPs were conjugated with HRP and Ab, and then incubated with limited amount of CTX1B to produce immunocomplex. The immunoreactive sample was injected into capillary for CE separation and EC detection. Enhanced sensitivity was obtained by adopting the AuNPs as carriers of HRP and Ab at high HRP/Ab molar ratio. The calibration curve of CTX1B was in the range of 0.06–90 ng/mL. The detection limit was 0.045 ng/mL, which is 38-fold lower than that of HPLC-MS method for CTX1B analysis. The proposed method was successfully applied for the quantification of CTX1B in contamined fish samples by simultaneously labeling Ab and HRP on AuNPs. The amplified IA with HRP and Ab labeled AuNPs probe hyphenated to CE and EC detection provides a sensitive analytical approach for the determination of trace ciguatoxin in complex samples.

1. Introduction

Ciguatera is a global disease caused by ingestion of fishes contaminated with the potent polyether toxins known as ciguatoxins (CTXs). These toxins are produced by the marine dinoflagellate *Gambierdiscus toxicus* (Yasumoto and Murata, 1993). Herbivorous fish accumulate these toxins in their musculature and viscera after ingesting dinoflagellates. The spread of ciguatera strongly impacts public health and considered a worldwide health problem. The symptoms of ciguatera begin with gastrointestinal problems, such as nausea, vomiting, diarrhea, and abdominal pain within 12 h of eating a toxic fish. CTX1B is the major toxin involved in ciguatera fish poisoning in the Pacific region, contributing to 90% of the toxicity of ciguateric carnivorous fish and posing a health risk at levels above 0.1 ppb (Dickey and Plakas, 2010). A major problem in avoiding the disease is that fish contaminated with CTXs look, smell, and taste normal. In addition, neither cooking nor freezing deactivates the heat-stable ciguatoxins, so an unwitting diner could easily consume a fatal meal if measures are not taken beforehand to test the fishes.

The traditional methods for the determination of CTXs involve testing lipid extracts by mouse bioassay (Lewis and Sellin, 1993), high performance liquid chromatography-mass spectrometry (HPLC-MS) (Lewis et al., 1999; Otero et al., 2010; Yogi et al., 2011) and enzyme-linked immunosorbent assay (ELISA) (Oguri et al., 2003; Tsumuraya et al., 2010, 2012), etc. Among the methods, immunoassay remains the most desirable method for accurate, sensitive, routine, and portable use. CE based immunoassay that combines the high separation power of CE and the high ligand specificity of immunoassay has proved to be a powerful technique for the separation and analysis of toxins (Zhang et al., 2012). By CE based immunoassay, the assay is faster because the immunoreaction occurs in solution. However, research on ciguatera has been severely hindered by the lack of labeled antigen or antibody. Therefore, it is necessary to develop a reliable and specific CE based immunoassay for detecting of CTXs in contaminated fish.

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Gold nanoparticles (AuNPs) have attracted great interest in biomolecular and toxin detection due to their facile synthesis and surface modification, making functionalized AuNPs an excellent candidate for bioconjugation (Lai et al., 2011; Yin et al., 2012). In particular, the large specific surface area and favorable biocompatibility of AuNPs can provide a suitable and promising platform for immobilizing enzyme moleculars for signal amplification in enzymelinkage reactions (Liu et al., 2010). All these made AuNPs been extensively used for the extraction and enrichment of analytes in complex matrices (Liu et al., 2008). Tseng's group employed AuNPs to extract and enrichment proteins (Lin et al., 2008) aminothiols (Shen et al., 2009; Chang and Tseng, 2010), indoleamines (Li et al., 2009) and melamine (Chang et al., 2010) from complex matrix followed by CE separation that demonstrated the AuNPs have capability to extracting a variety of molecules due to their high absorption capacity and facile functionalization. Ambrosi et al. applied AuNPs as multienzyme carriers to enzyme based immunoassay for the detection of biomarker (Ambrosi et al., 2010). The use of AuNPs allows the attachment of a multiple enzyme molecule which can generate an amplified optical signal. Lin et al. designed a triple signal amplification strategy for ultrasensitive immunosensing of cancer biomarker by using microbead carried AuNPs as tracing tag to label signal Ab and AuNPs induced silver deposition for anodic stripping analysis (Lin et al., 2012). Liu et al. employed membrane-based biosensing interface through the use of functional AuNPs in combination with in situ atom transfer radical polymerization reaction for detection of cell membrane binding proteins with high degree signal amplification (Liu and Cheng, 2012). Miao et al. reported an ultrasensitive sensing strategy for Ag^{+} assay based on the combination of AuNPs and smart enzyme cleavage mediated signal amplification with the detection limit as low as 40 pg/mL (Miao et al., 2013). Cui et al. combined an electrochemical immunosensor using AuNPs/carbon nanotubes hybrids with HRP-functionalized AuNPs for the sensitive detection of human IgG with a detection limit of 40 pg/mL (Cui et al., 2008). Recently, CE-based chemiluminescence immunoassay by using AuNPs as protein label reagent was reported for biological molecules determination (Liu et al., 2011; Jiang et al., 2013). AuNPs were conjugated with Ab or HRP labeled Ab, and then link to Ag to produce immunocomplex by noncompetitive immunoreaction. In a previous paper, we developed a CE-based EC immunoassay enhanced by AuNPs for the simultaneous determination of four shellfish toxins (Zhang et al., 2013). The AuNPs not only modified the mobilities of analytes to improve resolution but also used as multianalytes carrier to generate amplified EC signals. A dual amplification technique combining field-amplified sample injection and gold nanoparticles as multienzyme carriers was developed for detection of *E. coli* in scallop samples (Zhang et al., 2012). The assay resulted in the improved sensitivity of 1400 fold when compared with traditional CE using 10 kV electrokinetic injection for 10 s.

