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Avidin–biotin capped mesoporous silica nanoparticles as an ion-responsive release system to determine lead(II)

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ABSTRACT

We have developed DNAzyme-functionalized silica nanoparticles for the rapid, sensitive, and selective detection of lead ion (Pb^{2+}). The specific binding between avidin and biotinylated DNAzymes was used to cap the pore of dye-trapped silica nanoparticles. In the presence of Pb^{2+}, DNAzymes were catalytically cleaved to uncap the pore, releasing the dye cargo with detectable enhancements of fluorescence signal. This method enables rapid (15 min) and sensitive (limit of detection = 8.0 nM) detection. Moreover, the Pb^{2+}-responsive behavior shows high selectivity with other metal ions. The superior properties of the as-designed DNAzyme-functionalized silica nanoparticles can be attributed to the large loading capacity and highly ordered pore structure of mesoporous silica nanoparticles as well as the catalytical cleaving of DNAzymes with Pb^{2+}. The recoveries obtained by standard Pb(II) addition to real samples—tap water, commercial mineral water, and lake water—were all from 98 to 101%. Our design serves as a new prototype for metal–ion sensing systems, and it also has promising potential for detection of various targets in stimulus–release systems.

Lead is a common environmental contaminant. As a highly toxic heavy metal, lead is known to cause a diverse range of adverse health effects [1,2]. Because lead is not biodegradable, it accumulates in the environment and produces toxic effects in plants and animals, even at low concentrations [3–5]. Common symptoms of lead poisoning include behavioral problems, mental retardation, and hearing damage [6]. Although the accumulation of lead can be attributed to several sources, the contamination of water by water-soluble Pb(II) is the most common. The maximal contamination level of lead in drinking water defined by the U.S. Environmental Protection Agency is 72 nM. Therefore, the development of new lead ion (Pb^{2+})[1] detection methods that are sensitive, rapid, low-cost, and label-free systems has become an urgent need.

Traditional methods for detection of Pb(II) usually require complicated sample preparation processes and expensive instrumentation such as atomic absorption spectroscopy [7,8], inductively coupled plasma atomic emission spectrometry [9], and inductively coupled plasma mass spectrometry [10,11]. These instruments are available only in centralized laboratories, making sensors an attractive alternative. Different sensor concepts for analyzing Pb(II) have been a growing area during recent years, with the design of sensors using fluorescence [12–14], electrochemistry [15,16], colorimetric [17,18], nanoparticle [19], optical [20,21], and other detection techniques [22,23]. Among different classes of metal sensors developed, the DNAzyme nanotechnology-based sensor has witnessed an explosion due to the unique properties of nanomaterials and functional nucleic acids.

With this in mind, the direct, rapid, and sensitive detection of lead(II) based on a mesoporous silica nanoparticle (MSN) Pb^{2+}-responsive dye release system has been reported. The unique structure of MSNs is used as the dye carrier with biotinylated DNAzyme binding avidin as the Pb^{2+}-responsive cap. Their large load capacity, biocompatibility, high thermal stability, homogeneous porosity, inertness, adjustable pore size, and easy functionalization of the external and internal surfaces make MSNs very suitable as carrier vehicles [24–27]. In addition, the epoxy group on the surface provides a means of binding a biological linker to a large molecule that is able to cap the pores. By a specific stimulus, linkers

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[1] *Abbreviations used:* Pb^{2+}, lead ion; MSN, mesoporous silica nanoparticle; TEOS, tetraethylorthosilicate; CTAB, *n*-cetyltrimethylammonium bromide; GPOS, 3-glycidyloxypropyl trimethoxysilane; NaOH, sodium hydroxide; TEM, transmission electron microscopy; XRD, X-ray diffraction; PBS, phosphate-buffered saline; BET, Brunauer–Emmett–Teller; BJH, Barrett–Joyner–Halenda; RSD, relative standard deviation.

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can be cleaved to uncap the pore and release the cargo [28–30]. To date, many MSN-based controlled release systems have been constructed by various materials, including DNA [31], proteins [32,33], nanoparticles [34,35], and polymers [36–38] as capping agents. Triggered by various stimuli such as magnetic fields [36], pH [39,40], temperature [41,42], enzymes [38,43], target molecules [34,44], and competitive binding [45], the cargo can be released from the MSN system. Despite these burgeoning achievements, there has been very little application of a stimulus–release MSN system for the sensitive detection of metal ion.

In this study, we designed a facile and effective Pb^{2+}-responsive controlled release system using DNAzyme-functionalized MSNs. In this system, catalytic strands of Pb^{2+}-specific DNAzymes were covalently conjugated onto the surface of dye-trapped MSNs and combined with avidin to cap the pore. In the presence of target Pb^{2+} ions, catalytic cleavage of the DNAzymes resulted in opening of the molecular gates and release of cargo from the pores. Thus, Pb^{2+} acted as the stimulus to release the dye-trapped MSNs, which provided the detection signal in this stimulus–release process. The results show that the new design can achieve rapid and sensitive detection of lead(II) with no significant response to other environmentally important metal ions.

