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Quinone-mediated microbial synthesis of reduced graphene oxide with peroxidase-like activity

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HIGHLIGHTS

- First systematic study on quinone-/humic acid-mediated graphene oxide reduction.
- Detailed characterization of resultant quinone-mediated reduced graphene oxide.
- First peroxidase activity assay of bio-fabricated reduced graphene oxide.
- Glucose detection using quinone-mediated reduced graphene oxide.

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ABSTRACT

The effects of different quinones on graphene oxide (GO) reduction by *Shewanella oneidensis* MR-1 and the peroxidase activity of the resultant reduced graphene oxide (QRGO) were studied. The presence of 100 μM anthraquinone-2-sulfonate (AQS), anthraquinone-2,6-disulfonate and 5-hydroxy-1,4-naphthoquinone could lead to 1.6–2.8-fold increase in GO reduction rate, whereas anthraquinone-2-carboxylate slowed down the reduction. The stimulating effects of AQS increased with the increase of its concentration (10–100 μM). The mediated effects were proved by direct GO reduction by microbially reduced AQS. The mediated reduction of GO to QRGO was characterized by UV–vis, XRD, FTIR, Raman spectra, XPS, TEM and AFM, respectively. The as-prepared QRGO possessed peroxidase-like activity, which could catalyze the oxidation of 3,3′5,5′-tetramethylbenzidine by H_2O_2, and followed Michealis–Menten kinetics. A colorimetric sensor for quantitative determination of glucose based on the peroxidase activity of QRGO was developed over a range of 1–120 μM with a detection limit of 1 μM.

1. Introduction

Graphene, a two-dimensional carbon material with one-atom thickness, has attracted considerable interests due to its unique properties such as extremely huge surface area, extraordinary mechanical strength and high carrier mobility. These excellent characteristics make graphene potentially useful in fields of electronics, sensors, and solar and microbial fuel cells, etc. (Liu et al., 2012; Yuan et al., 2012). Moreover, peroxidase-like activity has recently been observed from graphene oxide, sonication-exfoliated few-layer graphene and graphene dots, which represent a new kind of peroxidase mimic (Song et al., 2010; Wang et al., 2013; Zheng et al., 2013).

Because of its low cost, simple procedure and easy control of morphology, chemical reduction of graphene oxide (GO) has received the most attention and believed to be the most promising method for large-scale preparation of graphene. Many chemical reducing agents, including hydrazine, phenylhydrazine, dimethylhydrazine, sodium borohydride, p-phenylene diamine, etc. could efficiently remove oxygenic functional groups and thus restore the conjugate structure of graphene (Bai et al., 2011). However, most of these reagents were toxic, unstable, dangerous and/or expensive, thus the finding of greener method for GO reduction has attracted much attention.

Recently, two groups has independently found that *Shewanella* strains, which are well-known for their great respiration capabilities and important roles in the biogeochemical cycling of metals and nutrients, could transfer GO to reduced graphene oxide (RGO) under ambient conditions through respiration (Salas et al., 2010; Wang et al., 2011a,b). After that, many other microbial species including *Escherichia* strains, *Halomonas* strains, *Pseudomonas aeruginosa*, *Bacillus marisflavi*, and baker's yeast have been

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successively found capable of reducing GO to RGO during the past 2 years (Akhavan and Ghaderi, 2012; Gurunathan et al., 2013a,b,c; Khanra et al., 2012; Raveendran et al., 2013). These studies offered more cost-effective and eco-friendly alternatives for RGO synthesis.

Besides its direct electron transfer from cell surface to extracellular substrates, *Shewanella* strains could also use self-secreted or exogeneous redox mediators (or electron shuttles) to promote extracellular reduction processes (Gralnick and Newman, 2007). Quinone compounds acting as redox mediators can be cycled back and forth from the reduced hydroquinone state to the oxidized quinone conformation, which results in acceleration of electron transfer processes, and improvement in the biotransformation of Fe(III) oxides, U(VI), Tc(VII), As(V), azo dyes and nitroaromatics, etc. (Borch et al., 2005; Fredrickson et al., 2000; Hong et al., 2007; Jeon et al., 2004; Royer et al., 2002; Yamamura et al., 2008). Previous study of Jiao et al. (2011) observed that anthraquinone-2,6-disulfonate could increase the GO reduction rate of *Shewanella oneidensis* MR-1. However, a detailed investigation of effects of different quinone mediators on microbial GO reduction and characterization of the resultant quinone-mediated RGO (QRGO) was not available.

Herein, mediated reduction of GO by *S. oneidensis* MR-1 was studied in the presence of different quinone compounds. The morphology and characterization of the obtained QRGO were studied in detail. In addition, the peroxidase activity of QRGO was investigated and applied for glucose detection. To our knowledge, this is the first systematic study on quinone-mediated microbial reduction of GO and peroxidase mimetic activity of the resultant QRGO.

2. Methods

2.1. Chemicals, bacterial strain and media

Graphite and other chemicals were all of analytical grade, purchased from Sigma–Aldrich, TCI or Sinopharm and used without further purification.

