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A novel fluorescent DNA sensor for ultrasensitive detection of *Helicobacter pylori*

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ABSTRACT

In this work, a novel fluorescent DNA sensor for ultrasensitive detection of *Helicobacter pylori* (*H. pylori*) DNA was developed. This strategy took advantage of DNA hybridization between single-stranded DNA (ssDNA, which had been designed as an aptamer specific for *H. pylori* DNA) and the complementary target *H. pylori* DNA, and the feature that ssDNA bound to graphene oxide (GO) with significantly higher affinity than double-stranded DNA (dsDNA). ssDNA were firstly covalent conjugated with $CuInS_2$ quantum dots (QDs) by reaction between the carboxy group of QDs and amino group modified ssDNA, forming ssDNA-QDs genosensor. In the absence of the complementary target *H. pylori* DNA, GO could adsorb ssDNA-QDs DNA sensor and efficiently quench the fluorescence of ssDNA-QDs. While the complementary target *H. pylori* DNA was introduced, the ssDNA-QDs preferentially bound with the *H. pylori* DNA. The formation of dsDNA would alter the conformation of ssDNA and disturb the interaction between ssDNA and GO. Thus, the dsDNA-QDs/GO system exhibited a stronger fluorescence emission than that of the ssDNA-QDs/GO system. Under the optimized conditions, a linear correlation was established between the fluorescence intensity ratio I/I_0 and the concentration of *H. pylori* DNA in the range of 1.25–875 pmol L^{-1} with a detection limit of 0.46 pmol L^{-1}. The proposed method was applied to the determination of *H. pylori* DNA sequence in milk samples with satisfactory results.

1. Introduction

Helicobacter pylori (*H. pylori*), first isolated from the gastric mucosa biopsy tissue of patients with chronic active gastritis in 1983, is a spiral micro anaerobic gram-negative bacteria pathogen. *H. pylori* is considered to be a crucial causative agent in the pathogenesis of chronic gastritis, gastroduodenal ulcer diseases, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma, one of the most common forms of cancer in human beings (Marshall and Warren, 1984; Parsonnet et al., 1991; Hansson et al., 1993; Bazzoli et al., 2000; Peterson, 2002; Megraud, 2012). About half of the world's population has been infected with *H. pylori* (Soni et al., 2003). Therefore, sensitive and selective detection of the *H. pylori* DNA sequence is of great clinical significance for monitoring and management of *H. pylori* infection associated diseases. In the past few years, several biosensors have been established for the determination of *H. pylori*, including immune-based biosensors (*e.g.* sandwiched and enzymatically amplified piezoelectric biosensor (Su and Li, 2001) and electrokinetically driven microfluidic immunoassay (Gao et al., 2005; Seia et al., 2012)), DNA-based biosensors (Del Pozo et al., 2005; García et al., 2008), electrochemical method (Sun et al., 2010). Among these developed detection strategies, the DNA hybridization assays have played a significant role in detecting and identifying *H. pylori* (Cui et al., 2015).

For high simplicity, sensitivity, efficiency and high-throughput screening, concerns have been raised on QDs-based fluorometric methods. QDs are semiconductor nanocrystals with unique electro-optical properties and photophysical features, such as high quantum yields, long fluorescence lifetimes, large extinction coefficients, higher brightness and stability against photobleaching, and broad absorption spectra coupled with narrow photoluminescent emission spectra (Freeman and Willner, 2012; Gao et al., 2012a, 2012b). However, the applications of QDs in the clinical field have been hampered due to the toxicity of QDs. Regular QDs-based reports are focused on the cadmium-based QDs that are toxic to biological systems, sensitive to heat, chemical and photochemical disturbances, and eventually would cause serious environmental problems due to the leakage of cadmium (Hardman, 2006; Liu et al., 2013). With regard to this, two main approaches have been employed to reduce the toxicity of QDs. One is to cover non-toxic substance, such as silica shell, and the other is to develop novel QDs without heavy metal ions. In recent years, the newly emerging I-III-VI $CuInS_2$ QDs are particularly impressive

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because they do not contain any toxic class A elements (Cd, Pb, and Hg) or class B elements (Se and As) (Xie et al., 2009).

Recently, GO has emerged as a fascinating material with one-atom-thick two-dimensional graphitic carbon system (Guo et al., 2011) and rich of oxygen-containing groups on its surface, including carboxyl, hydroxyl and epoxy groups. GO is attractive for its excellent electronic and photophysical features, good water-solubility, unique DNA adsorbing ability and wide-range energy transfer properties (Wang et al., 2010), which make it a wide prospect for biological application (Zeng et al., 2010; Dreyer et al., 2010; Gu et al., 2011). More recently, GO has been widely used in DNA analysis (Dong et al., 2010), protein assay (Li et al., 2011), ion detection (Zhao et al., 2011), live cell imaging (Wang et al., 2010), drug delivery (Sahoo et al., 2011), *etc*. Moreover, the sp^2 aromatic domains within GO can induce efficient fluorescence quenching *via* förster resonance energy transfer or non-radiative dipole-dipole coupling, which enabled GO to serve as an optical basis for fluorescence turn-on sensing (Lu et al., 2009; Song et al., 2010; Kim et al., 2010; Zhang et al., 2011).

