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Stimulus-response click chemistry based aptamer-functionalized mesoporous silica nanoparticles for fluorescence detection of thrombin

Zhonghui Chen, Mi Sun, Fang Luo, Kefeng Xu, Zhenyu Lin, Lan Zhang*

Ministry of Education Key Laboratory for Analytical Science of Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China

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ABSTRACT

In most aptamer based stimulus response mesoporous silica nanoparticles (MSN) systems, the aptamer is modified on the MSN via electrostatic interaction, however leakage might exist after a certain time in the system and hence the stability is not good. In this study, the pores of MSN were capped by aptamer through click chemistry reaction for the first time and the system was then employed to develop a fluorescence biosensor. Specifically, the aptamer of the target (thrombin in this study) was hybridized with its complementary DNA (which was initially modified with alkyne at the terminal) to form a double strand DNA (dsDNA) firstly, and then this dsDNA was modified on N_3 modified MSN via Cu(I) catalyzed alkyne-azide cycloaddition reaction. The guest molecules (fluorescein) were blocked in the pores of the MSN with high efficiency and nearly no leakage was detected. Upon the introduction of thrombin, thrombin specifically recognized its aptamer, so aptamer released from the MSN; and the single strand DNA(ssDNA) left could not cap the pores of the MSN efficiently and hence caused the releasing of fluorescein into the solution. The enhanced fluorescence intensity of the system has a good linear relationship with the thrombin concentration in the range of 50–1000 ng mL^{-1} with a detection limit of 28.46 ng mL^{-1}. The proposed biosensor has been successfully applied to detect thrombin in serum samples with high selectivity. The same strategy can be applied to develop biosensors for different targets by changing the adopted aptamer.

1. Introduction

Mesoporous silica nanoparticle (MSN) has sparked increasing interest in biomedical applications owing to the gradually increased requirements from clinical patients for high-performance therapeutic nanoformulations [1]. Many studies have focused on the application of MSN in controlled release system [2–6]. Such system normally adopts nanoparticles, polymers, small organic molecules, supramolecular assemblies, and biomolecules as capping agents to control the pores of MSN [7–11]. The release of guest molecules can be triggered by physical or chemical stimuli, such as pH, temperature, light, antibodies, enzymes, and nucleotides [12–17].

Aptamers are single-stranded DNA or RNA oligonucleotides that can specifically recognize their targets with high affinity and specificity. Many investigations have designed stimulus response controlled release system using aptamer as capping agent. For example, polyvalent MSN/aptamer bioconjugates were fabricated to target breast cancer cells (MCF-7) [18]. Zhu et al. reported a stimulus response fluorescence biosensor for adenosine triphosphate (ATP) [7]. MSN support was firstly loaded with fluorescein, aptamer-modified Au nanoparticles were used to cap the pores of MSN; upon the addition of ATP, the high affinity and specificity between ATP and its aptamer eventually caused the release of fluorescein from the capped pores. Tang et al. presented a novel target-aptamer-responsive controlled release system for ATP detection [19]; when glucose was loaded in the MSN pore, a commercially personal glucose meter was used to detect ATP based on the glucose released form the MSN. Zhang et al. designed a sensitive method for Hg^{2+} detection using DNA-capped MSN [20]. Two single-stranded DNA were cross-link on the MSN surface, they hybridized with T-base-rich linker DNA to cap dye in the MSN pore; in the presence of Hg^{2+}, T-Hg-T was formed, causing the releasing of dye from MSN. Our group reported a chemiluminescence biosensor for cocaine determination based on the stimulus response system [21]; glucose was initially loaded into MSN support, the interaction between positively charged MSN and negatively charged aptamer led to the capping of pores; the release of glucose was triggered via the introduction of cocaine. However, in most of reported stimulus response aptamer functionalized MSN system, the aptamer was modified on the surface of MSN to block the pores via

* Corresponding author.
E-mail address: zlan@fzu.edu.cn (L. Zhang).

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electrostatic interaction (specifically, negatively aptamer interacted with positively charged MSN to cap the pore, however, the pH of buffer in controlled release system notably affected aptamer binding to MSN), the leakage might exist after a long time so the stability was not high enough. It is necessary to build some simple capping way with higher stability and efficiency.

Since the first report of click chemistry in 2001 [22], the past years have witnessed an exponential increase in study regarding click chemistry [23–25]. One of the most studied reactions is Cu(I) catalyzed alkyne-azide cycloaddition reaction (CuAAC), it is simple and normally performed in mild conditions like H_2O solvent, even in the presence of many other functional groups with high efficiency [26]. CuAAC reaction has been successfully employed in the process of functionalization and modification (such as protein, cell, nanoparticle, etc.) [27–29]. To the best of our knowledge, no study has been reported on capping pores of MSN via chick chemistry reaction in the controlled release system.

In this study, the pores of MSN were capped by double stranded DNA (dsDNA) through CuAAC to develop a controlled release system for the first time. More specifically, N_3 was modified on the surface of MSN firstly to form N_3-MSN, then an alkyne modified dsDNA containing thrombin aptamer was modified on N_3-MSN through CuAAC to cap the pores of MSN with high efficiency. The target (thrombin in this study) combined with its aptamer with high affinity [30] and hence caused the releasing of aptamer from the MSN. As ssDNA could not block the pores of MSN with high efficiency, the fluorescein (which was initially blocked in the pores of the MSN) was released into the solution system and the fluorescence of the system increased. The proposed stimulus response system was then applied to detect thrombin in the serum samples with satisfied results.

