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A novel and label-free biosensors for uracil-DNA glycosylase activity based on the electrochemical oxidation of guanine bases at the graphene modified electrode

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ABSTRACT

Uracil-DNA glycosylase (UDG) as an important base excision repair enzymes is widely distributed in organism, and it plays a crucial role in sustaining the genome integrity. Therefore, it is significant to carry out the analysis of UDG activity. In this present work, a novel and label-free electrochemical sensing platform for the sensitive detection of uracil DNA glycosylase (UDG) activity has been developed. Herein, the graphene modified glassy carbon (GC) electrode was prepared. And two complementary DNA strands were hybridized to form dsDNA (P1P2). In the presence of UDG, the uracil bases in P1P2 were specifically hydrolyzed, inducing the unwinding of the DNA duplex, and accompanied by the release of P1. Thus, the released P1 was adsorbed onto the graphene/GC electrode surface via π–π stacking. By investigating the electrochemical behavior of P1 at the graphene/GC electrode, the electrochemical oxidation of guanine bases in P1 was obviously observed. Therefore, using the current responses of guanine base in P1 as a signal indicator, UDG activity can be simply determined with high sensitivity, and the detectable lowest concentration is 0.01 U/mL. This present design does not need covalent attachment of redox indicator to DNA, preventing participation of redox labels in the background. Meanwhile, the proposed strategy for the assay of UDG activity also has a remarkable sensitivity due to the excellent properties of graphene, which could increase both the immobilization amount of released ssDNA and the conductivity of the sensing system. All these elucidate that this developed protocol may lay a potential foundation for the sensitive detection of UDG activity in clinical diagnosis.

1. Introduction

Until now, there are some specific DNA repair pathways, such as base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MR) [1–3]. Uracil-DNA glycosylase (UDG) as a kind of BER enzyme is distributed in all lives and has been extensively studied. And moreover, UDG can specifically catalyze the cleavage of N-glycosylic bond joining the uracil base to the deoxyribose, leaving an abasbic site [4]. This process of uracil excision repair plays a crucial role in sustaining genetic integrity [5]. In recent years, it has been reported that various diseases including human immunodeficiency [6,7], lymphoma [8] and bloom syndrome [9] may be caused by the abnormal UDG activity. Therefore, it is significant to monitor the UDG activity for preventing from or diagnosis of these related diseases.

Conventional methods for the detection of UDG activity mainly include radioactive label, gel electrophoresis, mass spectrometry [10–12]. These methods are mostly based on separation principle, which are not only time-consuming, low sensitivity, but also indirect. Besides, the fluroscence method for detecting the UDG activity has been widely employed. For example, Maksimenko et al. carried out the detection of UDG activity by the fluorescence resonance energy transfer (FRET) probe [12,13]. Zhou et al. detected UDG activity based on the interaction between a fluorophore-labeled hairpin DNA probe and graphene [14]. These strategies are effective for the lower detection limits. However, there is a large amount of samples required and many false-positive errors due to being performed in homogeneous phase. Therefore, it is crucial to develop a fast, simple, sensitive and economical technique for UDG activity assay.

Graphene, as a kind of one-atom-thick and two-dimensional (2-D) carbon nano material, possesses excellent mechanical, thermodynamic, electrochemical and catalytic properties and has

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been widely employed in many research fields [15]. It has been reported that graphene modified electrode can improve the electrochemical oxidation of guanine and enhance the detection sensitivity of guanine [16,17], comparing to other modified electrodes, such as carbon nanofibers modified electrode [18], PbO_2-carbon nanotube-ionic liquid composite film modified electrode [19], cobalt phthalocyanine modified carbon nanotubes paste electrode [20], and so on. Meanwhile, it has been known that unique 2-D structure of graphene is useful for forming π–π stacking between graphene and ssDNA, which is effective for the adsorption of ssDNA onto the surface of graphene. On basis of this, Lim et al. investigate the electrochemical oxidation behavior of bases in ssDNA and dsDNA at the graphene-modified electrode, respectively [21]. It was found that bases in ssDNA can be electrochemically oxidized at the graphene modified electrode, but the electrochemical oxidation of bases in dsDNA did not take place. In addition, Dubuisson et al. studied the electrochemical oxidation of bases in ssDNA at the graphene-modified electrode and realized the detection of single base's mutation in short ssDNA [22]. Therefore, it can be concluded that the electrochemical oxidation of bases in ssDNA, not in dsDNA could take place, and also can be improved at the graphene modified electrode.

In this present paper, we report a simple and label-free electrochemical approach for the detection of UDG activity based on the electrochemical oxidation of guanine bases in ssDNA at the graphene modified electrode. Using the electrochemical oxidation of guanine bases in released P1 at the graphene/GC electrode as the signal indicator, the detection UDG activity can be simply carried out with high sensitivity and selectivity. Comparing to reports in previous literature [13,14,23–25]. There are no requirements to label the DNA strand and to construct the complex substrate electrode. This proposed strategy may provide a simple and rapid method for successful monitoring UDG activity, and also lay a potential foundation for researches of UDG activity in the field of clinical diagnosis.

