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Protections of bovine serum albumin protein from damage on functionalized graphene-based electrodes by flavonoids

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ABSTRACT

A sensitive electrochemical sensor based on bovine serum albumin (BSA)/poly (diallyldimethylammonium chloride) (PDDA) functionalized graphene nanosheets (PDDA-G) composite film modified glassy carbon electrode (BSA/PDDA-G/GCE) had been developed to investigate the oxidative protein damage and protections of protein from damage by flavonoids. The performance of this sensor was remarkably improved due to excellent electrical conductivity, strong adsorptive ability, and large effective surface area of PDDA-G. The BSA/PDDA-G/GCE displayed the greatest degree of BSA oxidation damage at 40 min incubation time and in the pH 5.0 Fenton reagent system (12.5 mM $FeSO_4$, 50 mM H_2O_2). The antioxidant activities of four flavonoids had been compared by fabricated sensor based on the relative peak current ratio of SWV, because flavonoids prevented BSA damage caused by Fenton reagent and affected the BSA signal in a solution containing $Co(bpy)_3^{3+}$. The sensor was characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM). UV–vis spectrophotometry and FTIR were also used to investigate the generation of hydroxyl radical and BSA damage, respectively. On the basis of results from electrochemical methods, the order of the antioxidant activities of flavonoids is as follows: (+)-catechin > kaempferol > apigenin > naringenin. A novel, direct SWV analytical method for detection of BSA damage and assessment of the antioxidant activities of four flavonoids was developed and this electrochemical method provided a simple, inexpensive and rapid detection of BSA damage and evaluation of the antioxidant activities of samples.

1. Introduction

Many human diseases, immune function, aging and other biological processes are closely related to free radicals and other unstable species mediating biological macromolecule damages [1]. Proteins are most likely to be attacked as a result of their abundance in cells (proteins compose ca. 70% of the dryness of most cells) [2]. In particular, oxidative damage to proteins is involved in a number of pathologies including cancers and aging process [3,4]. Therefore, it is very important to develop a sensitive and simple method for determining the oxidative damage of protein in order to study the effects of free radicals on human health and quality inhibition effect of antioxidant enzymes and small molecule antioxidants.

As an important biomacromolecule, protein oxidative damages have been determined by HPLC [5,6], capillary zone electrophoresis [7], differential scanning calorimetry [8], fluorescence spectroscopy [9], mass spectrometry [10], horizontal Attenuation Total Reflection Fourier Transform Infra-red (ATR-FTIR) spectroscopy [11], UV–vis spectroscopy [12] and electrochemical methods [13]. By contrast, electrochemical method has attracted much attention due to its quick response, relatively high sensitivity and easy operation. Bian et al. had explored an electrochemical biosensor for analysis of Fenton-mediated oxidative damage to BSA using poly-o-phenylenediamine as electroactive probe [14]. Wang et al. had showed electrochemical study of bovine serum albumin damage induced by Fenton reaction using tris (2,2′-bipyridyl) cobalt (III) perchlorate as the electroactive indicator [15]. In these studies, the application of active probe solution significantly enhanced the detection signal of protein damage by electrochemical methods. However, the direct electron transfer is relatively difficult between electrodes and protein modified on electrode. Therefore, the key to electrochemical methods for determining protein damage is to find suitable functional materials which could promote electron transfer and amplify the electrochemical signal in electrochemical measurement.

Recently, graphene, a flat monolayer of sp^2 hybridized carbon atoms which tightly packed into a two-dimensional honeycomb lattice, retaining the feature of good mechanical strength, zero band gap, high carrier mobility, large specific surface area, outstanding electric conductivity [16–19] and representing a particular kind of graphene-related material, has attracted enormous attention in constructing electrochemical sensors. The modification or functionalization of graphene is

