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Poly(styrene sulfonate) and Pt bifunctionalized graphene nanosheets as an artificial enzyme to construct a colorimetric chemosensor for highly sensitive glucose detection

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ABSTRACT

In this work, we developed a highly sensitive and simple colorimetric method for glucose detection using poly(styrene sulfonate)/Pt-modified graphene nanosheets (PSS-GN-Pt) as peroxidase mimetics. First, in the presence of glucose, H_2O_2 was generated as the main by-product of glucose oxidase (GOx)-catalyzed reaction. Then, the obtained H_2O_2 triggered the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) to generate blue color with the aid of PSS-GN-Pt nanocomposites as peroxidase mimetics. Via this method, as low as 9.28×10^{-8} M glucose could be detected with a linear range from 2×10^{-7} M to 1×10^{-3} M. Besides absorbency measuring, the visual detection of glucose by naked eyes could also be realized very easily through the observable color changing from colorless to blue. In addition, this strategy was further used to determine the concentrations of glucose both in injection and serum samples with satisfying results. This proposed nanosystem holds great potential for diabetes mellitus research and can be easily expanded to the detection of various H_2O_2-involved analytes.

1. Introduction

Glucose is the major carbon and energy source in cellular metabolism and plays critical roles in the natural growth of cells. Glucose levels in blood are associated closely with diabetes or hypoglycemia. During the past decades, diabetes has become one of the biggest global threats to human health [1–3]. Thus, to accurately detect glucose is of immense scientific technological importance for clinical diagnostics in diabetes control and also for other analytical applications in biotechnology, environmental pollution control as well as food industry. To date, tremendous efforts have been directed toward developing glucose sensors based on fluorescence [4–7], colorimetric [8–10], chemiluminescence [11], high performance liquid chromatography (HPLC) [12], capillary electrophoresis [13], and electrochemical methods [14–16]. Among these analytical techniques, colorimetric assay has attracted considerable attention owing to its good sensitivity, low cost, simplicity, and practicality [17]. Furthermore, this method can be interpreted by the naked eyes to realize visual detection without any instrumentation.

For colorimetric analysis of glucose, the detection process is generally completed by monitoring the consumption of H_2O_2 with the aid of horseradish peroxidase (HRP). Here, H_2O_2 is the byproduct of glucose oxidation by GOx. However as a natural enzyme, HRP suffers from some serious drawbacks such as inherent instability and high cost, which has inevitably restricted its widespread applications [18,19]. In recent years, nanomaterial-based artificial enzymes have received great attention and allowed us to view conventional heterogeneous catalysts with a new perspective. To date, various nanomaterials as peroxidase mimics have been applied for colorimetric biosensing [20–27]. Comparing with natural enzymes, these peroxidase mimetics have significant advantages including simple synthesis, low cost, high stability and considerable catalytic activity. Recently, cubic Pt nanocrystals and active agent-stabilized Pt nanoclusters have been reported with outstanding peroxidase-like activity [28,29]. Although these Pt nanomaterials could catalyze H_2O_2-mediated oxidation of TMB with high catalytic activity, the wider adoption of these nanoparticles faces great challenge due to their spontaneous aggregation and instability. To address this problem, layered materials with supported Pt nanoparticles have been widely investigated to improve the stability of the catalyst [30]. Based on our previous works, graphene

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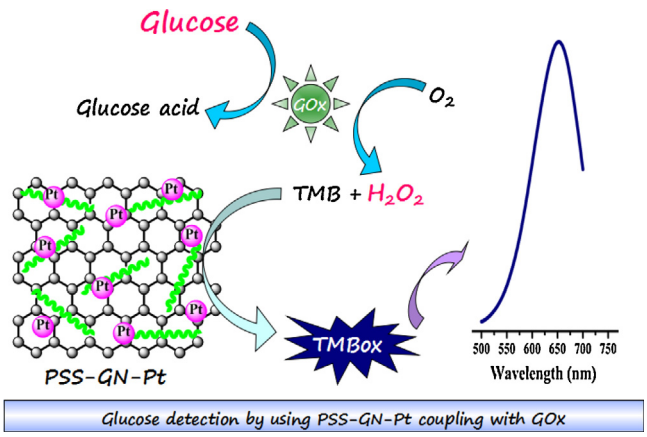

Scheme 1. Illustration of colorimetric platform for glucose detection by using glucose oxidase (GOx) and PSS-GN-Pt nanocomposites.

functionalized with PSS (PSS-GN) has shown superior water dispersability as well as favorable stability, which mainly attributed to the electrostatic interactions among negatively charge PSS units intercalated in graphene nanosheets [31]. Moreover, these PSS-GN nanosheets have also demonstrated intrinsic peroxidase-like activity [32]. All these properties suggest that PSS-GN has great potential to be used as a robust candidate for preparing nanosheet-supported metal or metal oxide nanomaterials.

