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Short communication

An electrochemical method to assay human 8-oxoguanine DNA glycosylase 1

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ABSTRACT

Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is an important DNA repair enzyme that can excise the 8-oxo-7,8-dihydroguanine (8-oxoG) from damaged DNA and is implicated in the etiology of many kinds of cancers. Here, we report an electrochemical method to assess the activity of hOGG1 by using a carefully designed DNA probe, which contains an internal 8-oxoG site and an electrochemical active tag. In this method, hOGG1 can selectively recognize the 8-oxoG site and cleave the probe DNA, releasing a methylene blue (MB)-labeled signal fragment. The signal fragment can then hybridize with the DNA molecules that have been previously immobilized on a gold electrode surface. Consequently, a sensitive electrochemical technique, square wave voltammetry, can be employed for the signal readout, thus characterizing the activity of hOGG1. This method is simple, sensitive and specific, and it has been used for human serum samples, so it may imply a potential new method for the assay of hOGG1.

1. Introduction

Reactive oxygen species (ROS) are produced as a result of aerobic metabolism or exposure to external oxidative environment, which may induce various oxidative damages of cells such as apoptosis and mutagenesis [1]. Normally, most of the ROS can be cleared by antioxidants, such as superoxide dismutase and glutathione peroxidase [2,3]. However, the antioxidant capacity of individuals is limited. Once ROS are overabundant, DNA may be oxidized to produce damaged bases, single- or double-strand breaks [4]. Oxidized bases can subsequently cause mutations in the replication process, which may be implicated in the development of cancers. Guanine is most easily oxidized to 8-oxo-7,8-dihydroguanine (8-oxoG) because of low redox potential [5]. If not removed, the 8-oxoG may cause a G: C→T: A transversion during replication by pairing with adenine [6]. Studies have shown that the presence of such mutation is often found in the coding sequences of oncogenes and tumor suppressor genes [7,8]. So, the increase of 8-oxoG abundance is associated with an elevated risk for cancers [9].

Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is a bi-functional N-glycosylase/DNA lyase. It can selectively bind and remove the 8-oxoG base to create an apurinic site (AP site) in the double-stranded DNA (dsDNA). It can then make a nick at the AP site for the following repairmen [10]. So, the 8-oxoG lesion of DNA can be repaired through this process. Current studies have shown that mutations of hOGG1 gene are closely related to the development of the cancer of lung and prostate [11,12]. So, to investigate the activity of hOGG1 is essential for evaluating the susceptibility of cancers in clinical practice.

Because of the significant relationship with cancer risks, hOGG1 has attracted much interest among many research groups. There are many methods to detect the activity of hOGG1 [13]. For example, to characterize the activity of hOGG1 in patients with pancreatic cancer, Li et al. [14] measured the level of 8-oxoG in the cancerous tissues through the high pressure liquid chromatography with electrochemical detection (HPLC-ECD). Kondo et al. [15] studied the expression of hOGG1 gene in colorectal carcinoma cells by quantitative–competitive RT-PCR. However, the use of these methods is limited by complicated and time-consuming procedures with high costing. Here, we report an effective and simple electrochemical method to analyze the activity of hOGG1. This method has shown advantages of low cost, fast response and easy operation, and it may have great potential in clinical applications in the future.

2. Experimental section

2.1. Materials and reagents

Human 8-oxoguanine DNA glycosylase 1 (hOGG1) and human apurinic/apyrimidinic endonuclease 1 (APE1) were purchased from New England Biolabs Ltd. (Beijing, China), together supplied with 10 × NEBuffer2 and bovine serum albumin (BSA). Ribonuclease A (RNase A),

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human serum albumin (HSA), 6-mercapto-1-hexanol (MCH), tris(2-carboxyethy) phosphine hydrochloride (TCEP), and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma. Human serum was purchased from Dingguo Biotech. Co. All solutions were prepared with deionized water, which was purified with a Milli-Q purification system (Bedford, MA). DNA oligonucleotides were synthesized by Takara Biotechnology Co., Ltd. The sequences of oligonucleotides used in the experiment were listed as follows:

DNA previously immobilized on the working electrode surface (E-DNA): 5′-AGGAGTAACTAACGG-SH-3′. DNA labeled with methylene blue (MB) (MB-DNA): 5′-MB-CCGTTAGTTACTCCT(8-oxoG)CTTCC-3′. Control DNA: 5′-MB-CCGTTAGTTACTCCTGCTTCC-3′. C-DNA: 5′-GGAAGC AGGAGTAAC-3′. The underlined sequences could hybridize with C-DNA to form a dsDNA.

The buffer solutions were as follows: the stock solution of E-DNA: 10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP and pH 8.0. The stock solutions of MB-DNA, control DNA and C-DNA: 10 mM Tris–HCl, 1 mM EDTA and pH 8.0. E-DNA immobilization solution: 10 mM Tris–HCl, 1 mM EDTA, 1 M NaCl and pH 7.4. Hybridization buffer: 10 mM phosphate buffer saline (PBS), 0.25 M NaCl and pH 7.4. Buffer for square wave voltammetry (SWV): 10 mM Tris–HCl and pH 7.4. Buffer for electrochemical impedance spectroscopy (EIS): 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KNO_3.

