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"On-off" switchable electrochemical affinity nanobiosensor based on graphene oxide for ultrasensitive glucose sensing

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ABSTRACT

A novel "smart" electrochemical affinity nanobiosensor with "on-off" switchable property was designed for the ultrasensitive determination of glucose. The sensing approach was based on the glucose-ConA-dextran competitive system induced charge evolution in the use of graphene oxide (GO) as transducer element, resulting in the enhancement of interfacial electron transfer kinetics between the redox probe and the electrode. As concanavalin A (ConA) constituent was pH-sensitive, when the ConA-DexP/GO film electrode switched in probe $Fe(CN)_6^{3-/4-}$ solution between pH 4.0 and 8.0, the film was cycled between the "on" and "off" states by the electrostatic attraction and repulsion of $Fe(CN)_6^{3-/4-}$ to and from the electrode surface. Upon introduction of glucose into the ConA-DexP/GO complex at the "off" state, glucose competed with DexP for ConA and displaced ConA from the GO platform, resulting in gradual decrease of the surface negative charge as well as the resistance of probe for electron communication on the sensor surface, and making the switching from "off" state to "on" state simultaneously. This ultrasensitive glucose nanobiosensor had a broad linearity between the decrease in electron transfer resistance (ΔR) and the glucose concentration over a range from 5.0 μM to 9.0 mM with a detection limit as low as 0.34 μM. The proposed method showed potential application for fabricating novel biosensors and bioelectronic devices.

1. Introduction

Graphene is a single-atom-thick and two-dimensional carbon nanomaterial that has attracted great attention because of its remarkable electronic, mechanical, and thermal properties (Geim and Novoselov, 2007; Lee et al., 2008; Novoselov et al., 2004; Service, 2008). Graphene, along with graphene oxide (GO), could serve as a good support for biomolecules immobilization due to its large surface area (theoretical limitation 2630 $m^2 g^{-1}$), flat surface, and rich π conjugation structure, and it has found important application in the development of biosensing (Bonanni and Pumera, 2011; Chang et al., 2010; Chen et al., 2010; Gulbakan et al., 2010; Lu et al., 2009; Swathi and Sebastian, 2008; Wang et al., 2010; Wen et al., 2010). However, the covalent immobilization techniques might disrupt the inherent sp^2 structure and π-conjugation of graphene, leading to compromised physical properties (Liu et al., 2010a, 2010b), while π–π stacking interactions can evade such drawbacks with minimized risk of permanently altering the intrinsic structures and properties of graphene (Liu et al., 2010b). Thus, immobilization of biomolecules on graphene interfaces via π–π stacking has attracted extensive attention in the development of affinity biosensors (Tang et al., 2011). It has been reported that graphene modified with bior-ecognition molecules such as antibody, aptamer or DNA can be employed to detect various targets, including biomacromolecules (proteins (Mao et al., 2010; Ohno et al., 2010), DNA (Dubuisson et al., 2011)) and biomicroparticles (bacterium (Mohanty and Berry, 2008), yeast cells (Kempaiah et al., 2011)). However, the detection of small biomolecules using the traditional affinity modes of graphene-based chemiresistive/field-effect transistor sensing might be ineffective, as their binding to the recognition molecule may not generate a measurable change in conductance/resistance (Cella et al., 2010; Tang et al., 2011). Thus, the fabrication of novel GO-based nanobiosensors that can detect small molecules with high sensitivity and selectivity constitutes the main challenge.

Glucose, as a small molecule (MW180.2), is the major energy source in cellular metabolism and plays an important role in the natural growth of cells. The glucose level in blood is usually used as a clinical indicator of diabetes, and the monitoring of glucose levels in blood with faster and more accurate methods has become an increasingly active area of research (Amato, 1992; Ben-Moshe et al., 2006; Koschinsky and Heinemann, 2001; Moschou et al., 2004;

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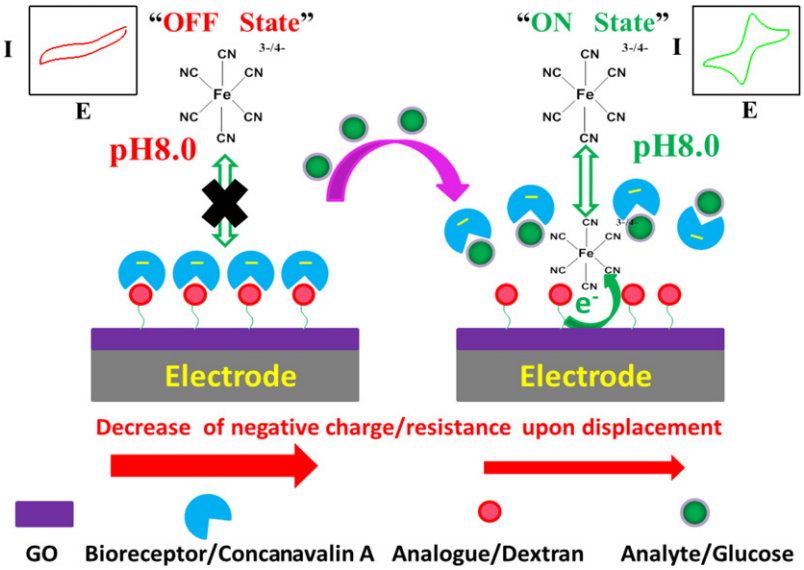

Scheme 1. Switching of the displacement-based electrochemical affinity biosensor between "OFF" and "ON" states before and after addition of glucose.

