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An electrochemical biosensor for the rapid detection of DNA damage induced by xanthine oxidase-catalyzed Fenton reaction

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ABSTRACT

Oxidative DNA damage is one of the most critical factors implicated in carcinogenesis and other disorders. Sensitive and reliable detection of oxidative DNA damage remains a significant challenge. In this work, a sensitive electrochemical biosensor based on a double stranded DNA immobilized on a xanthine oxidase (XOD)-modified glassy carbon electrode (denoted as DNA–XOD/GCE) has been developed to explore the rapid detection of DNA damage. $Co(bpy)_3^{3+}$ was used as a redox indicator to monitor DNA damage induced by hydroxyl radical (•OH), which is a reactive oxygen species generated by a XOD-catalyzed Fenton reaction in xanthine/$FeSO_4$ system. The produced •OH was validated by UV–vis spectroscopy. The electrochemical behavior of the underlying electrodes was characterized by square wave voltammetry and electrochemical impedance spectroscopy. Optimization of the concentrations of $FeSO_4$ and XA, and the incubation time in terms of DNA damage was explored. Moreover, the protection of DNA from damage by antioxidants, such as ascorbic acid, aloe-emodin, and rutin was investigated. The conclusions demonstrate that the proposed electrochemical method is expected to be of use in further application for DNA damage studies.

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1. Introduction

In recent decades, the detection of DNA damage has become one of the most important DNA research fields, because of the critical role of DNA in mutagenesis, carcinogenesis, and aging. It is well known that DNA in biological systems may be damaged by both exogenous and endogenous sources. Among them, the reactive oxygen species (ROS) have aroused great scientific interest. ROS play an important role in DNA damage, which is tightly related to mutagenesis and carcinogenesis [1]. If the damaged DNA cannot be repaired in time, the induced genetic mutation will result in a cancer or tumor during the DNA replication process [2]. Therefore, it is necessary to establish a sensitive, rapid, and inexpensive way to detect DNA damage.

Up to now, varieties of analytical methods have been developed for detecting DNA damage, including capillary liquid chromatography/mass spectrometry [3], fluorescence [4], ^{32}P-postlabeling [5], and capillary zone electrophoresis [6]. These methods are generally performed at centralized laboratories, requiring long assay times and high costs. Electrochemical methods have aroused great interest among researchers, mainly because of their simplicity, fast response, relatively low cost, and low power requirements [7,8], and much work have been carried out. Usually, two kinds of electrochemical methods are adapted to detect DNA damage, which are based on the direct electrochemical signals of guanine and adenine bases [9–11], or indirect electroactive indicators which could specifically interact with DNA [12,13]. Our groups have detected the oxidative DNA damage by hydroxyl radical (•OH) using $Co(bpy)_3^{3+}$ as electroactive probes, where •OH were generated by Fenton reagents [14]. In order to mimic the metal-mediated ROS generation pathway in vivo, H_2O_2 could be produced in situ through the enzymatic reaction. A large number of studies have been reported about the electrochemical detection of DNA damage by the enzymatic reaction [15,16]. Recently, our group had performed the electrochemical detection of in situ DNA damage induced by glucose oxidase-catalyzed Fenton reaction [17]. Some references have reported that xanthine oxidase (XOD) could catalyze the oxidation of xanthine (XA) under aerobic conditions, and the produced H_2O_2 reacted with ferrous ions in a Fenton-type reaction to generate •OH [16,18].

In this report, a sensitive electrochemical biosensor constructed from dsDNA and a XOD modified glassy carbon electrode (DNA–XOD/GCE) has been developed to explore the rapid detection

Abbreviations: XOD, xanthine oxidase; •OH, hydroxyl radical; ROS, reactive oxygen species; XA, xanthine; DNA–XOD/GCE, dsDNA and a XOD modified glassy carbon electrode; $Co(bpy)_3(ClO_4)_3$, Tris(2,2′-bipyridyl) cobalt(III) perchlorate; AA, ascorbic acid; AE, aloe-emodin; GCE, glassy carbon electrode; DNA/GCE, DNA modified GCE; XOD/GCE, XOD modified GCE; SWV, square wave voltammetry; MV, methyl violet.

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of DNA damage. Tris(2,2′-bipyridyl) cobalt(III) perchlorate ($Co(bpy)_3(ClO_4)_3$) was used as a redox indicator to monitor DNA damage induced by •OH in aqueous solutions. The results demonstrate that the proposed electrochemical method is likely to find further application in DNA damage studies.

2. Experimental

2.1. Chemicals and reagents

Calf thymus DNA was purchased from Sigma–Aldrich. Solutions of DNA were prepared by dissolving it in 0.01 M Tris–HCl/0.001 M ethylenediaminetetraacetic acid/0.05 M NaCl (pH 7.0) to form the 1.0 mg mL^{-1} stock solution and stored at 4 °C. XOD (EC 1.1.3.4, Type X-S, from *Aspergillus niger*) was obtained from Sigma–Aldrich. Solutions of XOD were dissolved in 0.1 M phosphate buffer solution (PBS, pH 7.0) to form 3.0 mg mL^{-1} solutions. XA were purchased from Sigma–Aldrich. $Co(bpy)_3(ClO_4)_3$ was prepared as described in the literature [19] and dissolved in 0.005 M Tris–HCl (pH 7.0) containing 0.05 M NaCl. Ascorbic acid (AA) was supplied by the Hubei University Chemical Factory (Hubei, China). Rutin and aloe-emodin (AE) were purchased from Shanghai Boyun Biotech Co., Ltd. (Shanghai, China). All solutions were prepared with doubly distilled water. Other reagents were analytical grade.