2.2. Preparation of E-DNA modified gold electrode

Firstly, the gold electrode (3 mm in diameter) was dipped in piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1) for 5 min followed by rinsing with deionized water. Then, it was polished to a mirror sheen using sand paper and 1.0, 0.3 and 0.05 μm alumina powder, respectively. After successive sonication in ethanol and deionized water for 5 min, the gold electrode was kept in 50% (v/v) nitric acid for 30 min. After that, it was electrochemically cleaned with 0.5 M H_2SO_4 followed by drying with high purity nitrogen. 10 μL E-DNA (0.5 μM) was then dropped on the gold electrode surface. After incubation for 16 h, the electrode was dipped in 1 mM MCH for 30 min to passivate the surface [16]. The electrode was rinsed with sufficient deionized water before the following experiments.

2.3. Detection of hOGG1

C-DNA and MB-DNA were firstly denatured in their stock solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) at 90 °C for 5 min with a ratio of 3:1 (3 μM:1 μM). Then the mixture was slowly cooled down to room temperature for annealing. After the formation of dsDNA, it was incubated with various concentrations of hOGG1 at 37 °C for 30 min in 1 × NEBuffer2 containing 100 μg mL^{-1} BSA. The reaction was terminated at 65 °C for 15 min. Then, the gold electrode was incubated with the reaction solution for 2 h at room temperature. Finally, the electrode was carefully rinsed and ready for electrochemical measurements.

2.4. Electrochemical measurements

All electrochemical measurements were carried out on a CHI660D Potentiostat (CH Instruments) work station with a three-electrode system, which included the above E-DNA modified gold electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum electrode as the counter electrode. The experimental parameters were as follows: SWV: potential range, −0.1 to −0.5 V; frequency, 100 Hz; and amplitude, 20 mV. EIS: bias potential, 0.224 V; amplitude, 5 mV; and frequency range, 0.01 Hz–10 kHz.

3. Results and discussion

The principle of our design is illustrated in Fig. 1. A methylene blue (MB) labeled oligonucleotide with an 8-oxoG site (MB-DNA) is hybridized with C-DNA to form a dsDNA through specific base pairing. In the presence of hOGG1, the enzyme selectively cleaves MB-DNA of the dsDNA at the 8-oxoG site, splitting MB-DNA into two fragments. The two fragments thus dissociate from C-DNA to form single-stranded DNAs as a result of the decreased base-stacking force. Then, the MB-labeled signal fragment can hybridize with the E-DNA and get access to the gold electrode surface. So, the electrochemical SWV signals of the MB can be recorded, which can be used to assay the activity of hOGG1.

The stepwise modification of the gold electrode can be recorded by EIS [17]. As shown in Fig. 2A, EIS of the bare electrode is similar to a straight line. After the modification of E-DNA through gold–sulfur chemistry [18], a large semicircle appears, indicating the increase

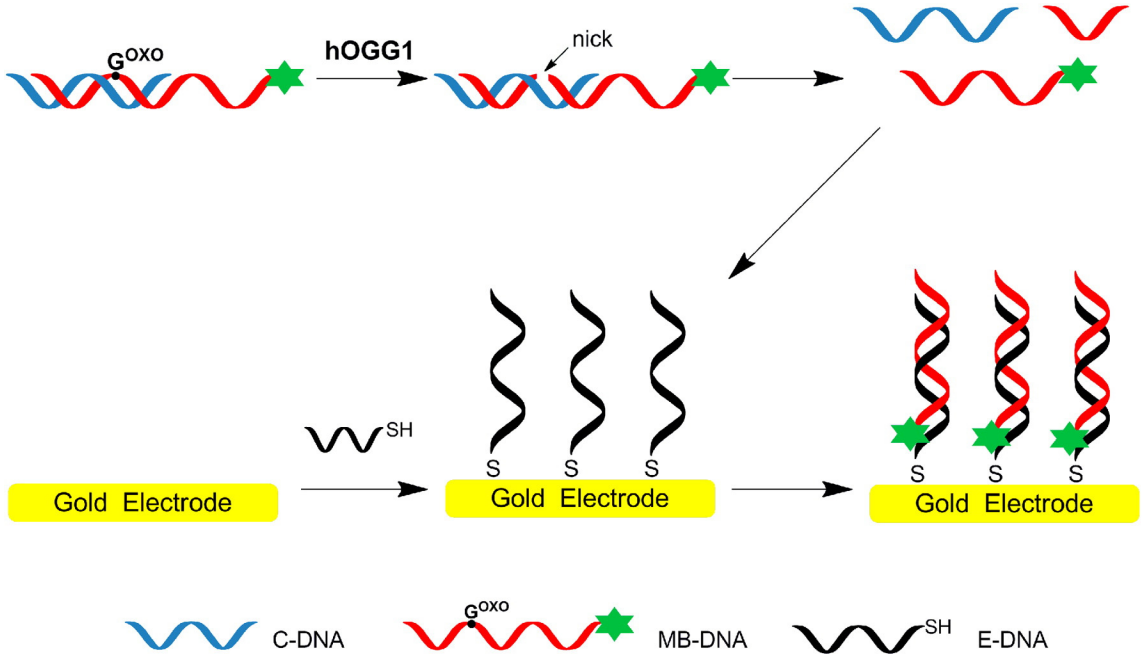

Fig. 1. The principle of the electrochemical assay for hOGG1.