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Onsite naked eye determination of cysteine and homocysteine using quencher displacement-induced fluorescence recovery of the dual-emission hybrid probes with desired intensity ratio

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ABSTRACT

Simple, inexpensive, portable sensing strategies for those clinically relevant molecules have attained a significant positive impact on the health care system. Herein, we have prepared a dual-emission ratiometric fluorescence probe with desired intensity ratio and demonstrated its efficiency for onsite naked eye determination of cysteine (Cys) and homocysteine (Hcy). The hybrid probe has been designed by hybridizing two differently sized CdTe quantum dots (QDs), in which the red-emitting CdTe QDs (rQDs) entrapped in the silica sphere acting as the reference signal, and the green-emitting CdTe QDs (gQDs) covalently attached on the silica surface serving as the response signal. When 1,10-phenanthroline with strong coordination ability to Cd atoms in gQDs was introduced, the fluorescence of the gQDs was effectively quenched, while the fluorescence of the rQDs stayed constant. Upon exposure to different contents of Cys or Hcy, the fluorescence of gQDs can be recovered gradually due to the displacement of the quencher. Based on the background signal of rQDs, the variations of the sensing system display continuous fluorescence color changes from red to green, which can be easily observed by the naked eye. The assay requires ~20 min and has a detection limit of 2.5 and 1.7 μM for Cys and Hcy, respectively. Furthermore, we demonstrate that this sensing scheme can be fully integrated in a filter paper-based assay, thus enabling a potential point-of-care application featuring easy operation, low power consumption, and low fabrication costs.

1. Introduction

Ratiometric fluorescent sensors, which allow the measurement of changes in the ratio of the fluorescence intensities at two well-resolved wavelengths, have received intense attention in recent years (Wu et al., 2012; Chen et al., 2013; Tyrakowski and Snee, 2014). In comparison with single-wavelength measurements, ratiometric fluorescence technique can provide built-in correction for environmental effects (Zhuang et al., 2014; Diaz et al., 2012; Lv et al., 2011), eliminates the fluctuation of excitation light intensity and the probe concentration (Dubertret et al., 2002; Deniz et al., 2001; Li et al., 2011), and thus possesses advantages in terms of improved sensitivity and accuracy. Till now, most reported ratiometric sensors using fluorescent organic dyes have been constructed based on the internal charge transfer (Das et al., 2012), native chemical ligation reaction (Lv et al., 2014), and fluorescence resonance energy transfer (FRET) (Long et al., 2011). However, there is difficult in choice of dyes, since many of them remain associated with some disadvantages including low fluorescence quantum yield, weak resistance to photo-bleaching, narrow excitation and broad emission bands, and especially the complexity of organic molecular synthesis and purification (Wu and Chiu, 2013; Zong et al., 2011). Therefore, there remains a great challenge to construct simple, low-cost, and high-efficient ratiometric fluorescence probes for practical use.

Fluorescent quantum dots (QDs) have been demonstrated to exhibit excellent optical properties, such as broad excitation spectra, high fluorescence quantum yield, and strong resistance to photo bleaching which are better suited as fluorescence probe than traditional organic dyes (Liu et al., 2014; Reiss et al., 2009; Wu et al., 2014). More importantly, size-dependent emission of QDs affords the convenience of incorporating differently sized QDs into a complex material to create a multicolor system, which is central to the ratiometric fluorescence detection (Gui et al., 2013; Yao et al., 2013). Generally, dual-emission is observed in core–satellite hybrid sphere containing two different species, in which one species is entrapped into the nanosphere serving as the

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internal standard, and the other is loaded on the surface of the nanosphere acting as the response signal. Following the "turn off" detection mode, several QDs-based dual-emission fluorescence probes with suitable intensity ratios have been demonstrated to be promising for environmental analysis, e.g., the visual detection of Cu^{2+} (Zhu et al., 2012; Wang et al., 2014) and Hg^{2+} (Sun et al., 2012), and the homeland security, e.g., the visual detection of trinitrotoluene (Zhang et al., 2011). In principle, such simple, inexpensive, portable sensing strategies are more suitable and affordable for point-of-care (POC) testing, especially in resource-limited settings such as developing countries and remote communities (Zhu et al., 2014; Yang et al., 2012; Mu et al., 2014). Displacement assay approach is the most popular way to detect analytes through "turn on" fluorescence signaling. It is reported that displacement directed chemosensing of analytes in fact improves the selectivity by many folds (Wang et al., 2012). By utilizing the dual-emission organic dyes, recent reports mainly focus on the displacement of quencher leading to the construction of efficient anion sensor (Kaur et al., 2013; Kumar et al., 2013; Singh et al., 2014). Due to the aforementioned disadvantages of the organic dyes, design and synthesis of the QDs hybrid probes and search for novel "turn on" detection principles, to perform the onsite visual detection of those clinically relevant molecules for POC diagnosis and disease screening, is of great significance.