Herein in this work, we developed a simple approach to construct Pt nanoparticles on the surface of PSS-GN nanosheets, and the resulting PSS-GN-Pt nanocomposites were found to possess superior water dispersability as well as excellent stability. More importantly, PSS-GN-Pt nanocomposites showed considerable mimetic enzyme catalytic activity, which may be attributed to the promotion of the electron transfer between the substrate and graphene-based carbon materials [23,33], and also the synergistic effect in simulated enzyme between metal nanoparticles and functionalized graphene [34]. Thus, the kinetic behavior and catalytic mechanism of PSS-GN-Pt were further investigated. Meanwhile, using PSS-GN-Pt as a peroxidase mimic, a novel colorimetric and visual method for glucose detection was developed (Scheme 1). A good linear relationship was obtained from 2×10^{-7} to 1×10^{-3} M with a detection limit of 9.28×10^{-8} M. Compared with the reported colorimetric methods for glucose detection, this proposed assay exhibited relative high sensitivity. In addition, this method was further used to determine the concentrations of glucose in injection and serum samples with satisfying results.

2. Experimental section

2.1. Chemicals and apparatus

Glucose, maltose, fructose, sucrose, galactose, 3,3′,5,5′-tetramethylbenzidine (TMB) and glucose oxidase (GOx) were obtained from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). Glucose kits were obtained from Shanghai Rongsheng Biotech Co. Ltd. (Shanghai, China). Proserum and hydrogen hexachloroplatinate (IV) hexahydrate ($H_2PtCl_6 \cdot 6H_2O$) were received from Aladdin (Shanghai, China). H_2O_2 (30%), sodium hypochlorite, dopamine and uric acid were purchased from Tianjin Chemical (Tianjin, China). Various amino acids, ascorbic acid, glutathione and poly(styrene sulfonate) (PSS) (70,000 g mol^{-1}) were purchased from Sigma-Aldrich. 0.2 M of acetate-acetic buffer (HAc-NaAc) was prepared with sodium acetate and acetic acid. Glucose injection was form Henan Kelun Pharmaceutical Co. Ltd. and serum sample was provided by the Zhengzhou University Hospital. All experiments were performed incompliance with the relevant laws and institutional guidelines and approved by Life-Science Ethics Review Committee of Zhengzhou University. All other reagents were of analytical grade without any further purification. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) with an electric resistance >18.2 MΩ. All glassware were soaked with saturated chromic acid solution for at least 24 h and rinsed with ultrapure water thoroughly before use.

Transmission electron microscope (TEM) images were obtained from JEM-2100 (JEOL, Japan) with a 200 kV accelerating voltage. Energy-dispersive X-ray spectroscopy (EDS) characterization was carried out on a JSM-7500F microscope (JEOL, Japan). The FT-IR spectra were obtained through a Bruker IFS 66 v/s infrared spectrometer. The X-ray diffraction (XRD) patterns were acquired on a D/max-IIIA diffractometer (Rigaku Co. Japan). The UV–vis absorption spectra were recorded on a T6 UV–vis Spectrophotometer (Purkinje General, Beijing, China). All of the pH values were measured by a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China).

2.2. Synthesis of PSS-GN-Pt nanocomposites

Graphene oxide (GO) was synthesized by natural graphite powder using the modified Hummer method [35]. PSS-GN-Pt nanocomposites were prepared as follows: Typically, 10 mg GO was dispersed in 20 mL water to form a 0.5 mg mL^{-1} GO dispersion by ultrasonication for 30 min. Then, 5 mg H_2PtCl_6 and 0.2 g PSS were simultaneously dispersed into the mixture via stirring. After stirring for 30 min, 60 mg $NaBH_4$ was slowly added as the reducing reagent and the above mixture was put into an oil bath (80 °C) for further reaction of 6 h (with constantly stirring). Finally, the obtained black product was separated by centrifugation, washed several times with water, and then dried in vacuum. The preparation of PSS-GN was similar to PSS-GN-Pt except no addition of H_2PtCl_6.

2.3. Procedure for peroxidase-like activity and kinetic analysis

To investigate the peroxidase-like activity of PSS-GN-Pt nanocomposites, the catalytic oxidation reaction of the typical peroxidase substrate of TMB in the presence of H_2O_2 was measured. Experiments were carried out using 15 μg mL^{-1} PSS-GN-Pt nanocomposites in 1 mL of 20 mM HAc-NaAc buffer with 0.2 mM TMB and 1 mM H_2O_2. The influence of the PSS-GN-Pt concentration, buffer pH, reaction temperature, and H_2O_2 concentration for the peroxidase-like activity of PSS-GN-Pt nanocomposites were also investigated.

The apparent steady-state kinetic measurements were carried out by varying concentration of TMB at a fixed concentration of H_2O_2 or vice versa unless otherwise stated for 2 min as a function of time. Experiments were carried out at room temperature in tube with 15 μg mL^{-1} PSS-GN-Pt nanocomposites in 1 mL of 20 mM HAc-NaAc buffer (pH 3.5) with 0.2 mM TMB or 1 mM H_2O_2. Immediately after the substrates were added, color reaction was observed. The Michaelis-Menten constant and maximal reaction velocity were calculated based on the Lineweaver-Burk plot.

2.4. General procedure for H_2O_2 and glucose analysis

In a typical process, the H_2O_2 solution with different concentrations was added to the mixture of TMB (final concentration of 0.2 mM) solution and PSS-GN-Pt nanocomposites solution (final concentration of 15 μg mL^{-1}) and HAc-NaAc buffer (20 mM, pH 3.5), and the mixture mixed uniformly by vortex was further