Herein, we established a new fluorescent sensing platform for sequence specific recognition of *H. pylori* DNA based on the DNA hybridization between ssDNA-QDs and the complementary target *H. pylori* DNA, and that ssDNA-QDs could bind to GO with significantly higher affinity than dsDNA-QDs. In the absence of target *H. pylori* DNA, GO could absorb ssDNA-QDs onto its surface and quench the fluorescence of ssDNA-QDs. However, in the presence of *H. pylori* DNA, the binding between the ssDNA-QDs and target *H. pylori* DNA (forming dsDNA-QDs) would alter the conformation of ssDNA-QDs, and disturbed the interaction between the ssDNA-QDs and GO. Such interaction will release the ssDNA-QDs from the GO, resulting in restoration of fluorescence intensity. By comparing the fluorescence intensity of ssDNA-QDs/GO system and the dsDNA-QDs/GO system, we could quantity analysis of *H. pylori* DNA sequence. To the best of our knowledge, the fluorescence "turn off-on" method for *H. pylori* DNA sequence detection based on self-assembled ssDNA-QDs/GO architecture have not been reported before.

2. Experimental section

2.1. Materials and apparatus

All chemicals and reagents were of at least analytical reagent grade and used as-received without any further purification. Copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$), indium (III) chloride tetrahydrate ($InCl_3 \cdot 4H_2O$), sulfourea (CS $(NH_2)_2$) and mercaptopropionic acid (MPA) were purchased from Sigma-Aldrich Corporation. Bovine serum albumin (BSA) and ascorbic acid were obtained from Sino-American Biotechnology Co. Ltd. Glucose, sodium chloride (NaCl), sodium hydroxide (NaOH), sodium dehydrogenized phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), sodium phosphate (Na_3PO_4), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and the other chemicals used were all purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}. All the oligonucleotide sequences used in the study were custom-made by Shanghai Sangon Biotechnology Co. Ltd. The target sequence of a 43-mer oligonucleotide specific in *H. pylori* was selected by restriction endonuclease analysis on the UreB gene sequence of *H. pylori*. And a 210 bp of UreB gene of bacterium *H. pylori* genome was amplified by PCR and used for real sample assay according to previous reports (Shanehsaz et al., 2013). The oligonucleotide sequences were listed in Table S1.

All the fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrofluorophotometer (Shimadzu Co., Kyoto, Japan) equipped with a xenon lamp using right-angle geometry. A 1 cm path-length quartz cuvette was used in all experiments. The hydro-thermal synthesis experiments were accomplished in a JCZ-JL intelligent digital display drum wind drying oven. UV–vis absorption spectra were obtained using a Varian GBC Cintra 10e UV–vis spectrometer. The ultrasonic processes was proceeded on a KQ2200 ultrasonic cleaner (Ultrasonic instrument Co., Kunshan, China). All pH measurements were completed on a PHS-3C pH meter (Tuopu Co., Hangzhou, China). The shaking processes were performed on a HY-2 variable-speed reciprocal shaker (Guo Hua Electric Appliance Co., Changzhou, China). The centrifugation processes were performed on a TGL-16G (Anke Technology Instrument Co., Shanghai, China).

2.2. Preparation of ssDNA-QDs

$CuInS_2$ QDs featuring carboxy groups were directly synthesized in aqueous solution *via* a hydrothermal synthesis method described in our previous report (Liu et al., 2012). In a typical experiment, $CuCl_2 \cdot 2H_2O$ (0.15 mmol) and $InCl_3 \cdot 4H_2O$ (0.15 mmol) were dissolved in distilled water (7.5 mL). Then, MPA (1.80 mmol) was injected into the above solution. The pH of the mixture was adjusted to 11.3 by adding 4 mol L^{-1} NaOH solution. 10 min later, CS $(NH_2)_2$ (0.30 mmol) was added into the solution. The Cu-to-In-to-S and Cu-to-MPA precursor ratios were 1:1:2 and 1:12, respectively. All the above mentioned experimental procedures were performed at room temperature. Then, the solution was transferred into a 15 mL Teflon-lined stainless steel autoclave. The autoclave was kept at 150 °C for 23 h and then cooled down to room temperature. The final concentration of $CuInS_2$ QDs was 1.36×10^{-4} mol L^{-1}.

The resulting QDs solution (1.36×10^{-4} mol L^{-1}, 0.2 mL) were firstly activated for 30 min in the presence of EDC and NHS (the mole ratio of QDs to EDC to NHS was 1:1500:1500) with vibrating at room temperature. The obtained N-hydroxy-succinimide-activated QDs solutions were then incubated with amine modified ssDNA for 3 h at room temperature with gently shaking. The mole ratio of ssDNA to QDs was 1:670.

2.3. Synthesis of GO

GO used in this study was synthesized according to modified Hummer's method (Gao et al., 2012a, 2012b). In a typical experiment, 2 g graphite powder was dispersed in 44 mL concentrated H_2SO_4, and incubated for 15 min in the ice-water bath. 6 g $KMnO_4$ was then added gradually into the solution under stirring with the temperature below 20 °C. This mixed solution was stirred at 15 °C for 20 min and reacted at 35 °C for 1 h. Subsequently, 160 mL deionized water was dropwise added to dilute the mixture. The resulting mixture was kept at 60 °C for 15 min, 200 mL water and 20 mL 30% H_2O_2 were then added to end the reaction. The product was washed with deionized water to remove the acid, and was further purified by dialysis for 2 week to remove the remaining metal species. Exfoliation was taken out by sonicating GO under ambient conditions. The obtained dispersion was centrifuged at 5000 rpm to remove any unexfoliated GO. The concentration of GO stock solution is 1.88 mg mL^{-1}.

2.4. Fluorescence quenching experiments

For fluorescence quenching experiment, 200 μL ssDNA-QDs and a series of different amounts of GO solution (from 0 to 10 μL) were separately mixed in 2.0 mL calibrated test tubes. Next, the mixed solutions were diluted to 1.5 mL with PBS solution. Each solution was mixed thoroughly and incubated for 20 min. The