



Antifouling properties of reduced graphene oxide nanosheets for highly sensitive determination of insulin



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ABSTRACT

In the present work, for the first time, excellent activity and antifouling properties of reduced graphene oxide (rGO) nanosheets toward the oxidation of insulin at physiological pH were reported. Electrochemical oxidation of insulin at the surface of rGO, graphene oxide (GO) and graphite (G) were investigated and compared. Cyclic voltammetry and hydrodynamic amperometry were used to investigate the analytical characteristics of the rGO-modified GC (GC/rGO) electrode toward the oxidation of insulin. While the electrooxidation of insulin on the bare glassy carbon (GC) electrode, G and GO-modified GC electrodes led to the deactivation of the surface after a short period of time, the GC electrode coated with rGO revealed highly stable insulin oxidation current. The effect of various experimental parameters on the electrochemical oxidation of insulin was investigated using cyclic voltammetric technique. The amperometric response of the GC/rGO electrode toward insulin under the optimum conditions was found to be linear over the concentration range of 4–640 nM with a sensitivity of 7.1254 nA/nM and a detection limit of 350 pM. The modified electrode offers considerable advantages of simple and fast electrode preparation, low cost, fast response time, signal stability, high sensitivity and insulin determination at physiological pH.

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1. Introduction

Human insulin is a globular protein with a molecular weight of almost 5800 Da, composed of two polypeptide chains, a 21-residue A-chain and a 30-residue B-chain. The molecule contains two interchain disulphide bonds between A and B and one intrachain bond within chain A [1]. This hormone controls the level of blood glucose by stimulating the uptake of glucose by muscles and fat tissues, and so has considerable effects on both metabolism and a number of other body functions [2]. Diabetes is due to either deficiency in production of enough insulin (type 1 diabetes) or action of insulin (type 2 diabetes). So, the analysis of blood insulin is clearly a basic subject for medical and physiological studies of diabetes. During the past decade, many methods for detection of insulin have been developed, including those based on chromatography [3], capillary electrophoresis [4] and electrochemical assay [5–8]. Many of methods used for insulin detection have the drawbacks of high cost, long time for detection processes and complex procedure [9]. So, the development of a sensitive, cheap and simple analytical device would be of considerable value for measuring insulin concentration. The direct detection of insulin by electrochemical techniques has already achieved considerable attention due to their intrinsic

benefits of high sensitivity, low cost, portability and simplicity, etc. [10, 11]. However, the electrochemical detection of insulin at most of the unmodified electrodes is limited by the slow oxidation kinetics, which results in surface fouling associated with the accumulation of reaction products, and hence, decreases the selectivity and operational lifetime of the sensor. A traditional solution to these problems is to modify the electrode with appropriate compounds [2,12] or use of mediators [13]. The increasing use of nanomaterials in recent years has led to substantial improvements in the efficiency of insulin electrochemical sensor. A wide range of nanomaterials, including cobalt oxide nanostructure [12], carbon nanotubes (CNTs) [5,7], nickel nanoparticles [14], guanine/nickel oxide nanoparticles [15], nickel oxide nanoparticles-MWCNTs [16], RuOx/CNT nanocomposite [17], silicon carbide nanoparticles [8], nanocarbon black electrode surface [18], CNT–nickel–cobalt oxide nanocomposite [10] and silica nanoparticle [11] have been used to accelerate the kinetics of insulin oxidation and to minimize the fouling of the sensor.

Graphene and related materials are currently, without doubt, the most widely used nanomaterial in the field of physics, chemistry, materials science and biotechnology. Graphene is a novel 2D nanomaterial with carbon atoms linked by sp^2 bonds in a honeycomb lattice network that possesses incomparable electrical and thermal conductivity, superior mechanical strength and large surface area. Owing to these unique chemical and physical properties as well as its biocompatibility, electrocatalytic activity and unresponsiveness to reactions, graphene has

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recently attracted substantial research efforts in biotechnology for numerous applications such as drug delivery [19–21], gene therapy [22], cancer diagnosis [23] and biosensing [24,25]. Furthermore, rGO is more electrochemically active compared to primitive graphene due to considerable density of reactive sites at sides and edge-plane-like defective sites, which has led to its use in developing highly efficient electrochemical sensors [26,27]. Graphene and graphene-based nanocomposite modified electrodes have been used successfully for determination of biomarkers, proteins, DNA, heavy metals, inorganic and organic analytes, etc. [28].

Here, we reported the development of an rGO-modified GC electrode for electrochemical determination of insulin for the first time. Antifouling and signal amplification characteristics of rGO toward oxidation of insulin were investigated and compared with bare glassy carbon electrode and glassy carbon electrodes modified with GO and G films using cyclic voltammetry and amperometric techniques. Experimental conditions for the amperometric detection of insulin were optimized and analytical performances of the modified electrode were evaluated.

2. Experimental

2.1. Chemicals and reagents

Insulin was obtained from Sigma-Aldrich (USA, 100 IU/ml). Graphite powder (G), hydrazine hydrate (85%, CR), hydrogen peroxide (H₂O₂ 30%), sulfuric acid (H₂SO₄), potassium permanganate (KMnO₄), disodium hydrogen phosphate (Na₂HPO₄), potassium chloride (KCl), potassium ferrocyanide (K₄Fe(CN)₆) and potassium ferricyanide (K₃Fe(CN)₆) were purchased from Merck. All chemicals were of analytical grade and used without further purification. The stock solution of insulin (0.42 and 2.5 mM) was prepared daily from its powder using phosphate buffer solution (PBS, pH 7.4). Insulin solutions were supplied by diluting the insulin stock solution to a convenient concentration in the same buffer. Human serum samples were obtained from a local plasma collection center. Double distilled water was used throughout the work.

