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A novel electrochemical biosensor for monitoring protein nitration damage affected by $NaNO_2$/hemin/H_2O_2

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ABSTRACT

A sensitive and facile electrochemical biosensor has been developed for monitoring the protein nitration damage affected by the nitro free radicals($NO_2^{\bullet}$). The $NO_2^{\bullet}$ radicals is generated from hemin-catalyzed oxidation of nitrite (NO_2^-) in the presence of hydrogen peroxide (H_2O_2). In this work, nanocomposite films of graphene-gold nanoparticles (EG-AuNPs) were modified on the glassy carbon electrode by electropolymerization. Bovine serum albumin (BSA) was then further assembled on EG-AuNPs film through Au–S bond. The damage of BSA molecule was caused by the $NO_2^{\bullet}$ radicals which was generated from the $NaNO_2$/hemin/H_2O_2 nitration reagent. The differential pulse voltammetry was used to detect the damage of BSA molecule. Fluorescence spectra and circular dichroism spectrum further confirmed the nitration damage of BSA. Moreover, the lowest concentration at which the BSA damage was detected is 28.9 μM NO_2^- or H_2O_2, and the volume ratio of NO_2^- and H_2O_2 was 1:1 in the hemin/NO_2^-/H_2O_2 nitration reagent. The results demonstrated that the proposed electrochemical method can be used to detect protein damage affected by nitration reagent. The developed electrochemical biosensor is envisioned to have promising applications in protein damage studies.

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important groups of free radicals which are capable of eliciting direct damaging effects or acting as critical intermediate signaling molecules, leading to oxidative and nitrosative stress and a series of biological consequences(Helen Wiseman 1996). The $NO_2^{\bullet}$ radical as one kind of RNS, is a potent oxidant and nitrating agent. Excessive generating of $NO_2^{\bullet}$ radical can damage protein, DNA and polyunsaturated fatty acids, and may lead to oxidative stress and a variety of diseases. The nitration of proteins modulates catalytic activity, cell signaling, and cytoskeletal organization (Chiappetta et al., 2009; Darley-Usmar and Halliwell, 1996; Fang, 2004). Therefore, the study about nitration damage induced by $NO_2^{\bullet}$ radical on protein plays a crucial role in the study of biological toxicity and pathogenic mechanism of $NO_2^{\bullet}$ radical.

NO_2^- widely existed in plants, water and was one of the key sources of RNS. It is widely believed that $NO_2^{\bullet}$ radical can be generated by hemin peroxidase/H_2O_2/NO_2^- system in situ and induce the enzymatic nitration of various compounds including bio-macromolecules, where the peroxidase includes myeloperoxidase (MPO), lactoperoxidase, and horseradish peroxidase (HRP). The immunization, High Performance Liquid Chromatography (HPLC), capillary electrophoresis, and UV spectrophotometry are usually used to detect protein nitration damage induced by RNS (Shiraiwa et al., 2012; Tsai et al., 2011; Yuan et al., 2012; Zhang et al., 2012). For instance, Bian et al. explored the nitration damage of $NaNO_2$/hemin/H_2O_2 on protein by Western blot (Bian et al., 2003); Lu et al. confirmed the different effects of DFO on hemin/NO_2^-/H_2O_2-induced protein nitration and oxidation with UV–vis spectra and Western blotting (Lu et al., 2008). Lu et al. demonstrated that EDTA-Fe(III), ferric citrate, ferritin, and heme (hemin and hemoglobin) showed higher efficiency in catalyzing protein nitration in the NO_2^-/H_2O_2 system (Lu et al., 2011). These approaches have confirmed the nitration effect of $NaNO_2$/hemin/H_2O_2 on protein, which provide the detailed molecular information on protein damage. Unfortunately, most of them are either time-consuming and labor intensive or require highly technical expertise and relative sophisticated instrumentation. Therefore, it is necessary to build a new, facile and simple method to evaluate the protein nitration damage induced by hemin/NO_2^-/H_2O_2.

In recent years, electrochemical methods aroused increasing interest among researchers in detecting the nitration and oxidation damage with the benefits of being simple, rapid, and convenient. Bian et al. analyzed Fenton-mediated oxidative damage on BSA using poly-o-phenylenediamine as electroactive probe (Bian et al., 2011). Wang showed a novel biosensor for the study of BSA damage induced by Fenton reaction using tris-(2,2-bipyridyl)

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cobalt(III) perchlorate as the electroactive indicator (Wang et al., 2012). Zhang et al. developed a novel electrochemical platform for detection of the DNA damage induced by the NO_2^-/H_2O_2 system (Zhang et al., 2012). To date, a few studies on using the electrochemical method for the detection of protein oxidation damage have been reported. However, there have been few reports concerning the electrochemical sensing of protein nitration damage induced by hemin/NO_2^-/H_2O_2.

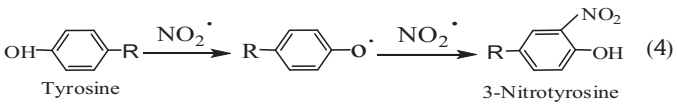
Graphene has recently attracted great attention due to its unique electrical, optical, and mechanical properties as well as its potential use in various fields, such as electronics, supercapacitors, sensors, and composite materials. Liu et al. have reported a simple method for the synthesis of graphene-AuNPs nanocomposite films by using a coelectrodeposition technique (Liu et al., 2011). The proposed EG-AuNPs exhibited better conductivity, larger surface area and better biocompatibility compared to the pure graphene film. Therefore, this can establish a foundation for fabricating electrochemical biosensor to detect protein nitration damage induced by $NO_2^{\bullet}$ radical.

