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A new label free colorimetric chemosensor for detection of mercury ion with tunable dynamic range using carbon nanodots as enzyme mimics

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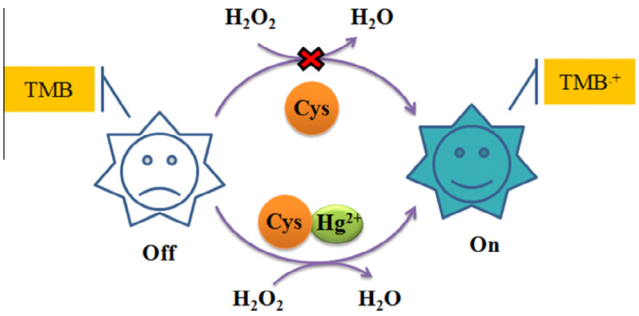
HIGHLIGHTS

- Preparation of a novel colorimetric chemosensor for sensitive and selective detection of Hg^{2+}.
- Use of carbon nanodots as a new generation of nanozymes, and cysteine as an antiradical biomolecule.
- H_2O_2 mediated oxidation of tetramethylbenzidine (TMB) into a blue colored cation radical.
- When cysteine is free, sensor is "on"; when it is complexed with Hg^{2+}, sensor is "off".
- Linear dynamic range of 0.00–0.31 μM with a limit of detection of 23 nM for Hg^{2+}.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a new label free colorimetric sensor was designed for highly selective detection of mercury ion. Carbon nanodots as a new generation of nanozymes, with their peroxidase mimetic activity could catalyze the H_2O_2 mediated oxidation of tetramethylbenzidine (TMB) into a blue colored cation radical. Cysteine (Cys) as a powerful antiradical biomolecule, could successfully impede the cation radical generation and prevent its emergence as the blue colored reaction product. Taking into consideration that mercury ions have strong affinity to thiol functionality, they entrapped the Cys molecules and provoke the typical oxidation reaction to occur in the presence of H_2O_2 and the catalyst. In fact, manipulation of the extent of catalytic oxidation of substrate by means of mercury/thiol mixture demonstrated to be a simple approach for construction of an on–off sensor. A major advantage of the present on–off system is the tunable dynamic range which was achieved simply by adjusting the Cys concentration. On the basis of this fact, two calibration ranges of 0.00–0.31 μM and 0.00–0.86 μM were obtained for Cys concentration of 5 and 10 μM, respectively. For Cys concentration adjusted to 5 μM, an excellent limit of detection of 23 nM was achieved. Moreover, this sensor is negligibly responsive to other metal ions, such as Ag^{+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Pb^{2+}.

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1. Introduction

Mercury ion is recognized as one of the most toxic transition metal ion in the world by the United States Environment Protection Agency (USEPA). There are various laws and regulations imposed by EPA to control the mercury emission to air, water, or from wastes and products. Once mercury in the air is washed into water, some microorganisms can convert it into highly toxic methyl mercury. Fish consumption is the main source of methyl mercury contamination for people worldwide [1]. Exposure to this pollutant, even at low concentrations, impact severe medical effects on human health [2–4]. Therefore, many research endeavors have been focused on probing mercury ion with high selectivity and sensitivity.

Many successful strategies based on optical sensing using new nanomaterials have been devoted for mercury detection in the past few years [5–13]. Functionalization of noble metal nanoclusters have been achieved mostly with thiols and DNA as the recognition elements [5–7]. Binding of potentially toxic metal ions to these functionalized nanomaterials induce significant changes in absorption or fluorescence spectra, mainly due to aggregation or formation of metallophilic complexes [14,15]. Luminescent semiconductor quantum dots [13,16] and carbon nanodots (CDs) [17,18] functionalized with chemical capping layers have also been implemented as nanosensors for mercury ion detection. The main strategy in these nanomaterials is the fluorescence quenching induced by mercury ions [19]. Development of metal ion sensors by exploiting the enzymatic property of nanomaterials is also a rapidly expanding field of study [20–23]. Li et al. [21] designed an oligonucleotide modified Au nanoparticles which catalyze the H_2O_2 mediated oxidation of nonfluorescent Amplex Ultra Red to the highly fluorescent oxidized products in the presence of Pb^{2+} through the formation of Au–Pb alloys and oligonucleotide–Pb^{2+} complexes. A recent study by Huang et al. [22] showed that the deposition of mercury ion on the surface of bimetallic platinum/gold nanoparticles can switch the peroxidase like activity of the nanostructure into the catalase like one. Such nanostructures, briefly called nanozymes [24], exhibit an enzyme mimetic activity. Nanozymes have been extensively explored in a wide range of applications including biosensing [25–27], immunoassays [28–30], cancer diagnostics [31,32], neuroprotection [33,34], stem cell growth [35], and pollutant removal [36–38].