2.2. Apparatus

Electrochemical measurements were performed on a model CHI 625A electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China). A standard three-electrode system, consisting of a film modified glassy carbon electrode (GCE) as the working electrode, a saturated calomel electrode as the reference electrode, and platinum foil as the auxiliary electrode, was used in the measurements. UV–vis spectroscopy was carried out with a UV 2300 spectrophotometer (Shanghai Tian Mei Scientific Instrument Co., Ltd., China). Atomic force microscopy (AFM) images were obtained on a PicoScan system (Molecular Imaging Inc.) operated in contact mode with commercially ultrasharpened Si_3N_4 tips (MAClever II, Molecular Imaging Inc.). The AFM measurement parameters were as follows: force constant, 0.12 N/m; number of scans, 8 times.

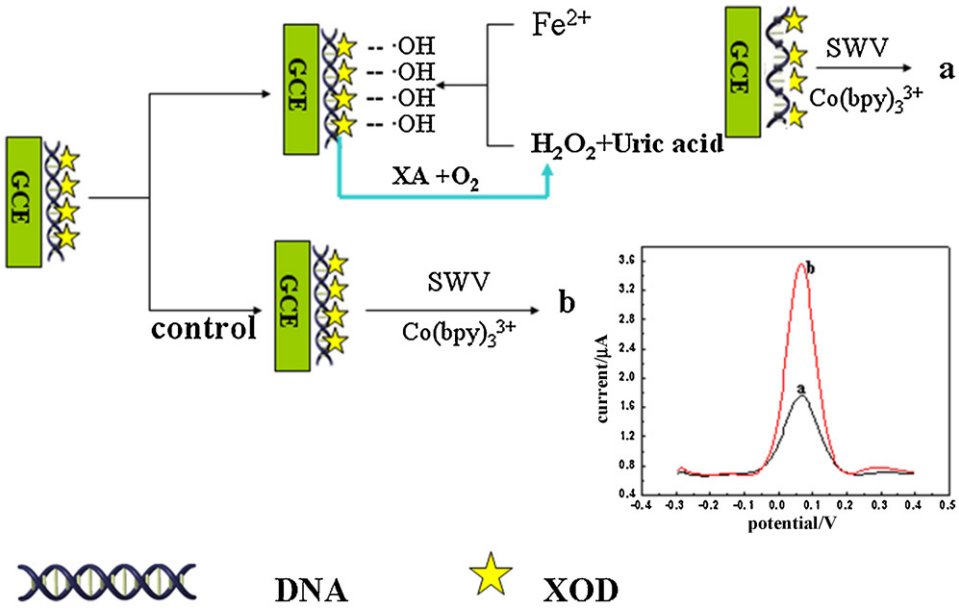
2.3. Preparation of the DNA–XOD/GCE

In our work, 1 mg mL^{-1} DNA and 3 mg mL^{-1} XOD with a volume ratio of 1:1 were mixed thoroughly, and 30 μL of this mixture was directly applied to the surface of the clean GCE, followed by air-drying overnight. Then, the modified electrode was incubated in a pH 7.0 PBS for 4 h to remove any non-adsorbed DNA. This electrode was hereafter referred to as DNA–XOD/GCE. For comparison, DNA modified GCE (DNA/GCE) and XOD modified GCE (XOD/GCE) were also prepared using a similar direct application technique.

2.4. Procedures

Scheme 1 shows the detection approach of DNA damage induced by XOD-catalyzed Fenton reaction in xanthine/$FeSO_4$ system. DNA–XOD film was incubated in pH 3.0 buffer containing 1 mM $FeSO_4$ and 1.7 mM XA, with stirring for the specified time for DNA damage (curve b). Control experiments were that DNA–XOD film was incubated in pH 3.0 buffer containing $FeSO_4$ or XA separately, and DNA film or XOD film were incubated in pH 3.0 buffer containing $FeSO_4$ or XA or $FeSO_4$/XA (curve a). To investigate the effects of antioxidants, aliquots of antioxidant samples were added into the above system. That is, DNA–XOD film was incubated with $FeSO_4$ and XA in a pH 3.0 buffer in the presence of AA, AE, or rutin. All incubations were conducted at room temperature with stirring for 20 min. After incubation, the modified electrode was rinsed with doubly distilled water and then transferred into pH 7.0 Tris–HCl containing 200 μM $Co(bpy)_3^{3+}$ for square wave voltammetry (SWV). Each measurement was repeated at least 3 times.

To correct the electrode-to-electrode or film-to-film variation for replicative experiments, the SWV oxidation peak current ratio (I_{pt}/I_{p0}), instead of the absolute peak current (I_{pt}), was used to evaluate the effect of DNA damage, where I_{pt} and I_{p0} are the peak currents after DNA–XOD film incubation with $FeSO_4$ and XA in a pH 3.0 buffer for t and 0 min.



Scheme 1. Schematic diagram showing working principle of SWV detection of in situ DNA damage on DNA–XOD film.

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