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Short communication

DNAzyme self-assembled gold nanorods-based FRET or polarization assay for ultrasensitive and selective detection of copper(II) ion

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ABSTRACT

In the paper, we have constructed a very simple, sensitive and promising assay for fluorescence biosensor detection of Cu^{2+} in aqueous solutions based on FRET between gold nanorods (AuNRs) and the 3′-TAMRA-labeled substrate strand (Sub) of DNAzyme. The fluorescence of the Sub is quenched when the substrate strand-DNAzyme strand (Sub-Enz) duplex is adsorbed on AuNRs surface through electrostatic interaction. In the presence of Cu^{2+}, the fluorescence is restored due to the decrease of FRET efficiency caused by the specific cleavage of the Sub by the DNAzyme (Enz), which weakens the electrostatic interaction between the AuNRs and short 3′-TAMRA-labeled DNA fragment. This method shows a high sensitivity for Cu^{2+} with a detection limit of 9.83 pM ($S/N=3$) and a linear range from 0.016 nM to 40 nM. At the same time, Cu^{2+} can be detected sensitively based on the significantly decreased Fluorescent polarization (FP). The detection limit of 8.40 pM is experimentally achieved for Cu^{2+}.

1. Introduction

Some metals are ubiquitous environmental contaminants, and their high toxicity makes their presence undesirable. Generally, metal ions can be classified into two categories: essential and nonessential. Nonessential metals like lead (Pb) and mercury (Hg) can cause a number of adverse health effects even at low-level exposure (Llobet et al., 2003). Essential metals such as copper (Cu) is required to support biological activities. However, even these essential metals are toxic in excess (Fox, 2003). The U.S. Environmental Protection Agency (EPA) has set the safety limits of copper in drinking water as 1.3 ppm(20 μM). At elevated concentrations, however, it may cause adverse health effects such as gastrointestinal disturbance and liver or kidney damage (Georgopoulos et al., 2001). Thus, monitoring the levels of copper ions, especially trace amounts of copper in the environment and biological samples, has become increasingly important.

Several sensor methods for the detection of metal ions have been developed over the recent years, including electrochemiluminescent sensors (Qiu et al., 2011), fluorescent sensors (Wu et al., 2010), and so on. Owing to the sensitivity and flexibility, the fluorescence resonance energy transfer technique (FRET) has been a powerful technique for characterizing and probing short distance-dependent interactions between the donor and the acceptor (Truong and Ikura, 2001; Xu et al., 2010). And the fluorescence resonance energy transfer technique (FRET)-based sensors is a homogeneous method in comparison with the electrochemical-based sensors, so it is more simple and convenient. For example, Liu et al. (2011) proposed a DNAzyme-based FRET sensors for Cu^{2+}, which not only provided convenient protocol but also effectively increased the sensitivity in environmental monitoring.

With the development of nanotechnology, many novel nanomaterials with unique optical and electrical properties are increasingly exploited. Recently, gold nanorods (AuNRs) achieved considerable attention owing to their anisotropic configuration and unique optical properties which are known to be dramatically affected by their size, shape, and surrounding surface environments. AuNRs have been widely exploited for chemical and biochemical sensing, such as DNA (He et al., 2008), antibodies (Yu and Irudayaraj, 2007), cancer cell imaging (Huang et al., 2006) and metal ions (Wang et al., 2010). Because of the tunable absorption spectrum and chemical properties of the AuNRs, they can be used for establishing a FRET system as a fluorescence quencher(Liang et al., 2009; Xia et al., 2011). In the paper, we developed a fluorescence method for ultrasensitive detection of Cu^{2+} by AuNRs-based FRET assay using the DNAzyme (Enz) as a recognition probe. In the absence of Cu^{2+}, the fluorescence of the

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3′-TAMRA-labeled substrate strand (Sub) is quenched by the efficient FRET between DNAzyme and AuNRs due to the stronger electrostatic interaction. In the presence of Cu^{2+}, the Sub is cleaved into two pieces by the DNAzyme, which resulted in the releasing of a short 3′-TAMRA-labeled DNA fragment. Therefore, the fluorescence of the sensing system is restored, which could be used to detect copper.

2. Experimental

2.1. Chemicals

Unless especially indicated, all reagents and solvents were purchased in their highest available purity and used without further purification or treatment. KCl, NaCl, $CdCl_2$, $CuCl_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $MgCl_2$, $Zn(NO_3)_2$, $CaCl_2$, $FeCl_3$ and $CoCl_2$ were of analytical grade and bought from Shanghai Chemical Reagent Co., Ltd (China). $HAuCl_4 \cdot 4H_2O$ was purchased from Sigma (USA). 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris), glacial acetic acid (HAc), HCl, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), ascorbate acid, Na_2HPO_4 and NaH_2PO_4 were obtained from Sinopharm Chemical Reagent Co., Ltd (China) without any further treatment. Phosphate buffer solution (PBS) was prepared using the stock solution of Na_2HPO_4 and NaH_2PO_4 with different volume ratios. All oligonucleotides used in this present study were also synthesized and purified by Sangon Biotech (Shanghai, China) Co., Ltd. The oligonucleotide stock solutions were prepared with PBS-3 buffer (10 mM, pH 7.4, 0.3 M NaCl) and kept frozen. Their base sequences are as follows: Sub: 5′–TTT TTT TTA GCT TCT TTC TAA TAC GGC TTA CC-TAMRA-3′; Enz: 5′–GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC-3′; Enz-BHQ: 5′–BHQ-GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC-3′. Ultrapure fresh water (resistivity ≥ 18.2 MΩ) was used throughout our experiments.