Schultz et al., 1982; Zhang and Anslyn, 2007). Among various glucose-assay methods, the widely used commercial glucose test employs electrochemical sensors, and is based on an amperometric electrode at which the glucose concentration is monitored by a change in current flow caused by the enzyme converting glucose into gluconolactone and hydrogen peroxide (H_2O_2). However, the corresponding enzyme is easily denatured during the immobilization procedures or leach out of the films, resulting in poor stability and perturbed function (Wang et al., 1999). As a result, much effort has been directed towards retaining enzymatic activity and improving the stable performance of the enzymatic biosensors (Hrapovic and Luong, 2003; Parthasarathy and Martin, 1994). In contrast, many non-enzymatic biosensors have been advocated, especially amperometric glucose sensors free from enzymes (Li et al., 2011; Park et al., 2003; Shoji and Freund, 2001; Sun et al., 2001; Ye et al., 2004). The technology that has been investigated extensively is based on a competitive binding reaction between the protein concanavalin A (ConA), dextran, and glucose (Aslan et al., 2005; Ballerstadt and Schultz, 2000; Barone et al., 2005; Blagoi et al., 2004; Cummins and Cote, 2012). However, electrochemical sensors based on competitive binding reaction are very sparse (Cella et al., 2010), and the majority of these sensors focused on the change of conductance in electrochemical channels since the blocking effect by biomacromolecules (ConA) could be effectively prevented after the competitive reaction occurs (Cella et al., 2010). As is well known, ConA, a metalloprotein with four carbohydrate binding pockets, can noncovalently and reversibly bind to some specific sugar groups such as dextran and glucose, forming a highly specific ConA-sugar complex (Anzai and Kobayashi, 2000; Becker et al., 1976; Welch et al., 2008). With the isoelectric point (pI) at about 5.0, ConA was positive charged at pH < 5.0 and negative charged at pH > 5.0. Utilizing this pH-sensitive property, Hu et al. (Yao and Hu, 2009, 2010, 2011) prepared H_2O_2 biosensors based on the ConA-confined layer-by-layer films mediated by $Fe(CN)_6^{3-}$. Up to date, there are few reports about the influence of pH values on the surface charge of ConA/graphene modified electrode, and no investigation concerning the effect of the competitive system induced charge evolution on the electrostatically controlling access of charged redox probes.

Herein, we propose a facile and sensitive electrochemical biosensor for glucose detection through a displacement-type mode on the ConA-DexP/GO-based resistive platform. The mechanism of glucose sensing is outlined in Scheme 1. By taking advantages of the competitive reaction between glucose and DexP for ConA binding sites, the as-prepared ConA-DexP/GO electrode exhibited excellent analytical performance towards the quantification of glucose, with a wide linear range, excellent sensitivity, good reproducibility, and long-term stability. Although the well-characterized glucose-ConA-dextran system is often evaluated as a model system to demonstrate the displacement mode of glucose sensing (Ballerstadt et al., 2006; Cella et al., 2010; Li et al., 2011; Tang et al., 2011), to the best of our knowledge, this is the first displacement-induced charge evolution on the GO platform for electrochemical glucose detection, and this electronic detection scheme will open new opportunity for the design of more novel sensing strategies for other small molecules.

2. Materials and methods

2.1. Materials

Graphite flake (99.8%, 325 mesh) was provided by Alfa Aesar China Ltd. (China). Concanavalin A (ConA, MW ≈ 104,000), dextran (MW ≈ 70,000), 1,2-epoxy-3-phenoxypropane (Epoxy), Tween20 and β-D(+)-glucose were purchased from Sigma-Aldrich (St. Loius, MO, USA). All other chemicals, such as potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) were of analytical grade. Phosphate buffer solution containing 0.1 M NaCl, 0.5 mM $CaCl_2$ and 0.1 mM $MnCl_2$ were used to prepare ConA, Tween20 and glucose solutions (Cella et al., 2010). $CaCl_2$ and $MnCl_2$ must be added to activate ConA conformation, which permitted the effective binding of ConA to carbohydrates (Loaiza et al., 2011; Shen et al., 2007). All solutions were prepared using doubly distilled water.

2.2. Characterizations

X-ray diffraction (XRD) patterns of the pristine graphite and GO were carried out using a Rigaku powder diffractometer equipped with Cu $K\alpha_1$ radiation ($\lambda = 1.5406$ Å) (Bruker Co., USA). Scanning electron microscopy (SEM) images were collected on a FEI QUANTA 200F scanning electron microscope (FEI, USA). The accelerating voltage was 20 kV. UV–vis absorption spectra were acquired with a UV-2450 spectrophotometer (Shimadzu, Japan). Fourier transform infrared spectra (FTIR) were recorded on a Nicolet 5700 FTIR spectrometer (Nicolet, USA). Zeta potential was