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Electrochemical detection of glutathione by using thymine-rich DNA-gated switch functionalized mesoporous silica nanoparticles

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ABSTRACT

A novel electrochemical sensor for highly sensitive and selective detection of glutathione (GSH) was developed coupled thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) coordination with mesoporous silica nanoparticles (MSN). Hg^{2+} ions could specifically interact with thymine bases to form strong and stable T-Hg^{2+}-T complexes. And the electroactive molecules-methylene blue (MB) was sealed in the inner pores of MSN with a unique sequential thymine (T)-rich DNA in the presence of Hg^{2+}. After the target was added, the formed duplex DNA T-Hg^{2+}-T complex could deform into single-stranded DNA by a competitive displacement reaction, leading to the release of MB entrapped in the MSN. Thus the target-dependent amount of released MB could be conveniently monitored with an *n*-dodecanethiol modified screen-printed gold electrode. Using GSH as a model target, the proposed assay showed a wide detection ranging from 1.0 nM to 1.0 μM with a detection limit of 0.6 nM. This strategy was simple and universal for various analytes with different competitive displacement ligands, possessing a great potential for convenient biotools testing and commercial application.

1. Introduction

Over recent decades, considerable researches were carried out on the bioengineering, bio-sensing, bio-nanotechnology, and drug delivery applications of different nanostructured materials. Various nanomaterials, as carbon nanotubes (Tran et al., 2016; Hernández-Ibáñez et al., 2016), magnetic beads (Reverté et al., 2016; Zhang et al., 2016), graphene (Zou et al., 2015; Park et al., 2016; Zhou et al., 2015), gold nanoparticles (Miao et al., 2016; Zhao et al., 2016), quantum dots (Vijian et al., 2016; Kokkinos et al., 2015) and mesoporous silica nanoparticles (Yu et al., 2016; Fan et al., 2016) were applied to design highly sensitive methods for bio-sensing. It was their specific surface area and unique electrical conductivity of nanomaterials that led to their extensive utilization in the design of trace nanoprobes and the construction of sensing interfaces. Apart from generally exhibiting merits of high ratio of signal-to-noise and high detection sensitivity, these nanomaterial-based electrochemical methods otherwise inevitably were performed in heterogeneous systems and required wash and separation steps, restraining their application in common point-of-care testing. Nevertheless, due to advantages of contamination risk mitigation and time-consuming operation steps elimination, homogeneous assay thus served as an attractive detection which was easy to design high-throughput and automation systems (Wang et al., 2015; Wang et al., 2016). Therefore, some nanomaterials were used for the construction of homogeneous electrochemical assay (Zhu et al., 2015).

Due to their unique properties, including biocompatibility, ease of functionalization, large surface area, large pore volume, controlled pore structure, and high thermal and mechanical stability, mesoporous silica nanoparticles (MSNs) attracted substantial research attention in the fields of biotechnology, biomedicine and nanomedicine (Yang et al., 2015a, 2015b; Zou et al., 2016). In particular, some signal molecules could be filled in the mesopores of MSNs and then sealed with gatekeepers such as organic molecules, nanoparticles, supramolecules, and biomolecules to construct stimuli-responsive MSN nanoprobes. While various stimuli, such as redox reagents (Trewyn et al., 2007; Kim et al., 2010), pH (Meng et al., 2010; Zhou et al., 2011), temperature (Aznar et al., 2011), enzymes (Patel et al., 2008; Coll et al., 2011) and

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photoirradiation (Angelos et al., 2009; Aznar et al., 2009) were implemented to trigger the opening of the pores and controllable release of the encapsulated substrates. Thus, these stimuli-responsive MSNs were frequently used as delivery vehicles and sensory nanoprobes. For example, in drug delivery, it was extraordinarily eye-catching for the progresses of stimuli-responsive nanoscopic hybrid gated materials involving biomolecules as caps with the ability to release entrapped guests upon application of an external stimulus. In contrast, examples of their use in bio-sensing were much less common. For bio-sensing, the carrier system was commonly loaded with an indicator and the capping mechanism was designed in a way that only a target analyte was able to trigger uncapping and the delivery of the cargo.

In past decades, because of its various advantages of simple fabrication, low cost, small size, disposability, portability and easily mass-produced, screen-printed electrode (SPE) were widely applied and challenged the conventional three-electrode system, modernizing the field of electroanalysis and bridging the gap between laboratory experiments and in-field applications. And it was of great interest in sensor development within many fields (Ahmar et al., 2014; Souza et al., 2015; Jiang et al., 2015) to replace conventional electrochemical cells with miniaturized SPE strips. Nevertheless, by selecting the screen-printing inks and designs, unique sensors could be worked out with different geometries such as screen-printed arrays, recessed electrodes and micro bands. Additionally, the convenience of the mass production of SPE made their applications one-shot sensors, enabling possible contamination to be avoided and relieving the demands for electrode pre-treatment as was the case for solid electrodes before their use. Therefore, in general, SPE supplied a simple, user friendly, fast and inexpensive rout for electrochemical measurements.