Thiol-containing amino acids (aminothiols), such as cysteine (Cys) and homocysteine (Hcy) which are structurally similar and metabolically linked, serve vital functions in human tissues including protein synthesis, detoxification, and metabolism (Chen et al., 2010). However, abnormal levels of aminothiols are relative to many human diseases. For example, a deficiency of Cys associated with hematopoiesis decrease, muscle and fat loss, psoriasis, slow growth in children, liver damage, skin lesions, hair depigmentation, edema, and so forth (Shahrokhian, 2001). The elevated Hcy level in blood is known to be directly linked to several disorders including cardiovascular and Alzheimer's diseases, neural tube defects, and osteoporosis (Klee, 2000; Seshadri et al., 2002; Refsum et al., 2004). Accordingly, the sensitive determination of these aminothiols in plasma and urine samples is of considerable importance and significant interest due to its promising application for the early disease screening and diagnosis (Yin et al., 2013; Jung et al., 2013). Classical determination of these aminothiols is generally accomplished by the high-performance liquid chromatography (HPLC) (Nolin et al., 2007), capillary electrophoresis (CE) (Kubalczyk et al., 2014), mass spectrometry identification (MS) (Vellasco et al., 2002), and electrochemistry (CE) (Wei et al., 2011). Of various sensing protocols, the color change observed by the naked eye is considered to be the most inexpensive and convenient way to determine those clinically relevant molecules for diagnosis onsite. To date, design of such sensors for Cys or Hcy still represents a great challenge.

Herein, we demonstrate a new concept for the onsite naked eye deterimination of Cys and Hcy. A dual-emission fluorescent hybrid sphere by using two differently sized CdTe QDs was prepared with desired intensity; the red-emitting CdTe QDs (rQDs) were entrapped in the silica while the green-emitting CdTe QDs (gQDs) were attached on the silica surface. Upon the introduction of 1,10-phenanthroline (Phen) with strong coordination ability to Cd atoms in gQDs, the green fluorescence of the attached gQDs was effectively quenched, while the red fluorescence of the entrapped rQDs stayed constant. In the presence of different contents of Cys or Hcy, however, the fluorescence of gQDs is recovered gradually because of the strong binding preference of gQDs for analytes, thus, exhibited continuous fluorescence color changes induced by Cys and Hcy without the need of elaborate equipment.

2. Experimental

2.1. Reagents

Tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTS), 3-mercaptopropionic acid (MPA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and amino acids were purchased from Sigma-Aldrich. Tellurium powder (99%), $CdCl_2 \cdot 2.5H_2O$, ammonium hydroxide ($NH_3 \cdot H_2O$, 25%), $NaBH_4$ (99%), tris(hydroxymethyl)methyl aminomethane, ethanol, and Phen were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Two differently sized CdTe QDs (gQDs and rQDs) capped with MPA containing carboxylic group were obtained according to a previous report (Zhang et al., 2010). All the reagents were used as purchased without further purification. Double-distilled water was used throughout the study.

2.2. Apparatus

The transmission electron microscopy (TEM) images were taken with a JEOL 2100 TEM (JEOL, Japan) at 200 kV. UV–vis absorption spectra were measured by UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). All the photographs were taken using a Canon digital camera (IXUS 230 HS, China) under the illumination of a 365 nm UV lamp.

2.3. Preparation of amino-functionalized rQDs@SiO_2 spheres

The rQDs were loaded inside silica spheres by a modified Stöber method (Zhang et al., 2011). Briefly, 0.5 mL of rQDs solution and 40 mL of ethanol were mixed in a 100 mL flask and stirred for 10 min, 100 μL of APTS was then added to the above solution and stirred for 6 h. Then, 0.5 mL of TEOS and 0.5 mL of $NH_3 \cdot H_2O$ was introduced, and the mixture was stirred for another 12 h. Finally, 100 μL of APTS was added under vigorous stirring and reacted for 12 h in order to modify the silica surface with amino groups. The as-prepared amino-functionalized rQDs@SiO_2 was subjected to several cycles of precipitation by centrifugation and washed with ethanol and water to remove the unreacted chemicals.

2.4. Preparation of dual-emission rQDs@SiO_2@gQDs Hybrid spheres

In a typical synthesis, 6 mL of EDC/NHS (2 mg mL^{-1} for each), 12 mL of H_2O and 2.4 mL of gQDs solution were added in a 50 mL flask. After stirring for 15 min at room temperature, the as-obtained amino-functionalized rQDs@SiO_2 spheres were added into the mixture which was stirred vigorously for 4 h in the dark. Finally, the rQDs@SiO_2@gQDs hybrid spheres with dual-emission fluorescence were collected by centrifugation and washing and re-dispersed in 10 mL of water for later use.

2.5. Procedures of fluorescence spectrometric and visual detection

50 μL of the as-prepared rQDs@SiO_2@gQDs hybrid probes, 90 μL of 1 mM Phen, a certain volume of Cys or Hcy were added into a 0.5 mL tube, the mixture was diluted with Tris–HCl buffer (pH 7.4, 50 mM) to a final volume of 200 μL and reacted for 20 min at room temperature to obtain solution A. To perform the visual fluorescence detections, solution A was transferred to a series of miniwells and the photographs were obtained using a digital camera under UV illumination (λ_{ex}=365 nm) in a dark box. To perform the spectrometric analysis, solution A was diluted to 3 mL with Tris–HCl buffer (pH 7.4, 50 mM) and the fluorescence spectra