Herein, protein nitration damage was investigated for the first time by the electrochemical method based on the attenuation of conductivity after damage caused by $NO_2^{\bullet}$ radical. Oxidation signal of $[Fe(CN)_6]^{4-/3-}$ was used as an indicator for the sensitive detection of BSA damage based on evidently attenuation of conductivity. The detecting mechanism of the exhibited electrochemical biosensor was shown in Scheme 1. GO–$HAuCl_4$ mixed solution can be direct reduced to yield EG-AuNPs nanocomposites on the electrode surface by electrodeposition. BSA was modified on EG-AuNPs composite film by Au–S bond. The $NO_2^{\bullet}$ radical produced by the $NaNO_2$/hemin/H_2O_2 system can induce the damage of protein, which could change the electroactivity of protein interface and realize the monitoring of the protein nitration damage. The nitration mechanism of hemin/NO_2^-/H_2O_2 on protein was as follows (Radi 2004, 2012):

$$Hemin(Fe^{3+}) + H_2O_2 \rightarrow Hemin^{\bullet\pi+}(Fe^{4+}) \quad (compound1) \qquad (1)$$

$$Hemin^{\bullet\pi+}(Fe^{4+}) + NO_2^- \rightarrow Hemin(Fe^{4+}) + NO_2^{\bullet} k \quad (compound2) \qquad (2)$$

$$Hemin(Fe^{4+}) + NO_2^- \rightarrow Hemin(Fe^{3+}) + NO_2 \qquad (3)$$



(4)

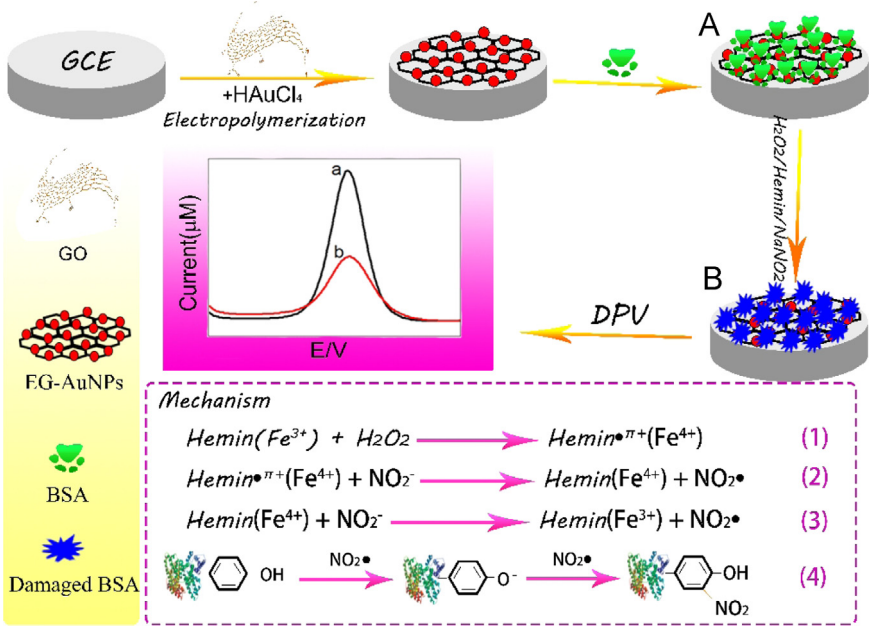
2. Experimental

2.1. Chemicals and reagents

AnnexinV (35–36 kDa), hemoglobin and bovine serum albumin (BSA) were purchased from Boisynthesis Biotechnology Co., Ltd. (Beijing, China) and used without further purification. Ascorbic acid, chlorogenic acid and resveratrol (RVL) were purchased from Lang Ze Pharmaceutical Technology Co., Ltd. (Nanjing, China). L-Tyrosine was purchased from Guangzhou Chemical Reagent Co. (Guangzhou, China). All other reagents were of analytical reagent grade. Double-distilled water was used in all experiments.

2.2. Apparatus

Electrochemical measurements were performed by a model CHI660A electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China). A three-electrode system was used in the measurements, with a bare GCE(4 mm in diameter) or nanobiocomposite film modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum foil as the auxiliary electrode. differential pulse voltammetry (DPV) and cyclic votammetry (CV) were used to make electrochemical measurements. The conditions of DPV were as follows: pulse amplitude 50 mV, and pulse period 0.5 s. The scan potential range was from 0 to 0.8 V (vs. SCE) in the present of 10 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. CV measurement was performed at scan rate of 0.1 V/s. Photoluminescence (PL) spectrum was performed on a 970CRT fluorescence spectrophotometer (Shanghai, China). The structure of protein was characterized by circular dichroism spectrum (CD spectra) (Applied Photophysics Ltd., England).



Scheme 1. Schematic diagram of BSA nitration damage induced by $NaNO_2$/hemin/H_2O_2.