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Graphene oxide-hairpin probe nanocomposite as a homogeneous assay platform for DNA base excision repair screening

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ABSTRACT

Uracil-DNA glycosylase (UDG) as one of the most important base excision repair enzymes plays a crucial role in protecting the genome from endogenous DNA damage and sustaining the genome integrity. Quantitative activity analysis of UDG is a central challenge and of fundamental importance in bioanalysis. Here, we proposed a novel biosensor constituted by adsorbing a fluorophore-labeled hairpin probe onto the surface of graphene oxide (GO) as a homogeneous assay platform for sensitive UDG activity assay. Active UDG could excise the uracil base in the hairpin probe, and further hydrolysis of the leaving abasic site gave rise to high fluorescence. Thus, it provided a convenient approach for UDG activity quantification. Because of the unique ability of GO in universal fluorescence quenching, a low background fluorescence signal can be obtained for the efficient fluorescence resonant energy transfer from the fluorophore-labeled on the hairpin probe to GO sheet. A quite wide dynamic range from 0.0017 U/mL to 0.8 U/mL was achieved for UDG assay and the detection limit was estimated to be 0.0008 U/mL. The results indicated that this strategy offers a simple, cost-effective, highly sensitive and selective homogeneous detection platform for UDG activity assay related biochemical studies.

1. Introduction

Base excision repair (BER) enzymes play crucial roles in protecting the genome from endogenous DNA damage and sustaining the genome integrity via initiating base excision repair pathway (Duncan and Weiss, 1982; Duncan and Miller, 1980). Uracil-DNA glycosylase (UDG) is one of the most important BER enzymes which widely distributes in almost all known organisms. It prevents uracil lesions of the genome by catalyzing the hydrolysis of the N-glycosylic bond joining the uracil base to the deoxyribose (Lindahl, 1974). The uracil residues in U:A pairs resulting from misincorporation of dUMP during replication and in mutagenic U:G mispairs resulting from the deamination of cytosine both can be recognized and excised by UDG (Tye et al., 1977; Wist et al., 1978; Lindahl and Nyberg, 1974), leaving an abasic site and triggering downstream damage repair pathways. Since the efficiency of DNA lesion repair is a determining factor for survival and variation of a damaged cell, the development of sensitive and selective methods for activity screening of UDG is of fundamental importance.

For activity screening of UDG, fluorescence-based strategies have been developed. Maksimenko et al. and Liu et al. separately performed UDG activity assay based on fluorescence resonant energy transfer (FRET) via using molecular beacon or linear double-stranded DNA (dsDNA) probes (Maksimenko et al., 2004; Liu et al., 2007). Quite recently, Kool et al. synthesized DNA sequences that contained a fluorescent base pyrene to acting as highly efficient reporters of UDG activity (Ono et al., 2012). These methods performed in a homogeneous assay format were generally robust, easily automated and scalable for parallel assays of hundreds of samples.

Graphene, a one-atom-thick nonmaterial with excellent mechanical, thermal, electrical, optical and catalysis properties, has attracted increasing interest in nanobiotechnology (Balandin et al., 2008; Service, 2009; Lee et al., 2008). For the construction of graphene-based biosensors, solubilization and bio-functionalization of graphene is a key challenge. Towards this point, most works rely on the use of oxidative chemistry, and the resulting graphene oxide can comprise abundant reactive and hydrophilic functional groups, which makes this material highly soluble and versatile for covalent conjugation of biomolecules (Sun et al., 2008; Gulbakan et al., 2010). Alternatively, the water-soluble graphene oxide possesses a basal aromatic plane allowing strong stacking interactions with aromatic molecules and universal fluorescence quenching of fluorophore, which constitute a useful platform for the development of biosensors. Significant improvements have been accomplished in many

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remarkable biological applications by using such a unique component to construct tailor-made composite materials, such as ions or small molecule detection (Zhao et al., 2011; Lu et al., 2010a; Gulbakan et al., 2010), DNA analysis (Lu et al., 2009, 2010b), enzyme activity sensing (Lin et al., 2011; Wu et al., 2011; Lee et al., 2011; Wang et al., 2011; De et al., 2011), drug delivery (Liu et al., 2008), etc. However, the exploration of GO for activity assay of base excision repair enzymes has not been reported yet.

Our group has made many researches in developing carbon material-based biosensors for diverse biological applications (Nie et al., 2009; Wu et al., 2009). Herein, a simple but effective strategy was developed for sensitive UDG activity assay by employing a fluorophore-labeled probe coupled with graphene oxide (GO) as a sensing platform. GO has shown unique abilities in single-stranded DNA (ssDNA) adsorbing and outstanding capability in universal fluorescence quenching, which hold great potential for constructing sensitive fluorescence-based platforms for UDG activity sensing. To test this hypothesis, a fluorophore-labeled probe with a long ssDNA tail which can be used as a substrate of UDG is designed. By this design, we expect to obtain low background in UDG assay and the activity of UDG could be instantly reported by the released fluorophore labels that are catalyzed by base excision repair proteins. In addition, fluorescence-based readouts permit a wide dynamic range and the use of simple instrumentation. The developed technique can be implemented in a homogeneous format without any washing and separation steps, thus affording improved assay robustness, simplicity and throughput in UDG detection, as desired in many applications.