Materials and methods

Reagents and apparatus

Tetraethylorthosilicate (TEOS) and *n*-cetyltrimethylammonium bromide (CTAB) were purchased from Bodi Chemical (Tianjin, China). 3-Glycidyloxypropyl trimethoxysilane (GPOS) was purchased from J&K Chemical (Beijing, China). Sodium hydroxide (NaOH), lead(II) nitrate [$Pb(NO_3)_2$], copper nitrate [$Cu(NO_3)_2$], manganese chloride ($MnCl_2$), nickel chloride ($NiCl_2$), calcium chloride ($CaCl_2$), barium chloride ($BaCl_2$), chromic chloride ($CdCl_2$), mercury(II) chloride ($HgCl_2$), zinc acetate ($ZnAc_2$), cobalt(II) acetate ($CoAc_2$), anhydrous toluene, methanol, and fluorescein were purchased from Ruijinte Chemical (Tianjin, China). Analytical reagent-grade chemicals and deionized, doubly distilled water (18.2 MΩ cm) were used throughout. The oligonucleotides were synthesized by Sangon Biotechnology (Shanghai, China). The sequences are as follows: substrate strand for Pb^{2+}-dependent DNAzyme (17DS), 5′-NH_2-ACTCACTAT<u>rA</u>GGAAGAGATG-3′ (the cleavage site of the substrate is shown by the italic font and underlining); catalytic strand for Pb^{2+}-dependent DNAzyme (17E): 5′-CA TCTCTTCTCCGAGCCGGTCGAAATAGTGAGTA-biotin-3′. Avidin was purchased from Sangon Biotechnology (Shanghai, China).

Transmission electron microscopy (TEM), zeta potential, X-ray diffraction (XRD) microscopy, and N_2 adsorption–desorption were employed to characterize the synthesized materials. TEM images were taken with a JEOL JEM-2100 instrument (Hitachi, Japan). Zeta potential and dynamic light scattering (DLS) were performed on a Malvern Zetasizer Nano instrument equipped with a 4-mW He–Ne laser (633 nm) and an avalanche photodiode detector. X-ray measurements were performed on a D/max2500PC diffractometer (Rigaku) using Cu–Kα radiation. Fluorescence spectra were measured using a Hitachi F-4600 fluorescence spectrometer controlled by FL Solution software.

Synthesis of MSNs

MSNs were synthesized according to the literature [46]. Briefly, 1.0 g of CTAB was dissolved in 480 ml of deionized water, and then 3.5 ml of NaOH (2.0 M) was slowly added to the CTAB solution, with stirring, over a 20-min period at 80 °C. TEOS (5.0 ml) was added dropwise into the mixture solution and vigorously stirred for 2 h until white precipitates were obtained. Following that, the prepared product was separated by centrifugation, washed with deionized water and methanol, and dried in air. To remove the CTAB, the MSNs were refluxed for 10 h in the solution composed of HCl (37%, 1.5 ml) and methanol (75 ml) and then washed with distilled water and methanol. The resulting nanoparticles were dried in a high vacuum container at 60 °C overnight to remove the remaining solvent from the pores.

Preparation of epoxy-functionalized MSNs and dye loading

MSNs (30 mg) were initially dried at 100 °C for 1 h and then reacted with 4 ml of GPOS (5%, v/v) in anhydrous toluene at room temperature under continuous stirring overnight. Then, GPOS-functionalized MSNs were separated by centrifugation and washed thoroughly with toluene and ethanol to remove the redundant GPOS. The MSN–GPOS was then dried under a nitrogen atmosphere at 100 °C for 1 h to acquire active epoxy groups on the surface. Next, 30 mg of epoxy-functionalized MSNs was suspended in 10 ml of fluorescein/anhydrous ethanol saturated solution to load the dye into the pores of the epoxy-functionalized MSN scaffolding. The mixture was shaken for 24 h to achieve maximum dye loading. Afterward, the dye-loaded MSNs were washed with ethanol three times and dried in an oven at 40 °C. The dye-loaded MSNs were kept dry until the next step.

Conjugation of catalytic strands with MSNs and capping

Substrate strands for Pb^{2+}-dependent DNAzymes were conjugated with the MSNs according to the previous report [32]. The epoxy-functionalized MSNs with the substrate strands were carried out using the amine–epoxy interaction. The epoxy-functionalized MSNs were dispersed into pH 7.4 phosphate-buffered saline (PBS) with a final concentration of 10 mg ml^{-1}. Substrate strands for Pb^{2+}-dependent DNAzymes (2.5 μl, 100 μM) were initially added to react with 250 ml of 10 mg ml^{-1} dye-loaded MSN suspension, and then the resulting mixture was shaken at 4 °C for 24 h. During this process, aminated substrate strands were covalently conjugated onto the surface of MSNs. The excess substrate strands were removed by centrifugation. Following that, 5 μl of catalytic strands (100 μM) was added into the mixture. The mixture was gently shaken for 2 h at 37 °C to make the substrate strands of DNAzymes hybridize with the catalytic strands. Then, 50 μl of avidin (6×10^{-7} M) was added into the mixture and incubated at 37 °C for 2 h to form the avidin–biotin capped MSNs. The obtained nanoparticles were then centrifuged and washed thoroughly with PBS buffer to remove the redundant avidin molecules.

Dye release experiment

To monitor the dye release of this system in the presence of Pb^{2+}, 0.5 mg of as-prepared MSN suspension conjugated with DNAzymes was suspended in lead(II) nitrate/buffer solution of different concentrations. The resulting mixture was incubated for 30 min at room temperature. During this process, the immobilized DNAzymes on the MSNs were catalytically cleaved by target Pb^{2+}, thereby resulting in the release of the entrapped fluorescein molecules from the pores. Subsequently, 0.20 ml of supernatant was taken from the suspension by centrifugation and merged into the buffer solution (~600 ml) for the fluorescence measurements. The release of dye from the pore voids to the buffer solution was determined by fluorescence emission spectroscopy (excited at 470 nm, emitted at 518 nm). Excitation and emission slits were all set for a 10.0-nm bandpass. In addition, the response of the DNAzyme–MSN to other metal ions was also investigated following the same procedures.