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An electrochemical biosensor for rapid detection of bovine serum albumin damage induced by hydroxyl radicals in room temperature ionic liquid

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ABSTRACT

Oxidative protein damage has been widely implicated in carcinogenesis and other disorders. Sensitive and reliable detection of protein oxidative damage remains a significant challenge. Reported herein is a novel electrochemical biosensor for direct detection of bovine serum albumin (BSA) oxidative damage in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]). Oxidation of BSA immobilized on the electrode was induced by hydroxyl radical produced from Fenton reaction. An electroactive indicator, Tris(2,2′-bipyridyl)cobalt(III)perchlorate, was employed in the detection. The oxidative peak current of $Co(bpy)_3^{3+}$ decreased with the incubation time of BSA film in Fenton reagents. Electrochemical impedance spectroscopy was used to study the interface properties of the protein modified electrode surface. More dramatic damage in [BMIM][PF_6] compared with that in an aqueous solvent media confirmed that [BMIM][PF_6] was suitable medium for the electrochemical detection of BSA damage. The influences of the incubation time, the concentrations of Fenton reagents and antioxidants on BSA damage were obtained. The method is promising for a rapid and sensitive detection of protein damage.

1. Introduction

Oxidative damage to proteins by reactive oxygen species has been linked to cancers and the aging process [1,2]. In recent years, scientists have focused on the mechanisms of protein oxidative damage and its prevention by antioxidants. Protein oxidation can occur on many amino acid residues. Most of these modifications could be used as useful markers for protein oxidation. For example, one of the most important irreversible protein oxidative modifications is carbonylation, the formation of ketone and aldehyde group in amino acid residues such as lysine, etc. Drazen Petrov and Bojan Zagrovic used classical molecular dynamics simulations to study the structure and dynamics of the carbonylated headpiece domain of villin [3]. Dityrosine, one important biomarker of protein oxidation, was confirmed by high performance liquid chromatography (HPLC) and identified by nuclear magnetic resonance [4]. 3,4-Dihydroxyphenylalanine has been confirmed as an index of L-tyrosine in proteins [5].

Up to now, considerable research has been focused on studies of protein damage, using methods including HPLC [4], HPLC and an electrical conductivity detector [6], capillary zone electrophoresis [7], differential scanning calorimetry [8], HPLC and a diode array detector [9], fluorescence spectroscopy [10], mass spectrometry [11] and radioimmunoassay [12]. Many of these methods are laborious, time-consuming, and often involve costly equipments. Electrochemical methods have great potential for detecting proteins due to their low cost, simple operation, and fast response. Kerman et al. have reported electrochemical detection of inhibition of enzyme-catalyzed tyrosine phosphorylation on magnetic beads by following the increase in the tyrosine oxidation current on a screen-printed carbon electrode [13]. Our group has explored an electrochemical biosensor for analysis of Fenton-mediated oxidative damage to BSA using poly-o-phenylenediamine (PoPD) as an electroactive probe in aqueous solution [14]. The electrochemical biosensor is proposed for detecting protein damage and for further application in protein damage studies.

Room temperature ionic liquids (RTILs) are attractive solvent for a wide range of applications because of their high chemical and thermal stability, negligible vapor pressure, high ionic conductivity, low melting point, and wide potential window [15,16]. RTILs have been used as supporting electrolyte [17,18] and modifying material [19,20] in electrochemistry. To date, the electrochemical application of RTILs in the study of biological macromolecules has mainly been concentrated on biocatalysis. RTILs are stable in water and air and belong to the aprotic and larger viscosity solvents, which make them attractive solvents for studying

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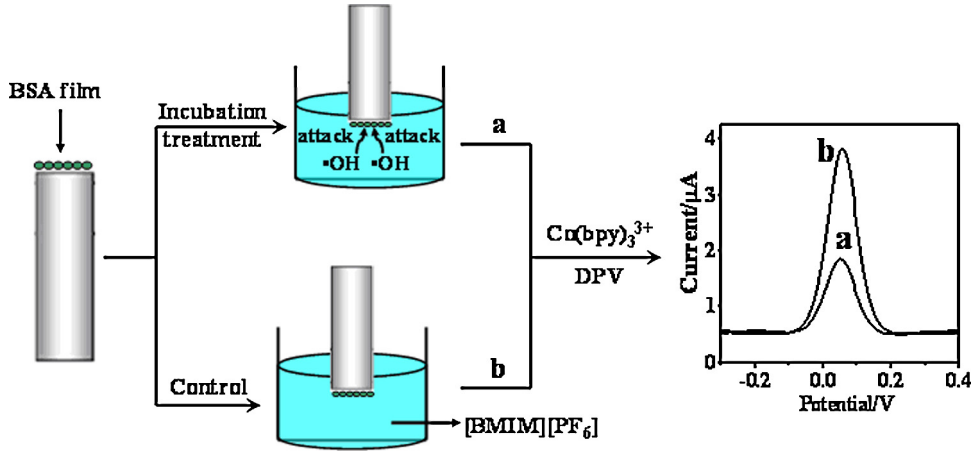

