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Constructing a novel 8-hydroxy-2′-deoxyguanosine electrochemical sensor and application in evaluating the oxidative damages of DNA and guanine

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ABSTRACT

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is commonly identified as a biomarker of oxidative DNA damage. In this work, a novel and facile 8-OHdG sensor was developed based on the multi-walled carbon nanotubes (MWCNTs) modified glassy carbon electrode (GCE). It exhibited good electrochemical responses toward the oxidation of 8-OHdG, and the linear ranges were $5.63\times10^{-8}-6.08\times10^{-6}$ M and $6.08\times10^{-6}-1.64\times10^{-5}$ M, with the detection limit of 1.88×10^{-8} M (S/N=3). Moreover, the fabricated sensor was applied for the determination of 8-OHdG generated from damaged DNA and guanine, respectively, and the oxidation currents of 8-OHdG increased along with the damaged DNA and guanine within certain concentrations. These results could be used to evaluate the DNA damage, and provide useful information on diagnosing diseases caused by mutation and deficiency of the immunity system.

1. Introduction

Reactive oxygen species (ROS), including hydroxyl radicals (•OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), are constantly generated in vivo during metabolic processes (Shigenaga et al., 1989; Zhang et al., 2015). Deoxyribonucleic acid (DNA), as an important biomolecule, can be attacked by ROS to cause oxidative damage, which could give rise to carcinogenesis and aging (Fojta et al., 2016; Sattarova et al., 2013; Zhang et al., 2015). Therefore, the investigation of oxidative DNA damage is crucial for human health. Nevertheless, the relationship between the degree of DNA damage and the corresponding diseases is still a concern in the medical field. Compared with other nucleobases in the DNA molecule, guanine is the most susceptible to oxidative damage since it possess the lowest oxidation potential, and could combine with the transition-metal ions to promote the catalyze oxidative processes (Holmberg et al., 1999; Zhang et al., 2013; Burrows and Muller, 1998; Muller et al., 1996). Study found that 8-hydroxy-2′-deoxyguanosine (8-OHdG) is formed first from •OH attack of guanine bases in the DNA molecule (Changenet-Barret et al., 2015; Zhang et al., 2013). Then it is typically excreted into urine without further change, and its excretion level is usually thought to reflect the extent of total body DNA oxidative damage (Liu et al., 2014a; Tuma et al., 2004). Thus, 8-OHdG is commonly accepted as a biomarker of oxidative DNA damage, and it is necessary to develop a sensitive 8-OHdG analytical technique and construct an oxidative DNA damage evaluation system.

Recently, various techniques have been employed to analyze the damaged DNA and 8-OHdG, respectively, such as gas chromatography-mass spectroscopy (Spencer et al., 1994; Teixeira et al., 1993), high-performance liquid chromatography (Esaka et al., 2003; Pilger et al., 2002; Fan et al., 2012), solid phase microextraction (Zhang et al., 2013), the coupling flow injection–chemiluminescent reaction system (Zheng et al., 2014), fluorescence (Viswesh et al., 2010) and CD spectroscopy method (Liu et al., 2016). However, these techniques usually require complicated pre-treatment steps, expensive equipments or skilled operators. In order to overcome these inherent disadvantages, electrochemical analytical technique may be a good option due to its fast response, excellent sensitivity, and simple apparatus. For example, Long et al. applied nanopore sensors to the discrimination of oligonucleotides, DNA damage and other single molecule (Cao et al., 2016; Ying et al., 2014). Wang et al. constructed an electrochemical sensor for the monitoring of DNA damage induced by ferric ions mediated oxidation of dopamine (Chen et al., 2013). Yu et al. assessed the total antioxidant capacity based on the immobilizing DNA on a poly L-glutamic acid doped silver hybridized membrane (Wang et al., 2014). Chen et al. evaluated of total antioxidant capacities in fruit juice based on electro-immobilization of guanine on graphene nanoribbon modified electrode (Yang

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et al., 2013). In these papers, they indirectly detected the damaged DNA by using the electrochemical responses of $[Co(bpy)_3]^{3+}$ or $Ru(NH)_6^{3+}$ as indicator, and discussed the influences of oxidation time and the molar ratio of Fenton reagents to current respond. Nevertheless, few researchers paid attention to the direct electrochemical study of oxidative DNA damage. On the other hand, some modified electrodes were developed for the determination of 8-OHdG. Recently, Wang et al. fabricated an electrochemical 8-OHdG sensor based on the poly (3-methylthiophene) modified electrode (Li et al., 2007). Galicia et al. investigated the oxidation of 8-OHdG at the polyethylenimine dispersed carbon nanotubes modified electrode (Gutiérrez et al., 2011). Unfortunately, all of the above work did not establish a relationship between oxidative DNA damage and the electrochemical responses of 8-OHdG. This will be a huge challenge for the further analysis of oxidative DNA damage process in living organisms. Hence, it is imperative to construct a highly sensitive electrochemical sensor to detect the 8-OHdG and assess the oxidative DNA damages. Furthermore, as well known, the abnormal change of guanine in DNA may cause deficiency and mutation in the immunity system, resulting in the presence of various diseases (Liu et al., 2014b ; Wallace, 2002). Therefore, the determination of the damaged guanine also has great significance to the biomedical chemistry and clinical diagnosis.

In this paper, we firstly fabricated an 8-OHdG sensor based on the multi-walled carbon nanotubes (MWCNTs) modified glassy carbon electrode (GCE). Then, the proposed sensor was applied to measure the 8-OHdG generated from the damaged DNA and guanine. The work will be potential to evaluate the DNA damage and provide useful information on diagnosing diseases in immunity system.

