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Fabrication of electrochemical interface based on boronic acid-modified pyrroloquinoline quinine/reduced graphene oxide composites for voltammetric determination of glycated hemoglobin

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ABSTRACT

A voltammetric sensor for determination of glycated hemoglobin (HbA_{1c}) was developed based on the composites of phenylboronic acid-modified pyrroloquinoline quinine (PBA-PQQ) and reduced graphene oxide. After the electrodeposition of reduced graphene oxide (ERGO) on the glassy carbon (GC) electrode, PQQ multilayer was decorated on the surface of the ERGO/GC electrode via potential cycling. Further modification with PBA would lead to the formation of the working electrode, namely PBA-PQQ/ERGO/GC electrode. PQQ on the electrode exhibited a quasi-reversible electrode process with 2-electron transfer and 2-proton participation, and the electron transfer efficiency was further enhanced by the introduction of ERGO layer. The complexation of PBA with HbA_{1c} through specific boronic acid–diol recognition could cause the change of the oxidation peak current of PQQ on the electrode, which was utilized for HbA_{1c} detection. Under the optimized conditions, the PBA-PQQ/ERGO/GC electrode provided high selectivity and high sensitivity for HbA_{1c} detection with a linear range of 9.4–65.8 μg mL^{-1} and a low detection limit of 1.25 μg mL^{-1}. The fabricated sensor was also successfully applied to determine the percentages of HbA_{1c} in whole blood of healthy individuals.

1. Introduction

Diabetes, which is a group of diseases characterized by high level of blood glucose due to the lack or inability of insulin in regulating the glucose level, devastates lives and burdens society (Heller and Feldman, 2008). For diabetic patient, the best possible way to lower the risk of complications and increase the longevity is by proper monitoring of glycemic control. Quantification of glycated hemoglobin (HbA_{1c}) is a very useful tool for the long-term glycemic control of diabetes without the influence of short-term fluctuations of blood glucose. The clinical reference range of HbA_{1c}, as a relative content of total hemoglobin (Hb), is 5–20%, and the range within 4–6% is considered as normal (Liu et al., 2006). Various techniques such as immunoassay (Stöllner et al., 2002), boronate affinity chromatography (Li et al., 2002), high performance liquid chromatography (Busto et al., 2008), and electrophoresis (Dong et al., 2013), are currently available for HbA_{1c} detection and provide good precision and accuracy. However, these methods are time-consuming and/or require special instruments.

Recently, electrochemical method is widely applied for the HbA_{1c} detection owing to its advantages of sensitivity, simplicity, low cost, and easy miniaturization (Pundir and Chawla, 2014). Among the electrochemical approaches, a few electrodes have been constructed based on the boronic acid–diol recognition, because the boronic acid could covalently bind to the cis-diol group of the surface sugar from glycated proteins under weak alkaline conditions. In previous study, an aminophenyl boronic acid-modified electrode was developed for capture HbA_{1c} and the catalytic reduction of H_2O_2 by HbA_{1c} itself was monitored as an analytical signal for HbA_{1c} detection (Kim and Shim, 2013). A potentiometric sensor for HbA_{1c} was also fabricated based on competitive binding of HbA_{1c} and alizarin red s (ARS) to phenylboronic acid and the resulting potential change of ARS (Liu and Crooks, 2013). In the boronic acid-based electrochemical sensors

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for HbA_{1c}, $[Fe(CN)_6]^{3-/4-}$ and ferrocenemethanol were also used as redox mediators for the signal generation (Park et al., 2008; Song and Yoon, 2009; Song et al., 2012). Scheller's group reported an electrochemical assay for HbA_{1c} by using bifunctionality of ferroceneboronic acid for both target recognition and signal producing (Liu et al., 2006). Compared with the conventional available methods, the above-mentioned electrochemical techniques are specific, simple and rapid. Nevertheless, most of these sensors require the use of additional redox probe in the solution because of the electrochemical inactivity of HbA_{1c}, and the sensitivity and stability need to be further improved. In our previous study, a potentiomentric sensor was fabricated for probing sialic acid through boronic acid–diol recognition, whereas the sensor needed the long-time stability of electrochemical potential prior to analysis. Thus, the electrochemical sensor based on interface response is still required to be constructed for the direct and sensitive detection of HbA_{1c} to meet the needs of various clinical requirements.

Pyrroloquinoline quinine (PQQ), which is originally found in the active site of methanol dehydrogenase, acts as a cofactor for mediating electron transfer in various enzymes (Gallop et al., 1993; Flexer et al., 2011; Neto et al., 2013). PQQ has a unique heterocyclic *o*-quinone structure with three carboxylic groups and exhibits a reversible electron transfer between the quinine and the quinol (Inoue and Kirchhoff, 2000; Young et al., 2013). Willner and coworkers reported that PQQ could be covalently bound on amine-modified electrode and acted as an electron relay for contact of active site of enzyme molecules and electrode surface (Willner and Riklin, 1994; Zayats et al., 2005). The enzyme-electrode produced an effective and direct electron transfer that was similar to the electrical communication of the enzyme with its native substrate. Therefore, the reversible redox process of PQQ on the electrode can be exploited to develop electrochemical sensors for inert substances through the electrochemical response change of PQQ. However, to the best of our knowledge, such strategy has not been reported yet. Importantly, the enhanced electrochemical response of PQQ has been obtained by simple immobilization of PQQ on a carbon nanotubes-modified electrode (Kanninen et al., 2010). Graphene with a two dimensional monoatomic thick has recently attracted increasing interest due to its high specific surface area and electron transport capabilities (Chen et al., 2012). In this regard, the composites of reduced graphene oxide and PQQ should have excellent electrochemical properties and can be utilized for the electrochemical sensor.