2. Experimental

2.1. Materials

E. coli Uracil-DNA Glycosylase (UDG), Endonuclease IV (EnIV), 8-Oxoguanine DNA Glycosylase (hOGG1), Bovine Serum Albumin (BSA), Uracil Glycosylase Inhibitor (UGI) and 10 × NEBuffer 3 (1000 mM NaCl, 500 mM Tris–HCl (pH 7.9), 100 mM $MgCl_2$ and 10 mM DTT) were purchased from New England Biolabs (Ipswich, MA, USA). Graphite (99.9%, 325 mesh) was purchased from Alfa Aesar. All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. Ultrapure water was obtained through a Millipore Milli-Q water purification system and had an electric resistance >18.3 MΩ. The hairpin structure probe used in the experiments was synthesized from Sangon Co. Ltd. (Shanghai), and the sequence is 5′–FAM-TXAATGAAGCTTTTTGCTTCATTAATCTAA-AAGCTG CGGAATTGT-3′ (X=dU, 2-deoxyuridine). Another ssDNA having the same sequence of the hairpin probe but without a fluorescein label was also synthesized from the same company for melting curve analysis.

2.2. Synthesis and characterization of GO

GO was synthesized from graphite powder according to previous reports (Hummers and Offeman, 1958; Tang et al., 2010). The GO suspension in ultrapure water (~2 mg/mL) was sonicated in an ice bath using a probe-type sonicator under a power of 40 W for 1 h. The resulting solution was centrifuged at 10,000 rpm for 10 min, and the supernatant was diluted further, yielding a stable dark yellow GO dispersion with the concentration about 0.2 mg/mL.

The transmission electron microscope (TEM) images were obtained with a field-emission high-resolution 2100 F TEM (JEOL, Japan) opened at an accelerating voltage of 200 kV. The sample films for TEM analysis were formed by dropping the diluted solution of GO suspension on a holey carbon mesh grids (400 mesh) and left to dry in air condition at room temperature. The AFM images were taken using a Veeco Dimension V scanning probe microscope (Veeco Instruments Inc., USA). For AFM characterization, the sample films for AFM imaging were prepared by dropping the diluted solution of GO suspension on a freshly cleaved mica sheet and left to dry in air condition at room temperature. The resulting mica sheet was rinsed with ultrapure water and left to dry in a nitrogen stream before the imaging. The infrared absorption spectroscopic measurements were operated with GO powders in KBr pieces with a Nexus 870 FT-IR spectrophotometer (Thermo Electron, USA) under continuous N_2 purge.

2.3. UDG activity assay and fluorescence measurement

The hairpin probe with final concentration of 1 μM in 1 × NEBuffer 3 (100 mM NaCl, 50 mM Tris–HCl (pH 7.9), 10 mM $MgCl_2$ and 1 mM DTT) was first heated to 95 °C for 5 min, followed by slow cooling to room temperature within 2 h. The obtained hairpin probe solution was stored at 4 °C for further use. The typical UDG activity assay was performed at 37 °C for 2 h in a 30 μL 1 × NEBuffer 3 containing 100 nM hairpin probe, 667 U/mL EnIV and a given concentration UDG. Then 15 μL 0.2 mg/mL GO and ultrapure water were added into the reactions with final reaction volume of 120 μL. The mixture was incubated at 37 °C for 30 min before allowing fluorescence detection. The fluorescence spectra were recorded at room temperature in a quartz cuvette on an F-7000 spectrofluorometer (Hitachi, Japan). The excitation wavelength was 494 nm and the emission wavelengths were in the range from 500 nm to 600 nm with both excitation and emission slits of 5 nm. For UDG activity quantification assay, varying concentrations of UDG (0, 0.0017, 0.02, 0.04, 0.08, 0.17, 0.2, 0.4, and 0.8 U/mL) in the first 30 μL reaction volume were used.

2.4. Liquid chromatography (LC) characterization of the biosensor in UDG activity assay

Ion-paired reverse-phase high-performance liquid chromatography was carried out on an Agilent 1200 Series Rapid Resolution HPLC analytical scale purification system equipped with a C18 column (4.6 × 250 mm). Eluent A was aqueous 0.1 M TEAA at pH 7.0 and eluent B was aqueous 0.1 M TEAA at pH 7.0 containing 50% (v/v) acetonitrile. The typical samples for LC were prepared as follows: a 30 μL aliquot of reagent solution containing 3 μL 10 × NEBuffer 3, 100 nM hairpin probe, 667 U/mL EnIV, 167 U/mL UDG and ultrapure water was gently mixed, and incubated at 37 °C. A volume of 10 μL sample was injected and the column was run at 30 °C with a following binary gradient (min, %A): 0, 100%; 5, 75%; 20, 50%; 21, 0% (flow rate 1.0 mL/min). The excitation and emission wavelengths of fluorescence detector were 494 nm and 516 nm, respectively.

2.5. Cell culture and sample preparation

Briefly, HeLa and A549 cells were cultured in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen) and 100 U/mL penicillin and 100 g/mL streptomycin. These cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells (1 × 106) were dispensed in a 1.5 mL centrifuge tube, washed twice with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) at 2000 rpm for 3 min, and thus suspended in 100 μL of lysis buffer (10 mM Tris–HCl with a pH value of 8.0, 150 mM NaCl, 1% NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice and vortex for 15–30 s every 5 min. Then