2. Experimental section

2.1. Apparatus and reagents

A CHI660A electrochemical workstation (Shanghai Chenhua Instrument Corporation, China) was employed for all electrochemical measurements. The electrochemical workstation has a conventional three-electrode mode. The modified glassy carbon (GC) electrodes (diameter: 3 mm) were used as the working electrode. The saturated calomel electrode (SCE) and a platinum wire were used as the reference and counter electrodes, respectively. Differential pulse voltammetry (DPV) was used to characterize the electrochemical oxidation of guanine (G) bases. DPV measurements were performed within the potential range from 0.7 to 1.05 V with the pulse amplitude of 50 mV and the pulse width of 50 ms. Raw voltammograms were treated with the automatic baseline correction method offered by computer-controlled instruments (CHI660A) [26]. All measurements were carried out in a phosphate buffer solution (PBS, 0.1 M, pH 7.0) as supporting electrolyte, and doubly distilled water was used throughout. All the potentials in this paper were with respect to SCE.

Uracil-DNA glycosylase (UDG), 10 × NEBuffer (200 mM Tris–HCl, 10 mM ethylene diamine tetraacetic acid (EDTA), 10 mM dithiothreitol (DTT), pH 8.0) and the stock solution of UDG (20 mM Tris–HCl, pH 8.0, 50% glycerol, 50 mM KCl, 1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA) were purchased from New England Biolabs (NEB, US). The synthetic oligonucleotides (DNA1 (P1): 5′-GTGAAGTTGAGGGAT-3′, DNA2 (P2): 5′-AUCCCUCAACUUCAC-3′) were obtained from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and used without further purification. Double distilled water was used throughout, which was obtained through a Millipore Milli-Q water purification system and had an electric resistance 18.2 M Ω cm^{-1}.

The graphene oxide (GO) was synthesized from natural graphite powder by the modified Hummers method according to previous reports [27]. Then the as-prepared GO powder was subjected to dialysis for 7 days to completely remove metal ions and acids [28]. Finally, the product was dried in air at room temperature for further use. The obtained GO powder was exfoliated in a 0.1 M acetate buffer solution (ABS, pH 5.0) by ultrasonication for 5 h to form GO colloidal dispersion (1.0 mg/mL).

2.2. Fabrication of the graphene modified electrode

The GC electrode was polished carefully to a mirror-like surface with 1.0 and 0.3 μm alumina slurry (CH Instruments) and then ultrasonically washed in ethanol and doubly distilled water for 5 min, respectively. Subsequently, the treated electrode was voltammetrically cycled in 0.5 M H_2SO_4 with the potential between −0.2 and 1.5 V at 50 mV/s until a representive cyclic voltammogram of GC electrode was obtained. And the cleaned electrode was dried under N_2 blowing for further use.

The graphene modified electrode was fabricated by referring the literature [29]. In brief, graphene nanosheets were directly deposited onto a glassy carbon electrode through cyclic voltammetric reduction of a graphene oxide colloidal solution (5 mL, 1.0 mg/mL) within the potential range from −1.5 to 0.5 V at a scan rate of 10 mV/s for ten CV cycles with a magnetic stirring and N_2 bubbling. Afterwards, CVs of the obtained graphene/GC electrodes in solution containing 0.1 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl were investigated to make sure that the current responses of these graphene/GC electrodes were almost reproducible. The obtained graphene/GC electrode was washed throughout and dried under N_2 blowing for later use.

2.3. Electrochemical detection of UDG activity

Both P1 and P2 solutions were prepared in 1 × NEBuffer (20.0 mM Tris–HCl, 1.0 mM EDTA, 1.0 mM DTT, pH 8.0). The hybridization reaction was carried out by mixing P1 (1.0 mL) and P2 (1.0 mL) at the same concentration. The mixture was first heated to 95 °C for 5 min, followed by slow cooling to room temperature within 2 h. The final reaction product 1.0 μM P1P2 (dsDNA) was kept in the refrigerator at 4 °C for further use.

The solution of 10.0 μL P1 (500.0 nM), P2 (500.0 nM), and P1P2 (500.0 nM) was dipped onto the graphene/GC electrode surface, respectively, and keeping for 12 min to fabricate the P1/graphene/GC, P2/graphene/GC, and P1P2/graphene/GC electrodes. And then, the electrodes were carefully rinsed with NEBuffer, respectively. Afterwards, the electrochemical behaviors of the obtained electrodes in 0.1 M PBS (pH 7.0) were investigated by DPV.

The cleavage of hybridized DNA (P1P2) by UDG was performed at 37 °C for 2 h in 1 × NEBuffer (1.0 mL, 20.0 mM Tris–HCl, 1.0 mM EDTA, 1.0 mM DTT, pH 8.0) containing 30.0 mM NaCl, 5.0 mM $MgCl_2$, 500.0 nM P1P2 and a specific concentration of UDG. Afterwards, 10.0 μL solution containing the prepared product was dropped on the graphene/GC electrode surface to keep for 12 min, leading to the adsorption of released ssDNA onto the graphene/GC electrode surface. And the electrochemical behavior of the obtained electrode (ssDNA/graphene/GCE) in 0.1 M PBS (pH 7.0) was investigated by DPV. A series of various concentrations of UDG solutions were prepared by diluting the stock solution of UDG (20.0 mM Tris–HCl, pH 8.0, 50% glycerol, 50.0 mM KCl, 1.0 mM EDTA, 1.0 mM DTT, 0.1 mg/mL BSA). DPV measurements were