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A novel potentiometric sensor based on a poly(anilineboronic acid)/graphene modified electrode for probing sialic acid through boronic acid-diol recognition

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ABSTRACT

A novel potentiometric approach was described for probing sialic acid (SA) using a poly(anilineboronic acid)/graphene modified glassy carbon (GC) electrode. The proposed electrode was prepared by electrodeposition of reduced graphene oxide (ERGO) at a GC electrode and then coated with a poly (anilineboronic acid) (PABA) film by electropolymerization of its monomer. Principle of SA detection at the PABA/ERGO/GC electrode was ascribed to a reversible and covalent boronic acid-diol binding which was sensitive to the electrochemical potential of the prepared sensor. The graphene layer introduced on the electrode surface was shown to dramatically improve the sensitivity of the sensor response. Under optimum conditions, the proposed sensor exhibited low detection limit of 0.8 μM with a wide linear range of 2 μM–1.38 mM, high stability, good regeneration, and remarkable selectivity. For the analysis of SA in human blood serum, the high accuracy and good recoveries revealed the great potential in the practical applications.

1. Introduction

Sialic acid (SA) is a generic name for the 9-carbon carboxylated monosaccharide, which is frequently located at the outer end of glycoconjugates on the surfaces of cells and soluble proteins (Villar and Barroso, 2006). One of the major types of SA is N-acetylneuraminic acid (Neu5Ac) in human serum and SA hereafter will imply Neu5Ac. The external and terminal positioning on the cell surface makes it an important mediator in various biological and pathological processes. SA has been regarded as a biomarker for some types of cancers and certain other diseases. Recent clinical and laboratory studies have shown that the SA concentration increased in the serum of patients with different malignant diseases such as, brain tumor, cardiovascular, leukemia, cancers of lung, breast, colon, and ovary (Gopaul and Crook, 2006).

Due to the important physiological role and the significance of SA level, several analytical methods based on the use of colorimetric, fluorescent, enzymatic, and chromatographic assays have been reported for the determination of SA (Massamiri et al., 1979; Marzouk et al., 2007; Marzouk et al., 2011; Matsuno and Suzuki, 2008; Tebani et al., 2011). The limitation of the colorimetric and fluorescent assays is the substance interference, especially for the hemolysed serum samples. The SA detection by enzymatic and chromatographic assays has high sensitivity and specificity, but these methods are time-consuming and need expensive instruments or enzymatic reagents. Sensing strategies based on the binding of boronic acid group with cis-diol of SA are an attractive alternative to enzymatic approaches. Kwak et al., (2012) described a micro-scaled thermometer using a phenylboronic acid modified layer to capture the SA-expressed erythrocytes. A density tunable dendrimeric array, which was assembled by 3-aminophenylboronic acid modified gold nanoclusters, was designed and used for in situ tracing of cell surface SA (Chen et al., 2013). A gold electrode modified with self-assembled monolayer containing phenylboronic acid was also employed for the noninvasive SA detection at cell membrane by potentiometric analysis (Matsumoto et al., 2009). These assays based on boronic acid chemistry show high specificity and are simple and "reagentless" for SA detection. However, some limitations including narrow detection ranges and/or low stability still exist because of low loading and instability of the self-assembled monolayer. Note that poly(anilineboronic acid) (PABA) film can be electrochemically polymerized from 3-aminophenylboronic acid (Shoji and Freund,

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2001). The formed polymer shows similar conductivity and redox behavior to those of polyaniline. The boronic acid groups remain reactive and have been utilized for an electrochemical saccharide sensor (Shoji and Freund, 2002). In addition, an exceptionally high binding constant of SA with boronic acid is obtained in physiological or acid buffer solution, compared to those of saccharides (Otsuka et al., 2003). Therefore, an electrochemical sensor based on PABA film is expected to recognize SA with high sensitivity, stability and selectivity.

From discovery to wide-ranging technological applications, graphene as a sp^2 carbon material with a two dimensional monoatomic thick, has received an increasing attention to biosensing applications owing to its unique electronic transport properties, high surface area, and low cost, during the past decade (Allen et al., 2010). Up to now, most of the graphene is prepared by chemical reduction of graphene oxide sheets and then is modified on electrode surface by drop-casting. These methods involve toxic reducing agents, and the formation of the graphene film needs a multistep coating process to keep its stability (Liu et al., 2012). Recently, electrochemical reduction of graphene oxide via two steps of predeposition of graphene oxide and further electrochemical reduction has been proved to be a promising approach due to its simple, fast and green nature (Deng et al., 2011; Haque et al., 2012; Mani et al., 2013; Wettstein et al., 2012; Yang et al., 2013; Zhou et al., 2009). However, the electrochemical method still suffers from lack of control of the film thickness. Chen et al. (2011) has demonstrated a one-step electrodeposition approach to form stable graphene films on electrodes from graphene oxide solution. Moreover, the electrodeposition technique can settle more graphene and the obtained graphene contain less oxygen compared to those by reducing pre-applied graphene oxide (Cui and Zhang, 2013). Even more important, one-step electrodeposited graphene modified on an ion-selective electrode exhibited excellent potentiometric performances, eg. fast response, high sensitivity, and long-term potential stability (Ping et al., 2012).

