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An amplified electrochemical strategy using DNA-QDs dendrimer superstructure for the detection of thymine DNA glycosylase activity

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ABSTRACT

A triple-signal amplification strategy was proposed for highly sensitive and selective detection of thymine DNA glycosylase (TDG) by coupling a dendrimer-like DNA label with the electrochemical method and quantum dots (QDs) tagging. The DNA-QDs dendrimer-like superstructure was designed by DNA hybridization and covalent assembling. Benefiting from outstanding performance of the amplification strategy, this assay showed high sensitivity, extraordinary stability, and easy operation. The limit of detection could reach 0.00003 U μL^{-1} with a splendid specificity. The TDG content in different concentration of HeLa cell was also determined. This assay opens a new horizon for both qualitative and quantitative detection of TDG, holding great promise for potential application in cancer cell research and clinical diagnostics.

1. Introduction

DNA glycosylases exist in almost all living organisms, from microbe to human (Dodson and Lloyd, 2002), which have two families of monofunctional and bifunctional glycosylases. Thymine DNA glycosylase (TDG) is a monofunctional enzyme, which plays a key role in the cellular defense against mutation (Solange et al., 2012). It excises thymine and uracil from G:T and G:U mismatched oligonucleotide substrates as well as 3′ N^4-ethenocytosine from double-stranded DNA (Ulrike et al., 2003). Recent studies showed that it has multiple functions, such as link DNA repair, the control of epigenetic DNA modification, and the regulation of gene expression (Gallinari, Jiricny, 1996; Cortazar et al., 2007). Furthermore, it could also act as either a co-activator or co-repressor for a variety of genes. These findings highlighted the significance using TDG as a tool to promote the basic biomedical research. Thus, it is highly desirable to develop rapid, sensitive, and specific methods to detect the activity of TDG.

To date, only a few approaches were presented for the analysis of TDG activity including gel electrophoresis analysis using radioactive isotopes or fluorescent labels (Li et al., 2007b; Hashimoto et al., 2012), enzymatic coupled fluorescence assay (Fitzgerald and Drohat, 2008) and repair-mediated firefly luciferase expression analysis (Li et al., 2010a,2010b). However, these methods have the limitations of low sensitivity, poor accuracy, and sophisticated operations. In order to circumvent these problems, fluorescence assay was selected to evaluate the activity of TDG (Chen et al., 2013). Nevertheless, the expensive probe limited their application. Thus, it is potentially promising to explore convenient, sensitive and selective approaches to determine the activity of TDG.

Nowadays, electrochemically amplified methods have been proven to be powerful analytical techniques due to moderate cost, instrumental simplicity, and high sensitivity (Kim et al., 1998; Zhang et al., 2013). For acquisitions of high sensitivities and preferable specificity, various electrochemical strategies have been explored including the use of nanoparticles (Liu et al., 2013a,2013b; Wang et al., 2013b), catalyst enzymes (Wang et al., 2013a; Zhu et al., 2011), and conducting polymers (Rowe et al., 2011; Das and Yang, 2009). Among them, quantum dots (QDs) were well recognized as electroactive species for signal amplification due to their unique properties such as versatility in surface modification and high sensitivity. Thus, new schemes based on coupling QDs probe with additional amplification processes are highly desired.

Recently, a wide variety of well-defined DNA superstructures in one, two, and three dimensions have been used as efficient amplifying tags for the development of biosensors due to their unique properties, such as simplicity and predictability (Tang et al., 2012;

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Lee et al., 2009; Bi et al., 2013; Lu et al., 2012; Liu et al., 2013a,2013b). Dendrimer is a regular tree-like and highly branched macromolecule (Jie et al., 2011), which is typically symmetric around the core, and often adopts a spherical three-dimensional morphology (Antoni et al., 2009). Hence, dendrimer-like DNA superstructure is an efficient structure to improve the sensitivity through capturing large amount of signal molecules. In 2006, Luo et al. (Um et al., 2006) firstly used dendrimer-like DNA-based fluorescence nanobarcodes for multiplex detection. Subsequently, Niemeyer et al. (Rabe and Niemeyer, 2009; Feldkamp et al., 2009) carried out extensive and excellent studies in the fabrication of dendrimer-like DNA. Tan et al. (Sun et al., 2011) and Zhang et al. (Bi et al., 2010) reported surface-enhanced Raman scattering and chemiluminescence biosensors based on DNA-Au dendrimer and DNA-RuNPs dendrimer. However, it has not been found the fabrication of DNA-QDs dendrimer based electrochemical biosensor until now.

Herein, we design a novel approach for the evaluation of the TDG activity based on triple-signal amplification by coupling DNA-dendrimer superstructure, QDs tagging with electrochemical technique. Several significant advantages can be observed. Firstly, a large number of QDs were assembled onto the dendrimer-like DNA superstructure, which could greatly amplify the electrochemical signal. Secondly, the hairpin structure and QDs tagging technique were employed for the improvement of selectivity. Thirdly, the stationary platform and anodic stripping voltammetry (ASV) technique could significantly improve the sensitivity and simplify the procedures. This strategy not only pioneered the development of TDG electrochemical biosensor but also played an active driving role on the aspects of cancer progression studies.

