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Visible assay for glycosylase based on intrinsic catalytic ability of graphene/gold nanoparticles hybrids

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ABSTRACT

A sensitive, rapid and label-free assay for colorimetric detection of human 8-hydroxyguanine glycosylase (hOGG1) was proposed based on the tunable catalytic ability of graphene/gold nanoparticles (graphene/Au-NPs) hybrids and the terminal protection of hOGG1. In presence of H_2O_2, the hybrids were capable of catalyzing the oxidation of color developing reagent, causing a concomitant change in color. Due to the excellent controllability, the capacity could be inhibited by adsorption of ssDNA onto the hybrids sheets and recovered when the adsorbed ssDNA was digested by exonuclease. The terminal protection of hOGG1 could irreversibly interrupt the digestion of the captured ssDNA (containing the oxidative damage site) by the exonuclease, thus preventing the catalytic ability of graphene/Au-NPs from being recovered. The original color change which related to the concentration of the protected ssDNA facilitated quantitative detection of hOGGl activity. Compared with conventional methods for hOGG1 detection, the presented assay without any labeling process greatly simplified the operation steps and reduced the analysis time. This approach performed a linear response for hOGG1 activity from 0.02 to 0.11 U/μL with a detection limit of 0.0016 U/μL, and realized the quantification of hOGG1 activity in real cell lines.

1. Introduction

Reactive oxygen species (ROS), which are generated by endogenous oxidation, are able to attack the biological macromolecule and cell wall. They produce various damages and lesions that seriously accelerated the caducity of a living organism (Valko et al., 2007; Wang and Jing, 2008). Being the most deleterious of ROS-induced adducts, 8-oxoguanine, which is formed by the oxidation of the guanine base, has aroused wide concern recently. On account of its highest frequency and strongest mutagenic ability in DNA (Grollman and Moriya, 1993; Michaels and Miller, 1992), this abnormal base associated closely with the occurrence and progress of tumors (Arizono et al., 2008). 8-oxoguanine, which could cause highly respective damages, is incapable of preventing a DNA chain from elongation, consequently affecting its genomic stability and DNA replication (Maehara et al., 2008; McWilliams et al., 2014; Obtułowicz et al., 2010). Specifically, due to space conformation variation, 8-oxoguanine residues can initiate a conversion from G●C to T●A by being mispaired with adenine (Kuchino et al., 1987) which is the frequent somatic mutation in human cancers (Agnihotri and Mishra, 2009; Bruner et al., 2000; Yadav and Mishra, 2014) during the DNA replication in vivo and in vitro. Because of its high content, significant mutagenicity and perdurability in human body, 8-oxoguanine is regarded as a sensitive marker of oxidative DNA damage (Au et al., 2002; Figueroa et al., 2007).

Excision repair is the main DNA damage repair mechanism in human somatic cell for a variety of DNA damage such as pyrimidine dimer, alkylation of bases and oxidative damage (Farmer et al., 2005; Goode et al., 2002; Sancar et al., 2004). During the base excision repair process, human 8-hydroxyguanine glycosylase (hOGG1), one type of DNA glycosylase expressed in all the tissues of human (Boiteux and Radicella, 1999), can effectively recognize and excise many abnormal bases including 8-oxoguanine when base paired with cytosine (Gao et al., 2013; Kohno et al., 1998). Therefore, as a vital repair enzyme of 8-oxoguanine, the enzyme activity of hOGG1 immediately impacts the ability of repairing 8-oxoguanine and the individual risk of cancer (Hernández et al., 2014; Sheehan et al., 2005). In consequence, the application value for rapid and accurate analysis of hOGG1 activity in clinical diagnosis and cancer research is quite critical.

Conventional methods for detecting the enzyme activity of hOGG1 such as instrumental testing (Rihs et al., 2012), biological assay (Park et al., 2009), biochemical and crystallographic analysis (Norman et al., 2004) are mostly time and labor consuming, or requiring expensive instrument. For example, western blot

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combined with immunohistochemistry and chemiluminescence (Gao et al., 2013; Xu et al., 2013) is employed to analyze the protein level and activity of hOGG1 in a tumor. Although this technology performs highly sensitively, it is susceptible to subtle structural differences caused by the conformation variation of enzymes (Anderson and Multiplex, 1999; Derenzini et al., 2002). Due to the poor selectivity, its application in samples containing various enzyme conformations is limited. The combination of flow cytometry (FCM) and fluorescence assay (Mirbahai et al., 2010) is employed to detect the hOGG1 activity with high accuracy, but the expensive high-tech equipment and tedious operation procedure limit its general use. In addition, a molecular beacon (MB) with hairpin structure (Liu et al., 2012a) or DNAzyme-based colorimetric assay with horse radish peroxidase (HRP) (Liu et al., 2013) is capable of realizing signal amplification to get high sensitivity or visualization; however, the labeling or hybridization process is complex and the natural enzyme and the hybridized probe cannot connect to the template completely leading to poor stability. In general, the practicability of these detection methods such as selectivity, stability, economical efficiency and operability still need to be improved. Hence, a sensitive, easy to operate and low-cost method for hOGG1 analysis with high selectivity is urgently required.

