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A dual-mode nanosensor based on carbon quantum dots and gold nanoparticles for discriminative detection of glutathione in human plasma

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ABSTRACT

Glutathione (GSH) plays key roles in biological systems and serves many cellular functions. Since biothiols all incorporate thiol, carboxylic and amino groups, discriminative detection of GSH over cysteine (Cys) and homocysteine (Hcy) is still challenging. We herein report a dual-mode nanosensor with both colorimetric and fluorometric readout based on carbon quantum dots and gold nanoparticles for discriminative detection of GSH over Cys/Hcy. The proposed sensing system consists of AuNPs and fluorescent carbon quantum dots (CQDs), where CQDs function as fluorometric reporter, and AuNPs serve a dual function as colorimetric reporter and fluorescence quencher. The mechanism of the nanosensor is based on two distance-dependent phenomenons, color change of AuNPs and FRET. Through controlling the surface properties of as-prepared nanoparticles, the addition of CQDs into AuNPs colloid solution might induce the aggregation of AuNPs and CQDs, leading to AuNPs color changing from red to blue and CQDs fluorescence quench. However, the presence of GSH can protect AuNPs from being aggregated and enlarge the inter-particle distance, which subsequently produces color change and fluorescent signal recovery. The nanosensor described in this report reflects on its simplicity and flexibility, where no further surface functionalization is required for the as-prepared nanoparticles, leading to less laborious and more cost-effective synthesis. The proposed dual-mode nanosensor demonstrated highly selectivity toward GSH, and allows the detection of GSH as low as 50 nM. More importantly, the nanosensor could not only function in aqueous solution for GSH detection with high sensitivity but also exhibit sensitive responses toward GSH in complicated biological environments, demonstrating its potential in bioanalysis and biodection, which might be significant in disease diagnosis in the future.

1. Introduction

Glutathione (GSH), a thiol-containing tripeptide (γ-Glu–Cys–Gly), plays key roles in biological systems and serves many cellular functions such as intracellular signal transduction, maintenance of intracellular redox activities, gene regulation, and xenobiotic metabolism (Dalton et al., 1999; Kanzok et al., 2000; Krauth-Siegel et al., 2005; Meister, 1988; Schirmer et al., 1995). The concentration of GSH ranges from millimolar inside the cells of all tissues and organs to micromolar in plasma and other biological fluids such as saliva, cerebrospinal fluid, and urine (Pastore et al., 2003). Abnormal levels of cellular GSH have been linked to a number of diseases, such as leukocyte loss, psoriasis, liver damage, cancer, aging, heart problems, and other ailments (Lu, 2009; Townsend et al., 2003). A decrease in GSH is a risk factor for chronic diseases that may be used to monitor the severity and progress of the diseases (Lang et al., 2000). Because of its important biological roles, research interests to develop efficient methods for the monitoring and determination of GSH under physiological conditions are growing unabated (Chen et al., 2010a; Monostori et al., 2009). In general, the conventional methods for determining GSH in biological samples include spectrofluorimetry (Mei et al., 2012; Niu et al., 2012; Yi et al., 2009), spectrophotometry (Chen et al., 2010b), high performance liquid chromatography (HPLC) (Cataldi and Nardiello, 2005; Patterson et al., 2008), capillary zone electrophoresis (Kubalczyk and Bald, 2009), nuclear magnetic resonance (Rabenstein et al., 1985) and electroanalysis (Calvo-Marzal et al., 2004; Jin and Wang, 1997; Ricci et al.,

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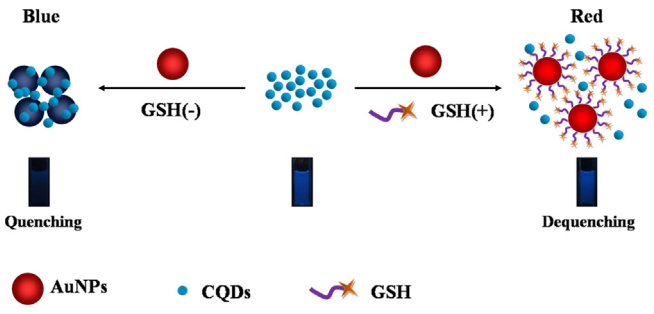
2006). However, discriminative detection of GSH over cysteine (Cys) and homocysteine (Hcy) is still challenging, since biothiols all incorporate thiol, carboxylic and amino groups. Therefore, it is of continuing interest to develop novel and facile GSH sensing strategies with improved selectivity and sensitivity to address biological, clinical, and medicinal requirements.

Advances in nanoscience and nanotechnology have resulted in various novel nanosensors which have been conditioned for biothiol detection both *in vitro* and *in vivo*. Among the various detection techniques, optical detections have proven most convenient methods of all. Colorimetric detection methods basing on the distance-dependent optical properties of gold nanoparticles (AuNPs) have been developed for the detection of GSH (Li et al., 2011; Lim et al., 2008; Zhang et al., 2002; Sudeep et al., 2005; Uehara et al., 2010; Xu et al., 2012). These colorimetric assays are based on color variation of AuNPs associated with the turnover process from dispersion to aggregation state induced by specific intermolecular zwitterionic interactions between GSH and surface modifiers attached onto AuNPs. Fluorescence resonance energy transfer (FRET) technique, which is a distance-dependent energy transfer technique, has also been utilized for GSH determination, where luminescent nanoparticles such as semiconductor quantum dots (QDs) (Banerjee et al., 2009; Gui et al., 2012; Liu et al., 2010), upconversion nanoparticles (UCNPs) (Deng et al., 2011), silver nanoclusters (AgNCs) (Zhang et al., 2013) and gold nanoclusters (AuNCs) (Park et al., 2013) are used as energy donors. To the best of our knowledge, all previously reported nanosensor systems have been focused on single readout, either colorimetric or fluorometric.