2. Experimental

2.1. Chemicals and materials

1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHS), and tris(2-carboxyethyl) phosphine hydrochloride) (TCEP) were purchased from Sigma-Aldrich. 3-Mercaptopropionic acid (99%), sodium borohydride (98%), and tris-(hydroxymethyl) aminomethane (Tris) were purchased from Nanjing Chemical Reagents Factory (Nanjing, China). Reagents for polyacrylamide gel electrophoresis and all DNA sequences were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). Prior to use, all the oligonucleotides solutions were heat-treated at 90 °C for 3 min and then cooled in ice for 10 min. Endonuclease IV (EnIV), 8-oxoguanine DNA Glycpsylase (hOGG1) were purchased from New England Biolabs Ltd (USA). Thymine DNA Glycosylase (TDG) was bought from R&D system (USA). 6-Mercapto-1-hexanol (MCH) was obtained from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Cell cultures were purchased from Nanjing KenGen Biotech Co. Ltd (Nanjing, China). All other chemicals involved in this work were analytical-grade. All solutions were freshly prepared using ultrapure water ($\geq$ 18 MΩ, Milli-Q, Millipore).

DNA sequences

MB-DNA: 5′-SH-TAT ATA TGG TAG TGA GTA GTG AGG TAG GTT GTA TAG TTG AGC CTC AGT AAA TTA TAC AAC CTA CC

Signal DNA1: 5′-NH_2-C6-AAAAAATTG AGC CTC AGT AAA-3′

Signal DNA2: 5′-NH_2-C6-AAAAAATTT ACT GAG GCT CAA-3′

2.2. Apparatus and characterization

UV–vis absorbance and fluorescence measurements were recorded on UV-3600 spectrophotometer and RF-5301PC (Shimadzu, Kyoto, Japan), respectively. Gel imaging was carried out with a Bio-Rad imaging system. Fourier-transform infrared (FTIR) spectroscopic measurements were performed on a Brukermodelvector22 spectrometer using KBr pressed disks. Electrochemical measurements were performed on a CHI 660B workstation (Shanghai Chenhua Apparatus Corporation, China) with a conventional three-electrode system composed of a platinum wire as the auxiliary, a saturated calomel electrode as the reference, and a gold (Au) electrode or a glassy carbon electrode (GCE) as the working electrode. Electrochemical impedance spectroscopy (EIS) was performed with an Autolab electrochemical analyzer (Eco Chemie, The Netherlands) in 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1.0 M KCl) as the supporting electrolyte at a bias potential of 0.18 V, within the frequency range of 0.01–100 kHz. Melting temperature (Tm) and the secondary structure of DNA were analyzed by OligoAnalyzer 3.1 (free online software from IDT).

2.3. Preparation of DNA1-QDs and DNA2-QDs

CdTe QDs were synthesized according to the literature (Qian et al., 2006; Zhang et al., 2006; Cui et al., 2009). The conjugation procedure for DNA1 and DNA2 to QDs was EDC/NHS method via the formation of amide between the carboxyl groups of QDs and the primary amine groups of DNA1 or DNA2. Briefly, QDs (100 μL), EDC (1 mg), and NHS (1 mg) were mixed in PBS (pH 7.4, 50 mM, 1 mL) and then incubated at room temperature for 30 min with continuous gentle mixing. Then, DNA1 or DNA2 solution (20 μL) was added and incubated for another 2 h under gentle stirring, then stored overnight at 4 °C. This allows the unreacted EDC to hydrolyze to lose its activity. After ultrafiltration with YM-30 ultrafilter, the DNA1-QDs and DNA2-QDs conjugates were redissolved in Tris buffer (pH 7.4, 50 mM).

2.4. Fabrication of the sensor

Before using, the Au electrode (3 mm in diameter) were cleaned with freshly made piranha solution (98% H_2SO_4:30% H_2O_2=7:3, v/v) for 10 min (CAUTION: piranha solution should be handled with great care) and then polished to a mirror using 1 and 0.05 μm alumina slurry followed by sonication in acetone, ethanol and water for 1 min each. After electrochemical cleaning in 0.5 M H_2SO_4, the electrode was allowed to dry at room temperature and a droplet of 10 μL MB (1 μM MB-DNA in 10 mM Tris–HCl (1 mM EDTA, 1 mM TCEP, 0.1 M NaCl, pH 7.4)) was dropped onto the electrode for 12 h at 37 °C in 100% humidity. Then the electrode was rinsed with deionized water and passivated with 10 μL MCH (1 mM MCH in 10 mM Tris–HCl, pH 7.4) for 2 h to remove non-specific DNA adsorption. After rinsed with water, 10 μL TDG solutions were dropped onto the above electrode for 2 h incubation at 65 °C to recognize G/T mismatch and excise the T base. Further rinsed with water, 10 μL EnIV (0.33 U μL^{-1}) were dropped onto the electrode for 2 h incubation at 25 °C. The electrode was consequently incubated in reporter DNA1-QDs and reporter DNA2-QDs for 30 min at 37 °C and then rinsed with buffer for 2 min. The reporters' incubation procedure was repeated 4 times, and the obtained electrode was washed with water.

2.5. Electrochemical detection of TDG

After bound with the reporter DNA-QDs, the electrode was rinsed with Tris–HCl buffer and immersed into HNO_3 (200 μL, 0.1 M) solution for 2 h to dissolve the residual CdTe QDs. Then, the concentration of TDG was determined by anodic stripping voltammetry (ASV) method. Briefly, the dissolved solution was added into a glass cell containing 4.8 mL acetate buffer (0.2 M, pH 5.2). The mercury film modified GCE was prepared by deposition at −1.0 V for 40 s and scanned from −0.9 V to −0.2 V in acetate