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Magnetic graphene oxide-supported hemin as peroxidase probe for sensitive detection of thiols in extracts of cancer cells

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ABSTRACT

Magnetic graphene oxide (GO)–hemin probes containing disulfide bonds are simply and effectively synthesized through amide reaction to covalently link magnetic particles to GO surface and π–π stacking interaction between hemin and GO to immobilize hemin on GO. Based on the strong nucleophilicity of sulfhydry, we have developed a colorimetric detection system for thiols by using glutathione (GSH) as a model analyte. Upon the introduction of GSH to the fabricated magnetic particle (MP)-GO–hemin probes, the disulfides can be readily reduced by thiols, resulting in the release of GO–hemin hybrids to solution. Due to the existence of hemin on GO surface, the released GO–hemin that has the intrinsic peroxidase-like activity can catalyze the oxidation of $ABTS^{2-}$ by H_2O_2 to form the colored radical product $ABTS^{\cdot -}$. A broad linear dynamic range of 10^{-10} M to 10^{-6} M GSH is achieved with a detection limit of 8.2×10^{-11} M (3σ). Moreover, the new probe is successfully applied to the detection of non-protein thiols and protein thiols in the extracts of Ramos cells, which shows favorable correlationship with the results obtained by electrochemical method. In addition, the MP-GO–hemin probe can detect non-protein thiols in Ramos extracts as low as 500 cells. In this assay, the prepared MP-GO–hemin conjugates are thoroughly characterized by SEM, AFM, UV–Vis, FT-IR, and Raman spectroscopy.

1. Introduction

Graphene with a one-atom thickness and two-dimensional plane structure has attracted considerable attention due to its unique physical, chemical, and mechanical properties (Georgakilas et al., 2012). Graphene is typically synthesized by mechanical cleavage or chemical methods (Guo and Dong, 2011). Particularly, using graphene oxides (GO) as the starting material to produce graphene provides an economic and efficient method for bulk production (Chen et al., 2012). GO can be well-dispersed in aqueous solution due to a rich variety of surface defects and abundant hydrophilic groups on its surface, such as hydroxyl, epoxide and carboxylic groups. As a novel fascinating material, graphene or GO shows many advantages, such as large specific surface area, excellent thermal conductivity, high electrical mobility, great mechanical strength, and low production cost, which has been widely used in synthesizing nanocomposites and fabricating microelectrical devices (Chung et al., 2013). Recently, GO which can be easily modified has been actively pursued in biotechnology (Huang et al., 2013; Parlak et al., 2013). For example, GO based magnetic composites have been used for drug delivery, biomedical engineering, and biomolecules detection (Shen et al., 2010; Yang et al., 2011, 2009). Moreover, noncovalent functionalization make GO as a perfect substrate to support molecular with improved catalytic activity and stability (Yang et al., 2010). The deposition of porphyrin on GO was achieved through π–π stacking interaction, which can take advantage of both superior properties of GO and the functionalizing molecules (Xu et al., 2009). As the active center of heme-protein, hemin (iron protoporphyrin) has been reported to conjugate with GO to act as a highly active biomimetic oxidation catalyst for bioassay (Deng et al., 2013; Guo et al., 2011; Xue et al., 2012).

Low molecular weight thiols are widely distributed in tissues and cells, which play a significant role in metabolism and celluar homeostasis (Zhang et al., 2004). Tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH), the most predominant and abundant cellular thiol, plays a crucial role in living organisms which can be found within human cellular system (Hwang et al.,

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1992; Yi et al., 2009). The reduced form, GSH, can be rapidly oxidized to its dimeric form GSSG in response to oxidative stress within cells (McMahon and Gunnlaugsson, 2012). Therefore, the altered level of intracellular GSH or the GSH/GSSG ratio has become an important indicator in monitoring the overall health of cells and their ability to protect cells against oxidative damage. So far, various analytical methods have been developed for the detection of thiols and thiol-containing peptides, including HPLC (Amarnath et al., 2003), mass spectrometry (Huang and Chang, 2007), electrochemical assay (Pacsial-Ong et al., 2006; Wang et al., 2008), and optical sensor (Wei et al., 2013a, 2013b; Yang et al., 2013; Zong et al., 2013). Recently, fluorescent probes containing S–S bond or Se–N bond were successfully synthesized for determination and imaging of celluar thiols with high sensitivity and selectivity (Pires and Chmielewski, 2008; Tang et al., 2007; Wen et al., 2011). However, these probes were often suffered from complicated synthesis and purification, high cost, photobleaching, and nonspecific signals caused by light excitation. Alternatively, design of simple and sensitive probe for thiols detection still needs to be developed. Moreover, example of convenient and accurate colorimetric method for thiols detection has been scarce reported (Liu et al., 2013; Ma et al., 2012; Yuan et al., 2013). In this assay, magnetic graphene oxide–hemin probes containing disulfide bonds are simply and effectively synthesized for colorimetric detection of thiols in vitro and in the extracts of Ramos cells with high sensitivity.