In this study, a voltammetric sensor for HbA_{1c} detection was fabricated based on the boronic acid-modified PQQ/reduced graphene oxide composites and the boronic acid–diol recognition. PQQ was immobilized on the reduced graphene oxide-modified electrode by electrochemical method, and the electrochemical properties were investigated. When HbA_{1c} bound to boronic acid linked to PQQ, the oxidation current of PQQ decreased. Quantitative determination of HbA_{1c} was based on measuring the current change of PQQ. This approach for HbA_{1c} detection is advantageous in terms of its simple equipment, need of no chromatographic separation, and requirement of cheap, non-biological, and commercially available reagents.

2. Experimental section

2.1. Chemicals and materials

Graphene oxide (GO) was synthesized from graphite nanopowders (Sinopharm, China) by a modified Hummers method (Hummers and Offeman, 1958; Ping et al., 2012). HbA_{1c} calibrator kits with three lyophilized Hb samples containing 5.67%, 6.97%, and 10.27% HbA_{1c}, respectively, were acquired from Aoke (Beijing, China). Lyophilized Hb standard was obtained from Sinopharm (China). PQQ, 3-aminophenylboronic acid, D-glucose, D-fructose, D-galactose, N-hydroxysuccinimide (NHS), and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Aladdin (Shanghai, China). Phosphate buffer (0.1 M) with various pH values were prepared by varying the volume ratios of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. 0.04 M Britton-Robinson (BR) buffer solution that contained 0.04 M each of H_3PO_4, HOAc, and H_3BO_3 was adjusted to appropriate pH by addition of 0.2 M NaOH. 0.1 M HEPES buffer solution was prepared by 0.1 M HEPES and 0.1 M NaOH. All other chemicals were obtained from commercial suppliers and used as received. Deionized water (18 MΩ cm, Milli-Q gradient system, Millipore) was used throughout the study.

2.2. Electrochemical measurements

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai CH Instruments, China). A conventional three electrode system, which consisted of a modified glassy carbon (GC) disk electrode (3.0-mm diameter) as working electrode, a platinum foil as counter electrode, and a saturated calomel electrode (SCE) as reference electrode, was used. Before all electrochemical measurements, the GC electrode was polished with aqueous slurries of α-alumina (1.0, 0.3, and 0.05 μm) on a polishing cloth, sonicated in deionized water for 10 min, and then dried under nitrogen. Unless otherwise stated, in differential pulse voltammetry (DPV) experiments, the amplitude was 0.005 V and the pulse width was 0.1 s. The 6.97% HbA_{1c} standard was used in the electrochemical analysis except plotting calibration curve for HbA_{1c} percentages. For the measurements of HbA_{1c} percentages, 5.67%, 6.97%, and 10.27% HbA_{1c} standards were used and 7.94% and 8.99% HbA_{1c} samples were obtained by the mixture of above standards. These samples were diluted 100-fold with 0.1 M phosphate buffer and the total Hb concentrations were 92.5 μg mL^{-1}.

2.3. Fabrication and characterization of the modified electrode

The phenylboronic acid modified GC electrode was prepared as follows. Firstly, direct electrodeposition of reduced graphene oxide (ERGO) was performed in a 0.5 g L^{-1} GO dispersion solution containing 0.1 M KCl by 15 consecutive cycles from 0.5 to −1.5 V (*vs.* SCE) at a scan rate of 50 mV s^{-1} (Chen et al., 2011). Secondly, the ERGO/GC electrode was subjected to potential cycling of 0.1 mM PQQ in HCl solution (pH 3.0) between −0.6 V and 0.8 V (*vs.* SCE) for 10 cycles at a scan rate of 80 mV s^{-1}. Thirdly, the prepared PQQ/ERGO/GC electrode was immersed into a solution containing 5 mM NHS and 10 mM EDC for 120 min, and then reacted with a 2 mM 3-aminophenylboronic acid solution in 0.1 M phosphate buffer (pH 7.4) for 20 min. Finally, the prepared electrode was washed with M-Q water, and thus phenylboronic acid modified electrode (PBA-PQQ/ERGO/GC electrode) was fabricated. For comparison, 5 μL of GO dispersion solution (0.5 g L^{-1}) was cast on the fresh GC surface and it was allowed to dry in the ambient condition. The above GO/GC electrode was further modified by PBA-PQQ following the same procedure to ERGO/GC electrode, and then the PBA-PQQ/GO/GC electrode was obtained. The fabrication processes were assessed using electrochemical impedance spectroscopy (EIS) and X-ray photoelectron spectroscopy (XPS). The XPS was carried out on an ESCALab220I-XL (VG Scientific) X-ray photoelectron spectrometer with a monochromatic Al Kα source (1486.6 eV photon energy).