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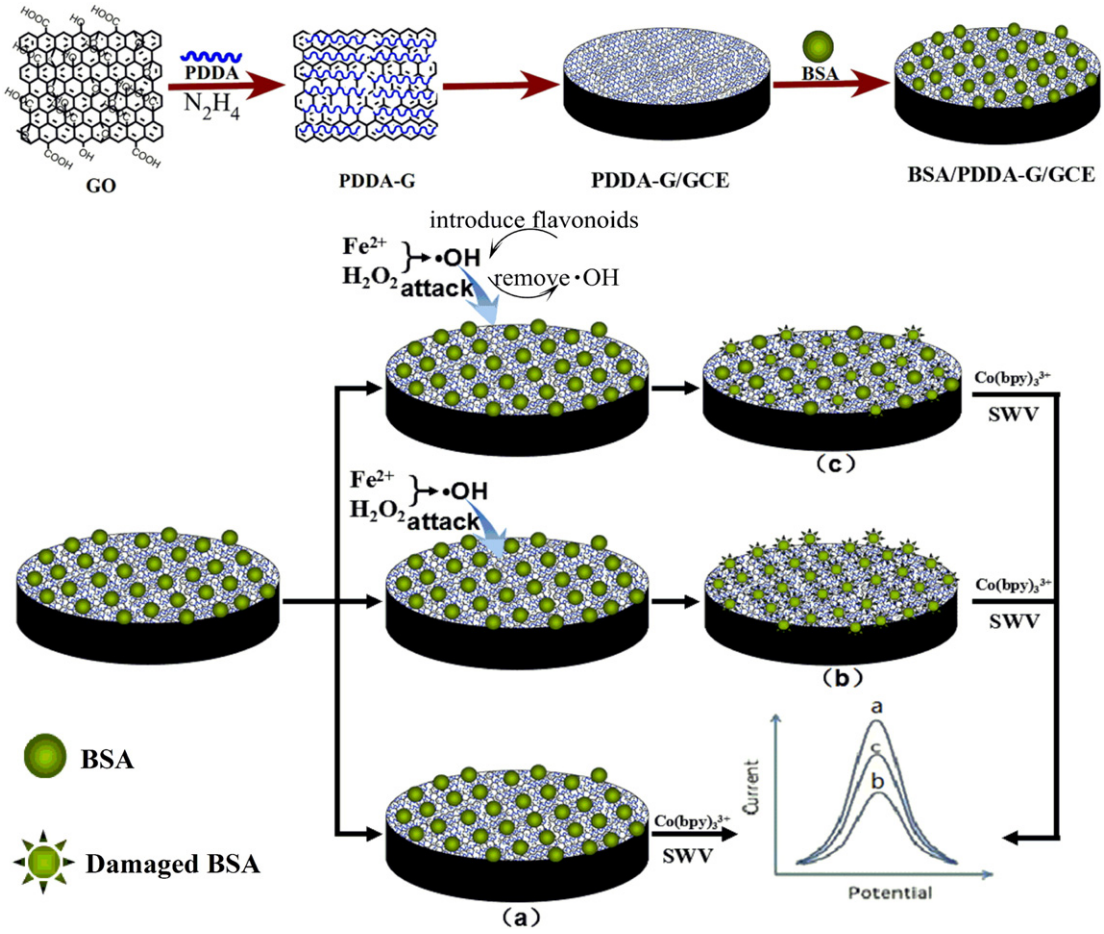

Scheme 1. Schematic diagram of BSA damage.

most widely used in the determination of drug molecules [20], DNA [21, 22] and biomacromolecules [23,24]. Poly (diallyldimethylammonium chloride) (PDDA) as functional agent, has been found to be an effective material for graphene functionalization without changing its electronic structure. Accordingly, the solubility of PDDA functionalized graphene is greatly increased, extending its application in electrochemical sensor [25]. Therefore, poly (diallyldimethylammonium chloride) functionalized graphene sheets (PDDA-G) can greatly increase the effective specific surface area of modified electrode and enhance the detection signal by accelerating the electron transfer on the electrode surface. An et al. fabricated PDDA-functionalized graphene sheets (PDDA-GS) based electrochemical sensor for shikonin determination [26]. However, there was no report using the PDDA-G modified electrodes for determining protein oxidative damage.

Flavonoids, the well-known ability to reduce free radical formation and scavenge free radicals, have been used to investigate the protections of protein from damage by antioxidants [27]. The flavonoids play an effective role in the prevention of free radical inducing diseases by being oxidized themselves by free radicals or oxidants. (+)-Catechin, kaempferol, apigenin and naringenin are not only the higher relative amount in flavonoids, but also they are the most important representative compounds in flavanes, flavonols, flavonoid and dihydroflavone, respectively, which their antioxidant activities have been compared by analyzing the structural features of four compounds with molecular

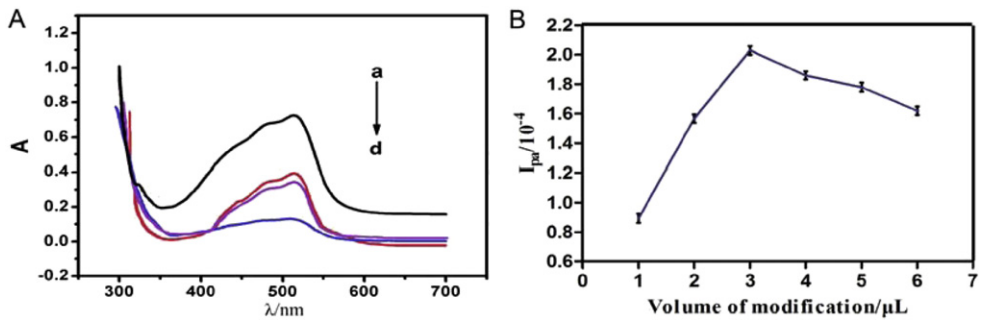

Fig. 1. (A) UV–vis spectroscopy of (a) 1,10-phenanthroline + Fe^{2+}, (b) 1,10-phenanthroline + Fe^{2+} + H_2O_2, (c) 1,10-phenanthroline + H_2O_2, (d) 1,10-phenanthroline. Concentrations of 1,10-phenanthroline, Fe^{2+}, and H_2O_2 were 0.006 mM, 12.5 mM, and 50 mM, respectively. (B) The plots of the anodic peak currents against the volume of modification.