In this work, we proposed the combination of HRP and Ab labeled AuNPs probe with CE and EC detection for sensitive determination of CTX1B. The use of AuNPs as carriers allows the simultaneous attachment of multiple HRP and Ab which can generate an amplified EC signal. On the basis of the noncompetitive immunoreactions, the formed immunocomplex, unbound HRP-Au-Ab and excess HRP can be efficiently separated by CE and sensitively detected by EC detection. The amplified sensitivity was enhanced by using bioconjugates featuring HRP labels and Ab linked to AuNPs at high HRP/Ab ratio. To the best of our knowledge hitherto, it is not found the report about the quantitative determination of CTX1B by EC immunoassay by simultaneously labeling Ab and HRP on AuNPs.

2. Material and methods

2.1. Instrumentation

Experiments were carried out using a laboratory-built CE based EC immunoassay system as described previously (Zhang and Zhang, 2012). Briefly, CE separation was performed in a model MPI-A CE setup (Remax Electronics Inc., Xi'an, China), equipped with a high-voltage power supplier (0–30 kV) for driving the electrophoresis and an EC potentiostat (0–2.5 V) for detection. Fused-silica capillaries (75 μm i.d., 375 μm o.d., Yongnian Chromatogr. Components Ltd., Hebei, China) with 30 cm and 5 cm were used as separation and reaction capillaries, respectively. The reaction capillary was coaxial along the separation capillary and working electrode. The buffer reservoir at the high voltage end was enclosed in a plexiglass box fitted with an interlock for operator safety.

A commercial UV visible spectrophotometer (Tianpu Analytical instrument Co., Ltd, Shanghai, China) was used to measure the absorbance of the AuNPs and immunocomplex. An H7100 transmission electron microscopy (TEM) (Hitachi High-Technologies Corp., Tokyo, Japan) operating at 75 keV was used to collect TEM images.

2.2. Chemicals and reagents

CTX1B (Ag) and anti-CTX3C Ab were purchased from Abraxis (USA). HRP (MW = 44,000) and lyophilized 99% bovine serum albumin (BSA) were from Sigma. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), trisodium citrate, polyvinylpyrolidone (PVP, Mr = 1,300,000), OAP and H_2O_2 were obtained from Shanghai Reagent Company (China). All buffer reagents and other chemicals were of analytical grade and supplied by local standard reagent suppliers, unless otherwise stated. All solutions were prepared in doubly distilled water. Britton–Robinson (BR) buffer of various pH were prepared by dissolving appropriate amount of H_3BO_3, H_3PO_4 and HAc, and then adjusting the pH with concentrated NaOH. The CE running buffer was 10.0 mM BR buffer with 1.0% PVP and 1.0 mM H_2O_2 at pH 5.0.

2.3. Preparation and characterization of gold nanoparticles

AuNPs were synthesized by sodium citrate reduction of $HAuCl_4$ in water (Jin et al., 2003; Liu and Lu, 2006). Precisely, 25.0 mL of 38.8 mM sodium citrate was rapidly added to 250.0 mL of boiling 1.0 mM $HAuCl_4$ solution under vigorous stirring. The solution changed color from pale yellow to deep red and then refluxed for another 30 min. After cooling, the synthesized AuNPs was filtered through a 0.22 μm cellulose membrane and stored at 4 °C. The size of AuNPs was verified by TEM, and their concentration was estimated by UV–vis spectroscopy. The concentration of the AuNPs was calculated to be about 5.6 nM.

2.4. Preparation of HRP and Ab labeled AuNPs probe

The HRP and Ab labeled AuNPs probe was prepared by following a published procedure (Cui et al., 2008; Liu et al., 2011). 3.5 μL of 5.0 mg/mL HRP and 1.5 μL of 5.0 mg/mL Ab (which corresponding to the molar ratio of HRP/Ab is about 9/1) were added in 1.0 mL of the AuNPs solution (containing 0.04% trisodium citrate, 0.26 mM K_2CO_3, and 0.02% sodium azide) under agitation, followed by gently mixing and incubation at room temperature for 2 h. Then 100 μL of 1% BSA solution was added with stirring for 30 min at room temperature for blocking. The conjugate was centrifuged at 15,000 rpm for 20 min at 4 °C. The oiled drop was washed with BR buffer (containing 1% BSA and 0.05% Tween 20) and resuspended in