Analytical Biochemistry 555 (2018) 50–54

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Coupling coumarin to gold nanoparticles by DNA chains for sensitive detection of DNase I

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ARTICLE INFO

Keywords:
Gold-nanoparticles
Coumarin
Fluorescence sensor
DNase I

ABSTRACT

A kind of coumarin-modified gold nanoparticle by the bridge of dsDNA chains was designed and synthesized for sensitive detection of DNase I. The fluorescence of coumarin 343 at emission wavelengths of 491 nm excited at 440 nm was quenched by the gold nanoparticles due to the energy transfer process after the coumarin 343 was connected on the gold nanoparticles by DNA chains. When dsDNA chains were cut off by DNase I, the coumarin 343 molecules were released from gold nanoparticles and the fluorescence of coumarin 343 would be restored. The DNase I activity could be detected by this fluorescence assay with a high sensitivity based on the change of the energy transfer efficiency. The intensity of restored fluorescence is linearly related to the quantity of DNase I in the range from 1.0 to 40 mU/mL with a detection limit of 0.22 mU/mL. This design idea could render a useful way to develop similar molecular or enzyme sensor in analytical or biological fields.

Introduction

Deoxyribonuclease I (DNase I) is an endonuclease enzyme that can cleave DNA preferentially at phosphodiester linkages to release phosphate-terminated polynucleotides with a free hydroxyl group [1]. This enzyme has been suggested to a waste-management nuclease and one of the deoxyribonucleases responsible for DNA fragmentation during apoptosis [2]. It is very important in clinical and biological field because its activity abnormity is the symptom of several serious diseases, such as some kinds of cancer [3], and it is used extensively in genomic studies [4,5]. There are some traditional analytical methods for DNase I such as single radial enzyme diffusion (SRED) [6], and enzyme-linked immunosorbent assay (ELISA) [7]. Recently, there are some reports on the gold nanoparticle based on colorimetry for evaluating the activity of DNase I [8,9] and fluorescent assay using luminescent materials polymer, inorganic or organic fluorophore for sensing of DNase I [10–13]. But the sensitivity of most of these methods was not good enough for the application. It is still urgent to develop a simple and sensitive method for the detection of DNase I.

As one of the most widely studied metal nano materials, gold nanoparticles (AuNPs) have many advantages such as small size, ease of manufacture, biocompatibility, high extinction coefficient and surface modification characteristics [14,15]. The appealing feature of the high extinction coefficients in the visible region enables them to function as efficient quenchers for most fluorophores in the design of biological sensors and biomedical applications. There have been a number of reports about nanosensor based on the AuNP as a quencher for the sensing of metal ions [16,17], biologically relevant molecules such as DNA [18] and enzyme [19] [20–23]. Conventional organic dyes coumarin has the characteristics of high fluorescent emission intensity. It can be used as sensing materials combined with nano materials to prepare photochemical sensors [24–26]. Because there is a large overlap between the fluorescent spectrum of coumarin 343 (CM343) and the absorption band of AuNPs, coumarin 343 could be used as a donor and AuNPs worked as an acceptor in the energy transfer (ET) process. Based on the specific activity of DNase I, a kind of DNase assay was developed by utilizing ET mechanism between a pair of a donor (CM343) and acceptor (AuNPs) in this work. A single-stranded DNA (ssDNA) was used as a stabilizer to functionalize on the gold nanoparticle surface by self-assembly technique based on Au-S covalent bond. Then coumarin 343 modified with this kind of complementary strand of ssDNA was functionalized on gold nanoparticles by hybridization. The fluorescence of coumarin 343 was efficiently quenched by the gold nanoparticles. After addition of DNase I, the dsDNA linkers were cleaved, leading to the releasing coumarin 343 molecules from AuNPs surface, and the quenched fluorescence were recovered. Based on the changes of the fluorescence, the DNase I could be sensitively and selectively detected. The principle of the sensor is shown in Scheme 1.

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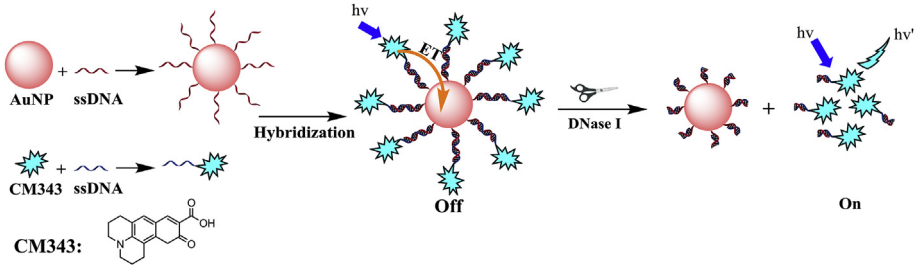
https://doi.org/10.1016/j.ab.2018.06.002
Received 28 April 2018; Received in revised form 1 June 2018; Accepted 1 June 2018
Available online 06 June 2018
0003-2697/



Scheme. 1. Schematic diagram of synthesis and principle of functionalized AuNPs.

Experiments

Materials

Coumarin 343 (CM343) was synthesized according to the previous report [27]. Single strand DNA and deoxyribonuclease I (DNase I, 2KU, ~2000 Kuntz Units) were purchased from Sangon Biotech (Shanghai) Co., Ltd. DNA1(OD's:5.0, %GC:66.67), DNA2(OD's: 5.0, %GC: 44.44) and DNA3(OD's:5.0, %GC:66.67) were purified by HPLC. Chlorauric acid ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich. Other chemicals were analytical reagent grade and used as received. DNA1 and DNA3 are complete complementary DNA chains. Single strand DNA chains are listed in Table 1.