2. Experimental

2.1. Chemicals and apparatus

Glutathione (GSH) was purchased from Solarbio Co., Ltd (Beijing, China). Graphene oxide (GO) was obtained from Beijing DK Nano Technology Co., Ltd. (China). S-2-pyridylthio cysteamine hydrochloride was purchased from Toronto Research Chemicals Inc. (Canada). Hemin, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt ($ABTS^{2-}$), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were ordered from Aladdin Chemistry Co. Ltd (China). Thiol modified magnetic particles (MPs) (3–4 μm) were obtained from BaseLine ChromTech Research Centre (Tianjin, China). Double-distilled, deionized ultrapure water was used in all experiments. All regents were of analytical grade and used without further purification.

2.2. Preparation of MP-GO–hemin composites

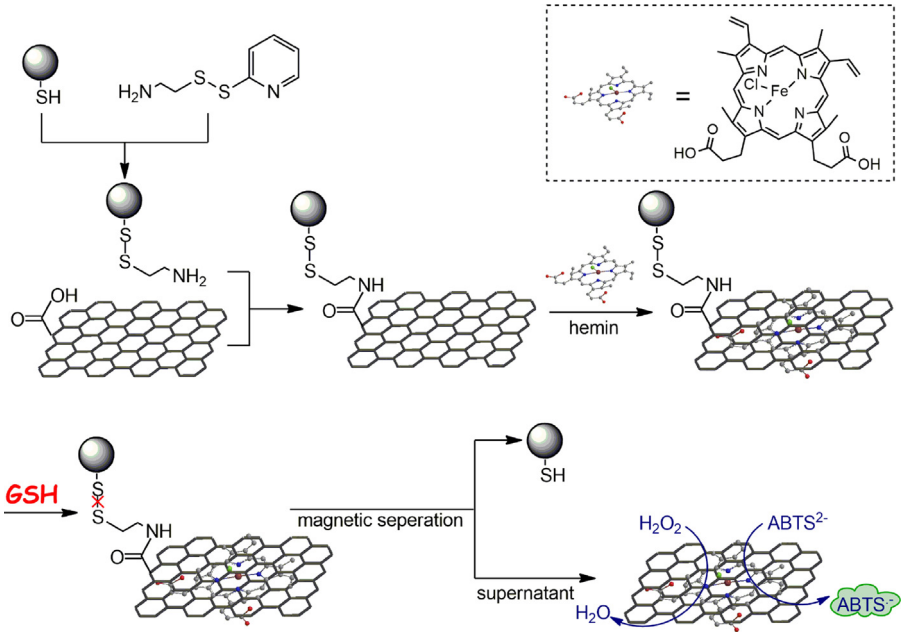
First, 50 μL of thiol-modified MPs were reacted with 100 μL of S-2-pyridylthio cysteamine hydrochloride solution for 2 h, which were washed with 0.01 M PBS buffer (pH 8.0) twice for further use. At the same time, 200 μL of GO suspension (450 μg/mL) was activated by 2 μL of EDC (400 μg/mL) and 2 μL of NHS (320 μg/mL) for 1 h. And then, the –COOH activated GO was added to the above $–NH_2$-coated MPs, followed by incubation overnight with gentle shaking at room temperature. Finally, 1 mL of hemin-methanol solution (5.0 μM) was added. The mixture was stirred mildly for 3 h to allow the conjugation between hemin and GO. The resulting MP-GO–hemin composites were magnetically washed three times with 0.01 PBS and then re-dipersed in Tris buffer (pH 7.4).

2.3. Characterization

Fourier transform infrared (FT-IR) spectra were performed on a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA). Scan electron microscopy (SEM) and atomic force microscopy (AFM) images were collected on a JSM-6700F microscope (HITACHI, Japan) and a Benyuan Nano-Instruments CSPM-5500 (Beijing, China), respectively. Raman spectra were conducted with a Renisaw Invia Raman spectrometer RamLab-010. The UV–Vis absorbance measurements were performed on a Cary 50 UV–Vis spectrophotometer (Varian, USA).

2.4. GSH assay

A 800 μL of different concentration of GSH in 0.01 M PBS buffer (pH 7.0) was added to the above prepared MP-GO–hemin composites. After reaction for 30 min at 25 °C in dark, the suspension and washings from MPs were combined through magnetic separation to a total volume of 1 mL. The resulting solution containing the released GO–hemin conjugates was reacted with 400 μL of $ABTS^{2-}$ (10 mM), 600 μL of H_2O_2 (7.5 mM) in a buffer solution consisting of



Scheme 1. Fabrication of MP-GO–hemin probe containing disulfide bonds for GSH detection.