Recently, great interest has been focused on the application of inorganic nanoparticles in biochemical quantitative analysis (Song et al., 2011; Wu et al., 2010) due to the excellent advantages over conventional methods, particularly in stability, reactivity and simple operation on the basis of electrochemistry, fluorescence and colorimetric technology, etc. (Gao et al., 2007; Han et al., 2012; Liu et al., 2012c). However, most of them require a complex labeling process or complex preparation. In our previous work, we demonstrated novel enzyme mimetics, graphene/gold nanoparticles (graphene/Au-NPs) hybrids, prepared through a one-step hydrothermal route (Zhang et al., 2011), exhibiting intrinsic peroxidase-like catalytic activity at its interface (Liu et al., 2012b). Due to the catalytic activity of the hybrids, remarkable color change of the solution caused by the catalytic oxidation of color developing regent could be visually observed without any further labeling process. In addition, the planar two-dimensional structure of supporter graphene provided a large specific surface area to load ssDNA (Liu et al., 2011), achieving flexible control of the catalytic activity of the hybrids. Combined with the strong affinity between ssDNA and its complementary single strand, other specific biological target (Hernández et al., 2014; Shi et al., 2011; Wang et al., 2010; Zuo et al., 2013) as well as the intrinsic stability of inorganic material, the mentioned graphene/Au-NPs hybrids were expected to be applied in bioassays, catalysis and biosensing.

In this study, we report that the graphene/Au-NPs hybrids could be used for rapid and quantitative detection of hOGG1 depending on the intrinsic peroxidase-like catalytic activity of the hybrids. Taking advantage of the spontaneous adsorption of ssDNA on the graphene sheets and the terminal protection of hOGG1 provide against the hydrolysis of exonuclease to the ssDNA, a label-free sensor protocol is proposed. The excellent catalytic activity of as-prepared hybrids, as well as the controllability and DNA-binding property of hybrids sheets (Liu et al., 2011), ensures the detection sensitivity. Together with the visualization and simplicity, we suppose that this strategy can develop a rapid and label-free platform for colorimetric detection of target enzyme hOGG1.

2. Material and methods

2.1. Materials

DNA oligomers (Sequence 1 (S1): 5′-GAGAATGGAGCACATCAGTT^{oxo}GAGCTCCATTCTC-3′, ^{oxo}G is 8-oxoguanine; Sequence 2 (S2, complementary ssDNA to S1): 5′-GAGAATGGAGCTCAACTGATGTGCTCCATTCTC-3′) were provided by Takara Biotechnology Co. (Dalian, China) and purified by high-performance liquid chromatography (HPLC). Exonuclease (Exo I and Exo III) and target enzyme hOGG1 were purchased from New England Biolabs (Ipswich, MA, USA). Cell lines (HL7702 and MCF-7) were provided by Cell Center of Shanghai Institutes for Biological Science, CAS. Non-denatured lysis buffer was purchased from KeyGEN BioTECH Co. (Nanjing, China). Graphite powder (<300 mesh) was obtained from Beijing Chemical Reagents Company. 3,3′,5,5′-tetramethylbenzidine (TMB) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (resistivity >18.0 MΩ cm^{-1}, Laikie Instrument Co., Ltd., Shanghai, China) was used throughout the experiments. Citrate buffer (20.0 mM, pH 4.0) was prepared by mixing the stock solution of Na_2HPO_4 and citric acid. Phosphate buffer solution (PBS, 20.0 mM, pH 7.4) was prepared by mixing the stock solution of Na_2HPO_4 and NaH_2PO_4. $HAuCl_4$, NaOH and other reagents of analytical reagent grade were purchased from Tianjin Bodi Chemicals Co., Ltd. (Tianjin, China). All the reagents were used as received without further treatment. All glassware was thoroughly cleaned with chromic acid and rewashed with the ultrapure water.

2.2. Instruments

UV–visible absorption spectra were recorded on a Jasco V-550 spectrometer. Transmission electron microscopy (TEM) images were obtained through a high-magnification TEM (FEI Tecnai G2 F30 S-Twin). Scanning electron microscopy energy and dispersive X-ray spectroscopy (SEM-EDS) images were obtained using a field emission Scanning Electron Microscope (SEM, Hitachi S-4800) equipped with EDS (IXRF systems 550i). The chemical structure and groups of prepared hybrids were characterized by fourier transform infrared spectroscopy (FT-IR) (Bruker VERTEX 70 FTIR) in transmission mode with a KBr window. The DNA stands were observed by agarose gel electrophoresis (AGE) analysis (DYY-6C).

2.3. Preparation of graphene oxide (GO)

GO was prepared by a modified Hummers method from graphite powders (Hummers and Offeman, 1958; Liu et al., 2008). In brief, 1.0 g graphite powder was firstly dispersed in concentrated 23.0 mL H_2SO_4 and stirred 12 h at room temperature. 3.0 g of $KMnO_4$ was gradually added with vigorous stirring at 0 °C. Then, the solution was sonicated in a water bath for 10 h to give a dark-green solution. Next, 46.0 ml ultrapure water was gradually added. The reaction was terminated by the addition of 140.0 mL ultrapure water and 10.0 mL H_2O_2 (30%), and the color changed to light yellow. The product was separated by centrifugation, and then washed with 5% HCl and ultrapure water for several times. Finally, the obtained solid was dried under vacuum.

2.4. Preparation of graphene/AU-NPs hybrids

In the typical experiment, a simple rapid hydrothermal route was used to prepare graphene/Au-NPs hybrids (Liu et al., 2012b). GO aqueous solution (2.5 mL, 0.5 g/L), $HAuCl_4 \cdot 4H_2O$ solution (1.6 mL, 10.0 mM) and NaOH solution (1.2 mL, 0.1 M) were mixed together and diluted to 25.0 mL. The final concentration of Au^{3+}