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Nanoporous gold assembly of glucose oxidase for electrochemical biosensing

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ABSTRACT

Nanoporous gold (NPG) is composed of three-dimensional (3D) bicontinuous nanostructures with large surface area. Nano-channels inside NPG provide an ideal local environment for immobilization of enzyme molecules with expected stabilization of the protein molecules. In this work, glucose oxidase (GOx) has been brought to assemble on NPG via surface chemical reactions to form enzyme modified NPG nanomaterial with promising sensitivity for glucose detection. Cyclic voltammetry and single-potential step chronoamperometry (SPSC) are employed to study the electrochemical behavior of both bare and enzyme-modified NPG. Two redox mediators, p-benzoquinone (BQ) and ferrocenecarboxylic acid (FCA) are used to shuttle electrons between the enzyme redox center inside of GOx and the NPG electrode. Diffusion patterns at the functionalized NPG electrode are found significantly different from those on planar gold electrodes. This is mainly caused by internal 3D single crystal-like structures of NPG. Electrostatically neutral BQ mediator gives much higher voltammetric sensitivity than negatively charged FCA for GOx modified NPG electrodes.This study provides insight into the understanding of the intrinsic properties of NPG materials aiming at evolving enzymatic biosensors with high performance.

1. Introduction

Nanoporous gold (NPG), a class of gold nanomaterials, is composed of three-dimensional bicontinuous nanostructures with large surface area [1,2]. Many unique properties on mechanical [3], catalytic [4], electrocatalytic [5–7] and optical [8,9] properties have been discovered recently. Nano-channels inside NPG provide an ideal local environment for immobilization of biological molecules such as proteins aiming at stabilization of the monolayers. NPG is versatile and can be prepared with controlled pore size via dealloying silver from Au-Ag alloys in concentrated nitric acid [10]. NPG shows potential applications in biomedicine [11], catalysis [12], energy storage [13–15], and sensors [16]. By its high specific surface area and biocompatibility, NPG offers excellent three-dimensional (3D) enzyme support.

Diabetes is a growing problem world-wide and development of glucose sensors dominates entirely biosensor research. Due to the increasing market, there is a current demand for improved detection of glucose in blood and urine samples, i.e. provide rich information for diagnostic of diabetes. Since the first glucose enzyme electrode was introduced by Clark and Lyons in 1962 [17], there have been tremendous efforts exploring robust and flexible approaches to fabricate glucose biosensors with high sensitivity and selectivity [18,19]. Developments of nanomaterials, such as gold nanoparticles [20], carbon nanotubes [21], quantum dots [22] and graphene [23], have stimulated updating glucose biosensors. Glucose sensors can be divided into two groups according to the way enzyme glucose oxidase (GOx) is used. In nonenzymatic glucose biosensors [24], transition metals and their oxides are used as key elements due to their catalytic function in glucose conversion. As examples, Co_3O_4 accommodated on NPG [25] and dendritic platinum-decorated gold nanoparticles [26] have recently been reported to offer good performance as supports for glucose sensing. Such sensors can reduce the cost and increase stability of the sensor, but with a high risk of losing selectivity. On the other hand, GOx has unique selectivity to glucose oxidation. Large numbers of glucose sensors based on electrochemical detection are therefore fabricated using this enzyme [19]. Introduction of NPG into bioelectrochemistry makes use of the high surface-to-volume ratio and bio-functionalized 3D NPG, which will ensure high loading of biological macromolecules, with a purpose to enhance the response signals of biosensors. Acetylcholine esterase [27], cytochrome *c*

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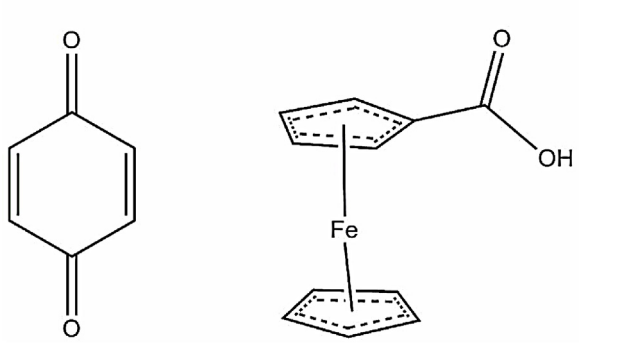

Scheme 1. Molecular structure of BQ (left) and FCA (right).

[28], azurin [29], laccase [29,30] and glucose oxidase (GOx) [31] have thus been immobilized on NPG surfaces for developing electrochemical biosensors as well as biofuel cells [32].

In this work, we have constructed a glucose sensor using NPG as a supporting material for immobilizing GOx. Mass transfer within the 3D nanoporous structures is a crucial factor for achieving high sensitivity and wide response range. Mass transport from bulk solution to the porous materials surface has been reported [33–35], but the diffusion mechanism inside the confined nanoporous NPG structures is unclear. Especially, the effect of internal electric fields in the NPG nanochannels, caused by high surface charge density of NPG at positive potentials, on the transport of charged molecules and ions is a major controlling factor in the performance of NPG based sensors. The exploration and mapping of the diffusion mechanism within the NPG films is a goal of this work, important for understanding the intrinsic properties of NPG and developing enzyme-modified electrodes. Although direct electrocatalytic oxygen reduction has been reported on laccase modified gold in the absence of mediator [36], our experiments show presence of mediators is essential for GOx. Two mediators, p-benzoquinone (BQ) and ferrocenecarboxylic acid (FCA) (Scheme 1), were used to transfer electrons between the enzyme redox center (flavin adenine dinucleotide, FAD) and the electrode surface. A GOx modified NPG electrode was fabricated by cross linking a self-assembled cysteamine monolayer (SAM) on the NPG surface and GOx with glutaraldehyde. The electrochemical behavior of the GOx modified NPG electrode was systematically explored and the diffusion behavior of active molecules from the solution into the NPG nanopores during glucose sensing have been tracked.