In present study, a dual-mode nanosensor with both colorimetric and fluorometric ('Off–On' fluorescence change) readout for discriminative detection of GSH over Cys/Hcy is thus developed (Scheme 1). The proposed sensing system consists of AuNPs and fluorescent carbon quantum dots (CQDs), where CQDs function as fluorometric reporter, and AuNPs serve a dual function as colorimetric reporter and fluorescence quencher. Due to their superior advantages in their favorable optical properties, excellent biocompatibility and green synthesis, CQDs recently attract considerable attention for biomedical applications, especially in which the size, cost and biocompatibility of the label are critical (Baker and Baker, 2010; Jaiswal et al., 2012; Lin et al., 2012; Wang et al., 2010, 2012; Zhai et al., 2012). AuNPs possess distance-dependent optical properties, and are known to have a superior quenching efficiency in a broad range of wavelengths (Kim et al., 2008; Shi et al., 2013a, 2013b; Xue et al., 2012; Zeng et al., 2012). We reasoned that the addition of CQDs into AuNPs colloid solution might induce the aggregation of AuNPs and CQDs through controlling their surface properties, leading to AuNPs color changing from red to blue and CQDs fluorescence quench. However, GSH demonstrates stronger affinity towards AuNPs due to the multidentate anchor together with the specific steric structure existing in GSH, thus rendering GSH to encapsulate AuNPs in priority. Therefore, GSH can protect nanoparticles from being aggregated and enlarge the inter-particle distance, which subsequently produces color change and fluorescent signal recovery (Scheme 1). Previous studies revealed that biothiols can induce the aggregation of AuNPs in the order of HCys ≫ Cys > GSH (Li et al., 2011; Zhang et al., 2002). Due to the tremendous difference in coordination capability and steric hindrance effects between the GSH and the competitive biothiols, discriminative detection GSH by the reported nanosensor is reasonably expected. The proposed dual-mode nanosensor demonstrated highly selectivity toward GSH over Cys/Hcy and allows the detection of GSH as low as 50 nM. The simultaneously colorimetric and fluorescent detections of GSH provide advantages of the high sensitivity of fluorescence with the convenience of a visual assay. The nanosensor described in this report reflects on its simplicity and flexibility, where no further surface functionalization is required for the as-prepared nanoparticles, leading to less laborious and more cost-effective synthesis. More importantly, the nanosensor could not only function in aqueous solution for GSH detection with high sensitivity but also exhibit sensitive responses toward GSH in complicated biological environments such as human plasma, demonstrating its potential in bioanalysis and biodection, which might be significant in disease diagnosis in the future.



Scheme 1. Schematic principle of GSH detection by using the dual-mode nanosensor with both colorimetric and fluorometric readout.

2. Experimental

2.1. Apparatus

Morphology of the synthesized nanoparticles was examined by transmission electron microscope (TEM, JEOL JEM-1400). DLS measurements were performed at 25 °C using a Malvern Zetasizer NanoZS90 instrument, fitted with a 532 nm laser at a fixed scattering angle of 90°. UV–vis and fluorescent spectra were obtained on a Beckman DU730 UV–vis spectrometer and a PTI Quanta-Master QM4CW spectrofluorometer, respectively.

2.2. Synthesis of luminescent CQDs and AuNPs

CQDs were prepared by microwave treatment with citric acid as the carbon source, and 2,2′-(Ethylene-dioxy) bis(ethylamine) as the co-reactant (Zhai et al., 2012). First, 2 g citric acid was dissolved in 10 mL distilled water and then mixed with 800 μL 2,2′-(Ethylenedioxy) bis(ethylamine) to form a transparent solution under ultrasonic vibration. The solution was then heated in a domestic 720 W microwave oven for 2.5 min, during which the solution changed from being a colorless liquid to a brown clustered solid, indicating the formation of CQDs. When cooled down to room temperature, the obtained solid was dissolved in 20 mL distilled water followed by centrifugation at 13,000*g* for 30 min. Then, the supernatant was dialyzed against pure water through a dialysis membrane (MWCO of 1000) for 2 days. Finally, the aqueous solution containing CQDs was centrifuged at 13,000*g* for 30 min, and the obtained supernatant was vacuum-dried to collect CQDs.

Aqueous dispersions of citrate-stabilized AuNPs were prepared using reagents supplied by Aldrich (Storhoff et al., 1998). Briefly, 120 mL of 1 mM $HAuCl_4$ solution was heated to boiling under vigorous stirring, followed by quickly adding 12 mL of 38.8 mM trisodium citrate solution. The reaction was allowed to continue for another 20 min. Then, the solution was cooled to room temperature and filtered by 0.45 μm cellulose acetate (CA) membranes. The as-prepared AuNPs were stored at 4 °C in the refrigerator for further use.