2. Experimental sections

2.1. Reagents

Thrombin was purchased from Sigma-Aldrich Chemical Co (USA); Copper iodide (CuI), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine (TBTA), and fluorescein isothiocyanate (FITC) were purchased from J & K Scientific Ltd. (Beijing, China). Trimethyl chlorosilane (TMCS), sodium hydroxide solution (NaOH), cetyltrimethyl ammonium bromide (CTAB), tetraeth-oxysilane (TEOS), and ethanol were purchased from Shanghai Aladdin biochemical Technology Co., Ltd (Shanghai, China). Sodium azide (NaN_3) was obtained from Fuchen Chemical Reagents Company (Tianjin, China). The DNA oligonucleotides (left to right: 5′ to 3′) were synthesized and purified by Shanghai Sangon Biotech. Co. Ltd (Shanghai, China). The sequence is shown below:

Thrombin-binding aptamer
AGTCCGTGGTAGGGCAGGTTGGGGTGACT
Alkynyl modified cDNA
AGTCACCCCAACCTGCCCTACCACGGACT-≡

All other chemicals were analytical grade and used directly without further purification. Aqueous solutions were prepared with Milli-Q system (18.2 MΩ cm). Unless otherwise pointed out, all studies were carried out at room temperature.

2.2. Instruments

The surface area, pore size, and pore volume were determined by N_2 adsorption-desorption isotherms obtained at 77 K on a Micromeritics ASAP 2020. The transmission electron microscopy (TEM) images were obtained with TecnaiG2 F20 S-TWINTEM (FEI, USA). Fourier transform infrared spectroscopy (FT-IR) was performed on a Nicolet 6700 (Thermo Fisher Scientific, USA) spectrometer by transmission mode. Fluorescence measurements were carried out on a 970-CRT fluorescence spectrometer (Hitachi Ltd., Shanghai, China) using a square quartz cuvette (200 μL). Excitation and emission slits were all set for a 10.0 nm band-pass. The excitation wavelength was set at 495 nm, and the emission spectra were collected from 520 to 600 nm. The fluorescence intensity at 525 nm was used to evaluate the performances of the proposed assay strategy. All nanoparticles were centrifuged on a TG16-W centrifuge (Hunan, China).

2.3. Preparation of N_3-MSN

MSN was synthesized according to the literatures [31,32]. Briefly, 0.5 g of CTAB was initially dissolved in 240 mL distilled water, and then 1.75 mL sodium hydroxide (2.0 M) was slowly added to the CTAB solution with vigorous stirring for 30 min at 333 K to get a clear and transparent solution. Afterwards, TEOS (2.5 mL) was dropped to the above prepared solution slowly, followed by vigorously stirring for 2 h until white precipitates were obtained. The product was filtered, washed with distilled water and methanol in order, and then dried in air. The mixture of synthesized MSN (700 mg) and TMCS (700 μL) was refluxed 20 h in the presence of anhydrous toluene (60 mL) to obtained the modified MSN with the template of chlorosilane (MSN-Cl-CTAB). To remove the CTAB, MSN-Cl-CTAB (500 mg) was dispersed in 50 mL of methanol containing HCl (0.50 mL, 37.2%) and the mixed solution was refluxed for another 24 h. Then the solid was washed with double-distilled water and anhydrous ethanol after centrifugation. To remove the solvent in the pore, the material without template was dried at 333 K and it was denoted as MSN-Cl. To finally produce N_3-MSN, MSN-Cl (100 mg) was dispersed in anhydrous DMF (40 mL), followed by slowly adding NaN_3 (100 mg) with stirring for 5 h at ice water.

2.4. Preparation of FITC-loading N_3-MSN capped with double stranded DNA

The loading of FITC into the pores of N_3-MSN was prepared according to the literature [33,34]. Thrombin aptamer (50 μM) was hybridized with complementary DNA (cDNA, 40 μM) to produce a dsDNA in phosphate-buffered saline (PBS, pH 7.4) for 1 h at 310 K. To ensure cDNA entirely hybridize with aptamer, the concentration of aptamer was higher than that of cDNA. N_3-MSN (4 mg) was initially dispersed into PBS (2 mL, pH 6.8) containing FITC (2 mg), and the resulting mixture was then gently shaken in the thermomixer for 16 h at room temperature. During this process, FITC was diffused into the pores of the N_3-MSN. Then dsDNA (60 μL), CuI (1 μL) and TBTA (2 μL) were added to the suspension. The mixture was gently stirred for 4 h at 310 K. Alkynyl modified dsDNA can be modified on the surface of N_3-MSN through the click chemistry reaction with high efficiency. Finally, the dsDNA capped N_3-MSN loaded with FITC (designated as dsDNA-FITC-MSN) was dispersed into PBS buffer and kept at 277 K for further using.

2.5. Detection of thrombin

dsDNA-FITC-MSN (10 mL) and different concentrations of thrombin were added to PBS buffer (180 μL), then the solution was gently shaken on the thermomixer for 40 min at room temperature. Then the mixture was centrifuged and the fluorescence of supernatant was detected (λ_{ex} = 490 nm, λ_{em} = 520 nm).

3. Results and discussion

3.1. The principle of biosensor for thrombin detection based on stimulus-response controlled release system

Scheme 1 shows the principle of the fluorescence biosensor for thrombin detection based on stimulus-response controlled release system. The biosensor consists of three parts: the controlled release material, the target recognition, and the signal transduction parts. Alkynyl modified cDNA was hybridized with the aptamer of thrombin to form dsDNA firstly and then reacted with the N_3-MSN through CuAAC,