Scheme 1. Schematic diagram about BSA damage. BSA/GCE were incubated in [BMIM][PF_6] containing $FeSO_4$ or H_2O_2 or both or blank for several minutes, and then transferred into $Co(bpy)_3^{3+}$ for DPV analysis. The produced •OH by Fenton reagents attacked the BSA in the film and induced the decrease of peak current of $Co(bpy)_3^{3+}$.

the free-radical production mechanism. Previous research on the free-radical lifetime by us and others showed that the lifetime of the free radical is significantly longer in ionic liquids (ILs). There has been very little work on biomolecule damage by the radicals in RTILs, however. Strehmel's group has validated that the degree of free radical polymerization increases with increasing viscosity of the ILs [21]. The electrochemical generation of stable superoxide radical has been studied in two kinds of ILs [22]. Our groups have detected the oxidative DNA damage using Tris(2,2′-bipyridyl)cobalt(III)perchlorate ($Co(bpy)_3(ClO_4)_3$) as an electroactive probe in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) [23] and BSA damage using PoPD as an electroactive probe in 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] [24].

In this study, because of its stability and suitable, $Co(bpy)_3(ClO_4)_3$ has been chosen as the electroactive indicator to investigate the BSA damage via differential pulse voltammetry (DPV). BSA was damaged by •OH produced from the Fenton reaction using [BMIM][PF_6] as the nonaqueous electrolyte. The mechanisms of protein damage were also investigated. The experimental results indicated that the proposed method was reliable for the detection of protein damage induced by •OH in [BMIM][PF_6].

2. Experimental

2.1. Reagents and materials

Bovine serum albumin (BSA, fraction V) was purchased from Sigma (St. Louis, MO, USA). The room temperature ionic liquid, [BMIM][PF_6], was purchased from Chengjie Chemical Ltd., Corp. (Shanghai, China). $Co(bpy)_3(ClO_4)_3$ was prepared as described in the literature [25] and dissolved in 5 mM pH 7.0 Tris–HCl containing 50 mM NaCl. 0.1 M pH 7.0 phosphate buffer saline (PBS) comprised of Na_2HPO_4 and KH_2PO_4 was purchased from TianJin Bodi Chemical Holding Co., Ltd. (Tianjin, China). Doubly distilled water was used throughout the experiments. Other reagents were analytical grade.

2.2. Apparatus

All the electrochemical measurements were performed on a CHI 660C electrochemical workstation. A standard three-electrode system, with a film-coated 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. The conditions of the differential pulse voltammetry (DPV) were as follows: pulse amplitude 50 mV; pulse width 0.05 s, and pulse period 0.1 s. The scan potential range was from −0.3 to 0.4 V. Electrochemical impedance spectroscopy (EIS) measurements were performed at an amplitude of 0.005 V and potential of 0.227 V in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl.

2.3. Biosensor construction and electrochemical measurements

The clean GCE was coated with 10 μl of 1 mg/ml BSA solution, followed by air drying overnight. Then, BSA/GCE was obtained. A schematic diagram and the electrochemical measurements of BSA damage induced by •OH are shown in Scheme 1. There was a good oxidative peak for the intact BSA film with peak potential at +0.056 V, which was attributed to the oxidative peak of $Co(bpy)_3^{3+}$. Then the modified electrodes were incubated in [BMIM][PF_6] containing 0.5 mM $FeSO_4$ and 8 mM H_2O_2 for 40 min, with stirring, followed by washing with water and transfer into 150 μM $Co(bpy)_3^{3+}$ for subsequent DPV analysis. During the course of the incubation treatment in [BMIM][PF_6] containing Fenton reagents, the Fe^{2+} and H_2O_2 reacted to produce •OH, so [BMIM][PF_6] was expected to be a suitable medium for free radicals, which was confirmed by Wang's group [23]. •OH attacked the BSA in the film and induced BSA damage. Then, the peak current of $Co(bpy)_3^{3+}$ decreased sharply, as can been seen in curve a. Control experiments (curve b) were that BSA film was incubated in [BMIM][PF_6] containing $FeSO_4$, H_2O_2, or blank. BSA film was also immersed in the Fenton system in the presence of ascorbic acid (AA) or catechin to investigate the effect of antioxidants. Considering the variation for replicative experiments, the relative peak current ratio I/I_0 [26] instead of the absolute peak current was employed to estimate the degree of damage to BSA. Here I_0 and I are the peak currents before and after BSA film incubation in the Fenton system. Each measurement was repeated at least three times.

3. Results and discussion

3.1. Electrochemical measurements of the BSA damage

Fig. 1A is a histogram presenting the effects of different reagents on BSA damage. The $Co(bpy)_3^{3+}$ oxidation signal was used as a probe to monitor BSA conductivity. It could been seen that the current response of $Co(bpy)_3^{3+}$ decreased to 40.6% with the relative standard deviation (R.S.D) of 4.0% of its original signal when