Carbon based nanomaterials such as carbon nanotubes [39], graphene oxide [40] and CDs [41] are suitable candidates for nanozymes. CDs have been demonstrated to act as peroxidase mimetic materials and, thus, are introduced as a new generation of nanozymes in recent reports [41]. In this work, we utilized the intrinsic catalytic property of CDs to catalyze the oxidation reaction of tetramethylbenzidine (TMB) as the most common peroxidase substrate. By oxidation of this substrate, a blue colored cation radical was generated. Glutathione could successfully cause a decrease in cation radical generation by means of its strong radical restoration ability. Inspired by this phenomenon, we designed a new label free sensor for highly selective and sensitive detection of mercury ion due to the strong affinity of thiolated compounds to mercury ion. The turn-on sensing mode in this work provides the feasibility to reduce background signals and enhance the system sensitivity.

2. Experimental

2.1. Reagents

Reagent grade TMB, %30 H_2O_2 and mercury(II) chloride were purchased from Merck. Cysteine (Cys) was obtained from Riedel-deHaën. Solutions of H_2O_2 and Cys were prepared daily prior to use. The buffer solution was prepared from 0.1 M acetic acid/sodium acetate (pH 3.5). Stock solution of TMB was prepared in DMSO and stored at 4 °C.

CDs were prepared as reported previously [42]. Briefly, a crucible filled with 0.5 g $Na_2EDTA.2H_2O$ was heated in a tube furnace using an argon flow at 400 °C for 2 h. The resulting black powder was dissolved in acetone (20 mL) and then centrifuged at high speed (14,000 rpm) for 15 min. Evaporation of the upper yellow solution resulted in the CDs powder. Finally, the powder was redissolved in 1 mL of deionized water for further studies.

2.2. Apparatus

A tube furnace (Azar Furnace-Iran) equipped with gas flow lines was utilized for the salt pyrolysis. Spectrophotometric measurements were performed on a Shimadzu UV–Vis spectrophotometer equipped with LO-Temptrol 154 thermostat.

2.3. Preparation of substrate working solution

Substrate working solution was prepared freshly as follows. A solution containing TMB (50 μM) and Cys (5 μM) in acetate buffer (pH 3.5) was prepared and kept at 4 °C during the experiment. To a cuvette containing the substrate working solution and an appropriate amount of CDs, different amounts of mercury ion was added and the solution was thermostated at 30 °C. After 5 min incubation, H_2O_2 was added so that its final concentration was 1 mM. The solution was mixed and the absorbance change at 653 nm vs. time was measured.

2.4. Analysis of real sample

A river water sample was analyzed as the real sample without any filtration or any other treatment. 5 mL of the water sample was diluted by acetate buffer (pH 3.5, 0.2 M) to 10 mL. To this solution were added TMB and Cys to the final concentrations of 50 μM and 5 μM, respectively, and the mixture was kept at 4 °C. To 300 μL aliquots of this solution, different amounts of Hg^{2+} standard solution were spiked. The resulting mixture was finally analyzed with the proposed method and the percent recovery values were obtained.

3. Results and discussion

Water soluble CDs were used as the catalyst in this work. CDs were synthesized with the simple procedure reported previously [42]. The near spherical CDs with the average size of below 10 nm were confirmed by the TEM micrographs (Fig. 1A). Typical absorption and photoluminescence spectra of CDs in aqueous solution are presented in Fig. 1B. As seen, CDs show obvious optical absorption in the UV region, with a tail extending to the visible range. When excited in 330 nm, CDs exhibited strong photoluminescence centered at about 410 nm with a full width at half maximum (FWHM) of about 80 nm.

The H_2O_2 mediated catalytic oxidation of TMB was used to examine CDs peroxidase mimetic activity through the convenient colorimetric reaction, which found to facilitate its detection even by the naked eye. There are two different oxidation products for TMB. A one-electron oxidation in the first step yields free cation radicals which absorb light at 370 and 653 nm. In the second step, a further-one electron oxidation of the cation radical leads to the formation of diimine derivative which absorbs light at 450 nm. Before the point where 50% of the initial TMB molecules are converted to the cation radical, the color of the reaction mixture is green and after that it changes to yellow. According to what is