Experimental apparatus

A PERKIN ELMER LAMBDA 750 UV–vis spectrometer and HITACHI F-4600 spectrofluorometer were used to obtain UV–vis and fluorescent spectra, respectively. Raman spectra were recorded on a RENISHAW INVIA Raman spectrometer. The decay curves were obtained on an Edinburgh FLS920 instrument with single-photon-counting system with an EPL405 source.

Functionalization of AuNPs

The detailed experimental procedure for the preparation and functionalization of AuNPs as described in detail elsewhere [24]. The single-stranded DNA (DNA sequences in Table 1) modified by mercapto group was connected to AuNPs by the Au-S bond as described in previous report [11]. The AuNPs modified by single-stranded DNA (DNA@AuNPs) remained red color even after addition of high concentrated salt (1 M NaCl), suggesting high steric stabilization.

The labelling ssDNA with coumarin 343 through carbodiimide chemistry was according to a literature method by a slight modification [28]. Firstly, coumarin 343 (1.75×10^{-4} M, 12.5 μL) were dissolved in phosphate buffer (10 mM, pH 7.2), and N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) was added to activate carboxylic groups (mole ratio: coumarin 343: EDC: NHS = 1:10:10) of coumarin 343. After shaking for 1 h, the solution of DNA3 with amino group were added to the mixture, and it was stirring for 12 h at room temperature. Then 20 mL DNA@AuNPs (5.9 nM) were added to the mixture, and it was hybrided at 37 °C for 12 h. Excess coumarin 343 was then removed by centrifugation at 12000 rpm (13,980 g) for 30 min. The AuNPs modified by coumarin 343 (CM343-DNA@AuNPs) were washed with 10 mM phosphate buffer (pH 7.2) twice and then the residue was resuspended in 10 mM phosphate buffer (pH 7.2). At last, the prepared CM343-DNA@AuNPs solution were stored at 4 °C in the refrigerator for further use.

Table 1
DNA chains.

DNA sequence 5′to 3′
DNA3: NH_2-CCTAGCCAGTGG
DNA2: SH-AAAAAACCACTGGCTAGG
DNA1: SH-CCACTGGCTAGG

Sensing application

The stock solution of DNase I was prepared in DNase buffer (25% Glycerin in H_2O) and stored at −20 °C in the refrigerator for further use. DNase I was activated in $CaCl_2$ (10 mmol/L) and $MgCl_2$ (10 mmol/L) solution for 10 min at 37 °C, then the activated DNase I (0.5 mL, 0.005–80.0 U) was incubated with CM343-DNA@AuNPs (2 mL, 1.4 nmol/L) in 10 mM phosphate buffer (pH 7.2) for different time (0–240 min). The fluorescence spectra were obtained on the spectrofluorometer excited at the wavelength of 440 nm. The fluorescence intensity was recorded at 491 nm. The optimum response time was about 120 min in this experiment. The control experiment was also carried out for the CM343-DNA@AuNP system in the absence of DNase I.

Results and discussion

Synthesis and functionalization of AuNPs

AuNPs with a diameter of ~14 nm were obtained according to our previous report [11,24]. ssDNA chains were assembled onto AuNPs surface by the Au−S bonds (DNA@AuNPs). The amino ssDNA were labeled with coumarin 343 fluorophore using carbodiimide chemistry, where the free carboxylic groups of coumarin 343 and amine groups on ssDNA were linked together by adding a solution of EDC and NHS [29]. Then the functionalized CM343-DNA@AuNPs was obtained by hybridizing ssDNA labeled with coumarin 343 with the complementary ssDNA.

The Raman spectra of coumarin 343 and AuNPs modified by coumarin 343 were shown in Fig. 1. The CM343-DNA@AuNPs probes show main features in Raman spectra similar to coumarin 343 [24]. The signals at 627-790 cm^{-1} are the phenyl ring breathing motions, the peaks at 1200 cm^{-1} are ascribed to the CH_2 rocking motions and C-C stretching vibrations, the signal at 951 cm^{-1} is the C-O-C symmetrical stretching vibration, the peaks at 1274-1578 cm^{-1} are the C=C、C-N and C-C stretching vibrations, and the peaks at 1657 and 1727 cm^{-1} are C=O stretching modes. The results showed that the coumarin 343 were successfully modified on AuNPs surface by hybridization of ssDNA.

The electronic absorption properties of AuNPs were investigated by UV–Vis absorption spectra. A characteristic surface Plasmon resonance (SPR) peak of citrate-stabilized AuNPs is located at ca. 520 nm as shown in Fig. 2. The CM343-DNA1@AuNPs and CM343-DNA2@AuNPs showed an unnoticeable shift in the UV–vis absorption peaks compared to the citrate-stabilized AuNPs. After addition of DNase I into CM343-DNA1@AuNPs and CM343-DNA2@AuNPs solution, the intensity of SPR peaks at ca. 523 nm decreased and a broad band between 700 and 800 nm appeared, suggesting there were some aggregation of AuNPs with the color changing from red to black purple.

The coumarin 343 fluorophore in phosphate buffer (10 mM,