2. Experimental

2.1. Fabrication of NPG electrodes

Cysteamine (98%, Aladdin, China), BQ (98%, Aladdin, China), glutaraldehyde (25% in H_2O, Shanghai Chemical Industrial Co., China), FCA (97%, Sigma-Aldrich, USA), ascorbic acid (AA, 99%, Shanghai Chemical Industrial Co., China), uric acid (UA, 99%, Shanghai Chemical Industrial Co., China) concentrated HNO_3 (65%, Shanghai Chemical Industrial Co., China) and GOx (GOx, EC 1.1.3.4, Type II from *Aspergillus niger*, ≥15,000 units/g, Sigma-Aldrich) were used without further purification. Phosphate buffer solutions (PBS) were made by mixing solutions of sodium dihydrogen phosphate (99.99%, Sigma-Aldrich, USA) anddisodium hydrogen phosphate (99.99%, Sigma-Aldrich, USA) and pH adjusted by potassium hydroxide (99.99%, Aladdin, China). Millipore water from UPH-IV ultrapure water purifier (Chengdu Ultrapure Technology Co., Ltd, China) or Milli-Q Synthesis A10^{TM} (France) were used throughout. Nanoporous gold (NPG) was prepared according to de-alloying method [10,16,37]. Briefly, a piece of Au/Ag alloy leaf (12-carat, Sepp Leaf Products, New York) of 100 nm thickness was chemically etched in concentrated HNO_3 for 30 min at 30°C. After rinsing several times in water, the floating NPG film was attached onto a mirror-polished glassy carbon electrode (GCE) with a diameter of 4 mm (Fig. 1A) or, for Atomic force microscopy (AFM), onto a freshly cleaved mica surface, dried in air at room temperature for at least 4 h.

2.2. Preparation of the glucose oxidase electrode

An electrochemical cleaning process was applied to create a NPG electrode with a high active surface area. The potential was cycled between -0.2 and +1.6 V vs. saturated calomel electrode (SCE) in 1 M H_2SO_4 until stable cyclic voltammograms (CVs) were obtained, Fig. S1. The NPG electrodes were then soaked in 50 mM cysteamine (CA) for 8 h at room temperature, and washed with water before it was transferred to other solutions. The presence of the self-assembled monolayer (SAM) of cysteamine was confirmed by a reductive desorption peak between -0.7 V and -1.2 V vs. SCE, Fig. S2, which is in agreement with previous report [38]. The CA modified NPG electrodes were reacted with 10% (v/v) glutaraldehyde (GA) aqueous solution for 1 h. After washing with 0.1 M PBS (pH 7.0), the modified electrodes were reacted with 15 mg mL^{-1} glucose oxidase in 0.1 M PBS (pH 7.0) for 6 h at 4°C and then treated again with glutaraldehyde solution for 30 min to cross link the GOx immobilized on the NPG electrodes [39]. Enzyme-modified planar polycrystalline gold electrodes with a diameter of 3 mm were prepared in a similar way for comparison. The resulting electrodes are denoted as NPG-CA-GA-GOx and Au-CA-GA-GOx, respectively.

2.3. Electrochemical characterization

A three-electrode system with a LK2005A electrochemical work station system (Lanlike Company, Tianjin, China) accommodating the enzyme electrode as working electrode, a Pt wire counter electrode, and a saturated calomel electrode (SCE) reference electrode was used for the electrochemical measurements. All potentials in this work are vs. SCE. CVs of the GOx-electrode in 0.1 M pH 7.0 PBS containing varying concentrations of BQ or FCA as mediator at room temperature were recorded. Amperometry of glucose was investigated by single potential step chronoamperometry in 0.1 M PBS (pH 7.0) containing a specific mediator with successive additions of 0.02 ml stock glucose solution (0.5 M) injected into the electrochemical cell containing 10 ml solution. All aqueous solutions were degassed by anitrogen stream for 20 min prior to electrochemical experiments and a N_2 flow above the solution was maintained during electrochemical measurements.

2.4. Morphology characterization

The morphology and chemical composition of NPG were characterized by scanning electron microscopy (SEM) (SU-70, Hitachi, Japan) at an accelerating voltage of 7 kV. AFM imaging was performed using a Scanning Probe Microscope (5500 AFM, Agilent Technologies, Chandler, AZ, USA) in air under ambient conditions. The clear porous morphology of NPG immobilized on mica was scanned using the contact mode.

3. Results and discussion

3.1. Morphology Characterization of NPG and NPG-CA-GA-GOx electrodes

Fig. 1 (B) shows representative AFM image of NPG film surfaces obtained by de-alloying 12 carat Au-Ag alloy in concentrated nitric acid. Height profile along a to b indicated in Fig. 1 (B) show