2. Experimental

2.1. Reagents

The multi-walled carbon nanotubes (MWCNTs) (diameter: 20–40 nm, length: $<5\,\mu m$, purity: $\geq 97\%$) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). 8-Hydroxy-2′-deoxyguanosine (8-OHdG) was bought from Sigma-Aldrich (Shanghai, China). Guanine was received from Aladdin. Double stranded calf thymus DNA was supplied from Shanghai yuanye Biochemical reagent (Shanghai, China). $FeSO_4$. 7 h_2O was obtained from Tianjin Chemical Reagent (Tianjin, China). Hydrogen peroxide solution (30 wt%) was provided by Beijing Chemical Reagent (Beijing, China). L-Asecorbic acid (L-AA) came from Shanghai zhongqin Chemical reagent (Shanghai, China). All the solutions were employed with deionized water and other reagents were of analytical grade.

2.2. Apparatus

Scanning electron microscopy (SEM) micrograph was acquired from JSM-6701F field emission SEM system operating at 5.0 kV (JEOL, Japan). Fourier transform infrared (FT-IR) spectra were recorded with a Fourier Transform-Infrared (FT-IR) spectrophotometer (USA). Electrochemical impedance spectroscopy (EIS) was measured by Multi-potentiostat (VMP2, Princeton Applied Research, USA). Electrochemical measurements were carried out on a CHI660C electrochemical workstation (Austin, TX, USA) with a conventional three-electrode cell. A bare or MWCNTs modified GCE (d=3.0 mm) was used as working electrode. An Ag/AgCl electrode (saturated KCl) and a platinum electrode were acted as the reference electrode and counter-electrode, respectively.

2.3. Preparation of the purified MWCNTs

The purified MWCNTs were prepared as described previously (Pilehvar et al., 2014). 100 mg of the pristine MWCNTs were dissolved in 200 mL mixture of 1:3 (V/V) concentrated HNO_3/H_2SO_4. The mixture solution was refluxed under magnetic stirring at 80 °C for 5 h. Then, the resulting solution was filtered and washed with deionized water to neutrality. Finally, the purified MWCNTs were obtained after dried in a vacuum at 38 °C. 5 mg of the purified MWCNTs were dispersed in 10 mL of doubly distilled water and sonicated for 30 min to obtain homogenous MWCNTs suspension.

2.4. Preparation of the MWCNTs/GCE

A glassy carbon (GCE) electrode was polished successively with 1.0, 0.3 and 0.05 μm alumina powder, rinsed thoroughly with doubly distilled water. Subsequently, 6 μL of 0.5 mg/mL MWCNTs suspension was dropped on the pretreated GCE surface and dried in the air. The obtained electrode was donated as MWCNTs/GCE.

2.5. Preparation of the damaged DNA and guanine

Preparation of the damaged DNA (Qu et al., 2011; Wang et al., 2012): First, 800 μL 0.01 mol L^{-1} $FeCl_2$ and 800 μL 0.05 mol L^{-1} L-AA were successively added into 4 mL 1 mg/mL calf thymus DNA solution. Subsequently, the mixture was continuously stirred for 5 min. After that, 800 μL 0.3 mol L^{-1} H_2O_2 was added and the resulting mixture was heated at 36 °C for 40 min. Finally, the damaged DNA was obtained and stored at 4 °C. During the course, DNA damage was induced by •OH generated from Fenton reaction (Fe^{2+}/H_2O_2). Especially, at high concentrations of L-AA and the presence of dissolved oxygen, L-AA can not only provide hydrogen ions, but also act as a prooxidant for the reduction of Fe^{3+} to Fe^{2+} (Samuni et al., 1983; Cross et al., 2003). For the control experiments, the undamaged DNA was prepared in blank solution, and Fenton reagents were composed of $FeCl_2$ and H_2O_2. Besides, the damaged guanine was obtained using the similar method as described previously.

3. Results and discussion

3.1. Characterizations of the modified electrode (MWCNTs/GCE)

Fig. 1A displays the SEM image of the purified MWCNTs. It is clear that the purified MWCNTs were less agglomerated and formed a disordered network-like structure. Fig. 1B shows the FT-IR spectra of the pristine and purified MWCNTs. Compared with the pristine MWCNTs (a), a new peak was observed at 1710 cm^{-1} in the purified MWCNTs (b), which corresponded to the C=O stretching vibrations of carboxylic groups. The particular peak confirmed that MWCNTs have been purified already (Zeng et al., 2007; Pilehvar et al., 2014). Fig. 1C depicts typical cyclic voltammograms of different electrodes in 5.0 mM $[Fe(CN)_6]^{3-/4-}$. A pair of redox peaks was observed in each CVs, which was ascribed to the redox of $[Fe(CN)_6]^{3-/4-}$. Relative to bare GCE (curve a), the current in MWCNTs/GCE (curve b) increased significantly, revealing that introduction of MWCNTs could accelerate the electron transfer and enhance the electric conductivity (Li et al., 2014a; Nie et al., 2011).

Electrochemical impedance spectroscopy (EIS) is also an effective tool to monitor the interface properties of modified electrode. The impedance spectrum includes a semicircle portion at higher frequencies representing the limited electron transfer process and a linear segment at lower frequency region corresponding to the diffusion controlled process. By using $[Fe(CN)_6]^{3-/}$