2.2. Apparatus and measurements

High resolution transmission electron microscopy (HR-TEM) images of graphene were obtained with a JEM-2100F transmission electron microscope. All electrochemical experiments were carried out with a computer-controlled μ -Autolab modular electrochemical system (Eco Chemie Ultecht, The Netherlands), run with NOVA software. A conventional three-electrode system, consisting of modified and unmodified glassy carbon (GC, 2 mm in diameters) electrode as a working electrode, an Ag/AgCl (3 M KCl) electrode as a reference electrode and a platinum wire as an auxiliary electrode was used for electrochemical experiments. Electrochemical impedance spectroscopy (EIS) experiments were performed in a 0.3 M KCl solution containing 10 mM Fe(CN)₆^{3-/4-} (1:1). The spectra were recorded over a frequency range from 10 kHz to 0.1 Hz, at a potential of 0.22 V, with an oscillation potential of 5 mV. All electrochemical experiments were performed at room temperature. The EIS data were approximated by using the modified Randles circuit model. More appropriate fit was selected based on criteria of the fit, such as number of model parameters, their physical meaning, and quality of the fit (Chi-squared, χ^2) and then, electron transfer kinetics parameters (R_{ct} , R_s and n) were extracted.

2.3. Synthesis of GO and rGO

Graphene oxide (GO) was synthesized from graphite powder according to the modified Hummers method [29]. To prepare reduced graphene oxide (rGO), the dried GO powder was transferred to a flask containing purified water (3 mg/ml) and sonicated for 1 h in the

sonicator to achieve a uniform suspension. 1 μ l hydrazine monohydrate (1 μ l for 3 mg of GO) was added immediately and the flask was heated in an oil bath at 80 °C. The mixture was continuously stirred for 12 h. The resulting black powder was then centrifuged and washed with water and finally, dried in an oven under vacuum (5 mbar) at 25 °C for 24 h [30].

2.4. Modification of GC/rGO electrode

Glassy carbon (GC, 0.03 cm²) electrode was polished on a soft polishing tissue with 1, 0.3 and 0.05 μ m alumina slurry, respectively, rinsed thoroughly with water and sonicated in a water bath for 5 min to remove any adsorbed particulates. The desired amount of rGO was dispersed in 1 ml of deionized water followed by ultrasonication for 30 min to yield a homogenous black suspension. The rGO modified GC (GC/rGO) electrode was prepared by dropping 5 μ l of the rGO aqueous suspension (usually containing 10 mg/ml rGO) on the mirror polished GC electrode and drying at room temperature for 2 h. For fabrication of GC/G and GC/GO modified electrodes, 5 μ l of the G or GO suspensions (10 mg/ml rGO) were placed on the surface of GC electrode and dried at room temperature for 2 h.

To investigation of the batch-to-batch reproducibility of the modified electrode, five individual modified electrode were prepared and their corresponding cyclic voltammograms in a 0.3 M KCl solution containing 10 mM Fe(CN)₆^{3-/4-} (1:1) were investigated (Fig. S1). As can be seen all of voltammograms are similar and only have very small difference in anodic potential and currents. The relative standard deviation (RSD) of 7.3% and 5.1% were obtained for anodic current and potential, respectively. These data revealed a very good batch-to-batch reproducibility of the fabricated modified electrodes.

3. Results and discussions

3.1. Characterization of rGO

Fig. 1A shows the high resolution transmission electron microscopic image of rGO film. As can be seen, rGO was homogeneously exfoliated into individual sheets during synthesis process [31,32]. So, the synthesized rGO sheets can provide a uniform film on the electrode surface for further immobilization of aptamer biomolecules.

The electron transfer property of synthesized rGO was investigated using EIS technique (Fig. 1 B). EIS is a powerful, non-destructive technique for monitoring the microscopic interfacial processes that occur on the electrode surface [33]. The complex plane plots contain a semicircle part (in the high frequency range), corresponding to the charge-transfer resistance, followed by a Warburg diffusion trend line (in the low frequency range) indicating domination of mass diffusion limiting process. The R_{ct} is approximately proportional to the semicircle diameter of the EIS spectra. Electron transfer resistances (R_{ct}) of the GC electrode (plot a) clearly decreased from 154.2 to 4.5 Ω (Table S1) after modification with rGO (plot b), which indicated the significant enhancement of electron transfer kinetics at the rGO-modified GC electrode. So, this modified electrode provides a very suitable environment for transfer of electrons in electrochemical process.

3.2. Voltammetric study of insulin oxidation at the GC/rGO electrode

The effect of rGO on the electrochemical behavior of insulin oxidation was examined using cyclic voltammetry method. Fig. 1 C shows the cyclic voltammograms (CVs) of GC and GC/rGO electrodes in the absence (voltammograms a and c, respectively) and presence of 9 μ m insulin (voltammograms b and d) in 0.1 M PBS (pH 7.4). For both electrodes, an anodic peak was observed in the presence of insulin, which is attributed to the electrochemical oxidation of insulin. However, the oxidation of insulin at the GC/rGO electrode gave an anodic peak at the potential of 0.56 V (started at 0.50 V), which is more negative compared to

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