GO was prepared from graphite powder by modified Hummers method (Hummers and Offeman, 1958). In brief, 1.0 g graphite powder was mixed with 10 ml HNO_3 and 46 ml H_2SO_4 in an ice bath under stirring for 30 min. Then, 6.0 g of $KMnO_4$ was slowly added to the mixture with stirring in 20 min and kept in an ice bath for at least 120 min. The solution was heated at 35 °C overnight, and subsequently diluted with 46 ml of ultrapure water (the temperature went up to about 98 °C) and kept for 120 min. After that, the solution was further diluted by the addition of 200 ml ultrapure water, followed by adding 20 ml H_2O_2 dropwise. The obtained bright yellow solution was centrifuged at 5000 rpm for 30 min to isolate graphite oxide precipitate, which was washed with 10% HCl for three times and ultrapure water for several times till the supernatant became neutral, and finally re-suspended in ultrapure water. The aqueous graphite oxide solution was then sonicated for 3 h to facilitate the exfoliation of stacked graphite oxide sheets into mono- or multi-layered GO sheets.

S. oneidensis MR-1 (ATCC 700550) obtained from ATCC was routinely cultured in Luria–Bertani (LB) broth medium aerobically at 30 °C. GO reduction studies were performed in modified M-R2A medium (Liu et al., 2013) containing (mg l^{-1}) KH_2PO_4, 250; K_2HPO_4, 400; KCl, 505; NH_4Cl, 800; $CaCl_2{\cdot}2H_2O$, 15; $MgCl_2{\cdot}6H_2O$, 20; $FeSO_4{\cdot}7H_2O$, 7; Na_2SO_4, 5; $MnCl_2{\cdot}4H_2O$, 5; H_3BO_3, 0.5; $ZnCl_2$, 0.5; $CoCl_2{\cdot}6H_2O$, 0.5; $NiSO_4{\cdot}6H_2O$, 0.5; and $CuCl_2{\cdot}2H_2O$, 0.3. Lactate (50 mM) was added as electron donor and the pH of the medium was adjusted to 7.0.

2.2. Quinone-mediated reduction of GO by MR-1

The MR-1 cells cultured aerobically overnight were harvested by centrifugation (10,000*g*, 5 min) and washed thrice with sterile phosphate buffer solution (20 mM, pH 7.0). The resulting cell pellets were suspended with M-R2A medium and held in the anaerobic chamber before use in the following studies.

The experimental systems were 100-ml serum bottles containing 96 ml deoxygenated sterile medium, 0.2 g of GO l^{-1} and 100 μM of anthraquinone-2,6-disulfonate (AQDS), 5-hydroxy-1,4-naphthoquinone (JQ), anthraquinone-2-carboxylate (AQC) and anthraquinone-2-sulfonate (AQS), respectively. After MR-1 cell inoculation (0.4 g l^{-1}), samples were periodically taken with sterile needle and syringe and analyzed spectrometrically as described below. Control systems with heat-killed cells or without inoculation were also performed.

To study the effects of quinone concentration on GO reduction by MR-1 cell, varied concentrations of AQS (10–100 μM) were added to the reduction system.

To confirm the reduction of GO by microbially reduced quinone compounds, harvested MR-1 cells were incubated in 96 ml M-R2A medium containing 100 μM AQS in serum bottles and held anaerobically in the anaerobic chamber overnight to guarantee the complete reduction of AQS to its reduced form AH_2QS, which was monitored and confirmed spectrometrically at 398 nm (Rau et al., 2002). The microbially reduced AQS prepared as described above were anaerobically taken out of the vials, filtered to remove cells, and mixed with 0.2 g of GO l^{-1} to study the chemical reduction of GO by reduced redox mediator. As a control, MR-1 cells were also incubated overnight in normal M-R2A medium without quinone, and then equal volume of filtered metabolite was added to GO solution.

GO reduction was monitored through the constructed UV–vis spectrometric method, which detected the increase of OD_{600} after correcting for bacterial cells (Jiao et al., 2011; Wang et al., 2011a,b). Quinone mediators used here had no impact on the OD_{600} value.

2.3. Characterization of QRGO

Mediated reduction of GO by MR-1 in the presence of AQS was performed as described above. After overnight incubation, the harvested QRGO was used for further characterization.

The UV–vis spectra of GO and QRGO were obtained with Jasco V-560 spectrophotometer. X-ray diffraction (XRD) studies were performed with a Rigaku D/max 2400 X-ray diffractometer (Cu Kα radiation, $\lambda = 0.1541$ nm). Fourier transform infrared (FTIR) spectra of GO and QRGO were recorded by a Bruker Equinox 55 FTIR spectrometer over the wavenumber range of 4000–400 cm^{-1}. Raman spectroscopy was carried out at room temperature using a Renishaw inVia Raman microscope in backscattering configuration and an excitation line of 632.8 nm provided by an He–Ne laser. The calibration was initially made using a silicon reference at 520 cm^{-1} and gave a peak position resolution of <1 cm^{-1}.The chemical characteristics of GO and QRGO were analyzed using X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha). XPS peaks were deconvoluted by using Gaussian components after a Tougaard background subtraction. Transmission electronic microscopy (TEM) image of QRGO was obtained with Tecnai G2 spirit transmission electronic microscope operating at 120 kV. Surface topography of QRGO was measured using tapping-mode atomic force microscopy (AFM, Veeco, DI 3100).

2.4. Peroxidase activity assay of QRGO

The peroxidase-like activity of QRGO was investigated through detecting the blue color formation of the catalytic oxidation of the