This work used MSNs and DNA biogate to design a homogeneous electrochemical assay for direct and rapid detection of GSH based on n-dodecanethiol modified SPE. Firstly, the DNA biogate were used for electrochemical assay by using the specific reaction to displace the MSN and open the molecular gate for release of MB. Then, the immobilized n-dodecanethiol could not only avoid nonspecific protein adsorption but also enhance the currents response due to accumulation of MB molecules that penetrated into the alkanethiol layer. Next, by tremendously shortening the time without the complicated pretreatment steps, this method proved to be a promising new technology for rapid detection. Lastly, it employed SPE, enabling the detection of GSH in a disposable, cost-effective, reagentless and washing-free fashion. In this way, the target-responsive controlled release system-based electrochemical assay thus offered a promising scheme for the development of advanced homogeneous assay without the sample separation and washing procedure.

2. Experimental section

2.1. Reagents and materials

N-Cetyltrimethylammonium bromide (CTAB, 99%) and tetraethylorthosilicate (TEOS, 28%) were supplied by Heowns Biochem Technologies. LLC. (Tianjin, China). Sodium azide (NaN_3, 99%), copper (I) bromide (CuBr, 99.9%), methylene blue (MB), *n*-dodecanethiol and 3-chloropropyltrimethoxysilane (ClTMS, 97%) were purchased from Alfa-Aesar. $Hg(NO_3)_2$ and glutathione (GSH) were purchased from Dingguo reagent company (Beijing, China). Sodium hydroxide (NaOH), t-butyl alcohol (tBuOH), dimethyl sulfoxide (DMSO), ammonium hydroxide solution (25%), and N, N-dimethylformamide (DMF) were purchased from Xilong reagent company (Guangdong, China). All other chemicals not mentioned here were of analytical reagent grade and were used as received. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ, Milli-Q, Iowa, USA) was used in all assays. All buffers were prepared with ultrapure water. The oligonucleotides were obtained from Sangon Biotechnology Inc. (Shanghai, China), and the oligonucleotide sequences were as follows:

T-rich DNA: 5′-alkyne-$(CH_2)_4$- GCTTCTGTTCTCTC–3′.

T-rich DNA contained rich thymine (T) bases, thus enabling double-stranded structure complexation with Hg^{2+} via the T-Hg^{2+}-T interaction.

2.2. Instrumentations

Thermogravimetric analysis (TGA), Dynamic light scattering (DLS) analysis, Transmission electron microscopy (TEM), N_2 adsorption-desorption and UV–visible (UV–vis) spectrum were employed to characterize the synthesized materials. TEM images were obtained on a JEOL 3010 transmission electron microscope and an accelerating voltage of 100 kV (JEOL Ltd., Japan). UV–vis spectra were collected using a UV-1601 spectrophotometer (Shimadzu, Japan). DLS analysis was performed on a Zetasizer (Nano-Z, Malvern, UK). N_2 adsorption/desorption isotherm was obtained at 77 K on a Micromeritics ASAP 2010 sorptometer by static adsorption procedures (Micromeritics, Norcross, GA, USA). The specific surface areas were calculated from the adsorption data in the low pressure range using the Brunauer-Emmett-Teller (BET) model. Pore size distribution was estimated from the adsorption branch of the isotherm by the Barrett-Joyner-Halenda (BJH) method.

The electrochemical measurements were performed on a CHI 660E electrochemical workstation (CH Instruments Inc., shanghai, China) at room temperature with a portable screen-printed electrode (DRP-220AT, Metrohm China Limited), which consisted of a gold working electrode (diameter 4 mm), a gold counter electrode, and an Ag reference electrode. All laboratory experiments were conducted at room temperature and measurements on SPEs were performed by placing a 50 μL drop of the corresponding solution to the working area. The potentials were with respect to Ag reference electrode. All the measurements were performed by independent experiments with repetition for at least five times.

2.3. The Synthesis of MSN-DNA

The MSN was first synthesized with the following procedure. In a typical synthesis, 0.50 g of CTAB was dissolved in 240 mL of deionized water. 1.75 mL NaOH (2.0 M) was then introduced into the CTAB solution and the temperature of the mixture was adjusted to 95 °C. Under continuous stirring, 2.5 mL of TEOS (0.25 mol) was added dropwise to the surfactant mixture. The mixture was allowed stirring for 3 h to give a white precipitate. Then the solid product was centrifuged, washed with deionized water and ethanol, and dried at 60 °C overnight. The obtained white powder was finally calcined at 550 °C using oxidant atmosphere for 5 h to remove the template phase to yield the as-synthesized MSN. Subsequently, 0.70 g of MSN was then treated for 20 h in 60.0 mL of anhydrous toluene with 0.70 mL of ClTMS to yield the MSN-Cl material. To remove the surfactant template, 0.50 g of the as-prepared MSN-Cl was refluxed for 6 h in amethanolic solution of 0.50 mL HCl (37.2%) in 50 mL methanol. The resulting material was filtered and extensively washed with nanopure water and methanol. The surfactant-free MSN-Cl material was placed under high vacuum with heating at 60 °C to remove the remaining solvent from the mesopores.

0.10 g of MSN-Cl was added to a saturated solution of sodium azide in N, N-dimethylformamide (DMF) solution (20 mL) and stirred at 90 °C for 12 h. The resulting mixture was then separated