Combination of the specificity of boronic acid-diol reaction and the excellent electrochemical properties of graphene film is potentially an alternative strategy for SA determination. Here, we demonstrate a novel potentiometric sensor based on a PABA/graphene modified electrode for SA detection. The graphene and the PABA polymer are immobilized on the electrode by direct electrodeposition of reduced graphene oxide and electropolymerization of 3-aminophenylboronic acid, respectively. The parameters of this sensing system are optimized and the analytical characteristics are investigated under the optimized conditions. The further determination for serum samples is also studied to test the effectivity of the method for clinical use.

2. Experimental section

2.1. Chemicals and materials

Graphene oxide was synthesized from graphite nanopowders (Sinopharm Chemical Reagent Co, China) by a modified Hummers method (Hummers and Offeman, 1958; Ping et al., 2012. Supporting information). 3-Aminophenylboronic acid was supplied by Aladdin Reagent Ltd. (Shanghai, China). N-acetylneuraminic acid (SA) was purchased from Sigma-Aldrich. 0.1 M phosphate buffer solution (PBS) at various pH values was prepared by mixing the stock solutions of Na_2HPO_4 and NaH_2PO_4. 0.04 M Britton–Robinson (BR) buffer solution was obtained from an acidic solution which contained 0.04 M H_3PO_4, HOAc, and H_3BO_3 by adjusting to pH 4.75 using 0.2 M NaOH. 0.1 M acetate buffer solution at pH 4.75 was prepared using NaAc and HAc in a certain proportion. All other chemicals were of analytical grade, and the water used was obtained from a Millipore M-Q purification system ($>$ 18 MΩ cm).

2.2. Preparation of PABA/graphene modified electrode

Before modification, glassy carbon (GC) surfaces were polished with 1.0, 0.3, and 0.05 μm alumina powder with pure water on polishing cloths, and rinsed thoroughly with pure water between each polishing step. After polishing, the GC electrodes were sonicated in acetone and pure water in succession for 5 min. Electrodeposition of reduced graphene oxide (ERGO) was carried out by 15 consecutive cycles from 0.5 to -1.5 V (vs. saturated calomel electrode, SCE) in a 0.5 g L^{-1} graphene oxide dispersion solution containing 0.1 M KCl at a scan rate of 50 mV s^{-1} (Chen et al., 2011). Thus, the graphene modified GC (ERGO/GC) electrode was obtained, and then the PABA film was further coated by electropolymerization of its monomer on the ERGO/GC electrode (Shoji and Freund, 2001). The monomer solution contained 20 mM 3-aminophenylboronic acid and 100 mM NaF in 0.5 M HCl solution. The polymer film was prepared via repeated cycling between -0.1 V and 1.1 V (vs. SCE) at a scan rate of 100 mV s^{-1}. The cycle numbers applied to electrodes were used to regulate properties of the polymer film. The prepared PABA/ERGO/GC electrode was washed with pure water, and then soaked in 0.1 M PBS (pH 4.75) for 24 h to allow the electrochemical potential to stabilize. The surface morphologies of the modified electrodes were observed by scanning electron microscopy (SEM; Quonxe-2000, FEI). The PABA film was examined by Fourier Transform Infrared (FT-IR) spectrometer (Avatar 360, Thermo Nicolet) for possible functional groups.

2.3. Electrochemical measurements

A CHI660D electrochemical workstation (Shanghai CH Instruments, China) was used for electrochemical analysis. Potentiometric measurements were performed in a two electrode cell system with a modified GC disk electrode (3.0-mm diameter) as the working electrode, and a SCE as the reference electrode. Unless otherwise noted, the open circuit experiments were carried out in a stirred PBS (5 mL, 0.1 M). The change in open circuit potential (E_{oc}) was recorded as a function of time before and after the addition of SA. ΔE was the E_{oc} value of 0.1 M PBS with SA after subtracting the background signal of 0.1 M PBS without SA. Cyclic voltammogram (CV) and electrochemical impedence spectroscopy (EIS) were conducted in a conventional three-electrode configuration. A Pt wire and a SCE served as counter and reference electrodes, respectively. All electrochemical measurements were performed at room temperature.

2.4. Determination of SA in human blood serum

Blood samples supplied by Shangqiu First People's Hospital were collected from normal individuals. The blood samples were centrifuged at 3000 rpm at 4 °C for 10 min (KA2200 centrifuge, Kubota, Japan), and then the plasma was separated. The serum was transferred into a 2 mL polypropylene test tube and stored at -20 °C up to the time of electrochemical analysis. The serum samples were 1,000-fold diluted with 0.1 M PBS (pH 4.75).

3. Results and discussion

3.1. Fabrication and characterization of the PABA/ERGO/GC electrode

Scheme 1 shows the fabrication procedure of PABA/ERGO/GC electrode and the sensing mechanism of SA. Electrochemical reduction of graphene oxide by the one-step electrodeposition