2.2. Apparatus

UV–vis absorption spectra were obtained by using a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd). Photoluminescence (PL) measurements and the fluorescence polarization assay were performed at room temperature using a LS-55 luminescence spectrometer (Perkin Elmer, USA). Transmission electron micrographs and scan electron micrographs of the system were taken with a TEM-1230 (JEOL, Japan)

2.3. Fluorescence experiments

First, the fluorescence spectra of 20 nM Sub and 20 nM Sub-Enz duplex were recorded with excitation at 540 nm and an emission range from 570 to 680 nm, respectively. Then, a certain volume of AuNRs solution was added into the mixture and incubated in darkness. For the assay of Cu^{2+}, appropriate concentrations of Cu^{2+} solution was added into the above mixture. And 20 μL of 0.01 M ascorbate acid was added to the mixture with the final concentration of 200 μM. After reaction for 1.5 h at 37 °C, the fluorescence spectrum was recorded. All experiments were repeated twice. Each sample was measured three times.

2.4. Fluorescence polarization experiments

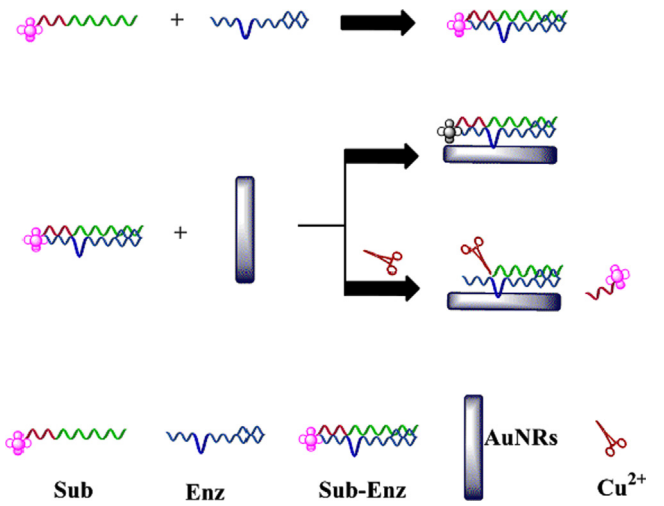
In homogeneous solution, the fluorescence polarization signal of the Sub-Enz duplex and Sub-Enz duplex with AuNRs were measured with excitation at 540 nM and emission at 580 nM. To study the interactions between Cu^{2+} and DNAzyme, the corresponding concentration of Cu^{2+} was added into the above solution for the fluorescence polarization measurement. All experiments were repeated twice. Each sample was measured three times.

3. Results and discussion

3.1. Sensing mechanism

In this contribution, we have developed a FRET strategy for detection of Cu^{2+} using gold nanorods (AuNRs) as the fluorescence quencher. Cu^{2+}-dependent DNAzyme composed of a 3′-TAMRA-labeled ribonucleotide-containing substrate strand (Sub) and an enzyme strand (Enz) were used as probe DNA. And the schematic illustration of our strategy is shown in Scheme 1. It is well-known that DNA can be adsorbed onto the surface of positively charged AuNRs because of the electrostatic interactions between the anionic backbone phosphates of oligonucleotides and the cationic surfactant bilayer around the nanorods (Wang and Li et al., 2010; Gou et al., 2010). And this assay is based that positively charged AuNRs have higher affinity for double-stranded DNA (dsDNA) than single-stranded DNA (ssDNA) because the surface charge density of dsDNA is much larger than that of ssDNA (Rosa et al., 2005). So, the decrease of fluorescence intensity is due to the dsDNA adsorbed to the AuNRs (Fig. S1). In the absence of Cu^{2+}, the electronic attraction between AuNRs and the Sub-Enz duplex solution is strong because the conformation of Sub-Enz duplex is similar to dsDNA, which bring the labeled TAMRA and AuNRs into close proximity. Therefore, the fluorescence of TAMRA is significantly quenched. However, upon the presence of Cu^{2+}, the DNAzyme is activated and cleaved the substrate strand at the guanine base site into two parts, which release a short 3′-TAMRA-labeled DNA fragment, a related long DNA fragment, and the DNAzyme strand. The cleaved short TAMRA-labeled DNA fragment has a lower charge density than the Sub-Enz duplex, which weakens the electrostatic interaction between DNA and AuNRs, then the fluorescence of TAMRA dye is restored. Based on the decrease of FRET efficiency Cu^{2+} can be detected sensitively.

To demonstrate the feasibility of our design, we investigated the FRET protocol by adding Cu^{2+} into the mixed solution of Sub-Enz duplex and AuNRs. Briefly, when there is appreciable overlap between the emission spectrum of the donor and the absorption



Scheme 1. Schematic illustration of the strategy of Cu^{2+} ions detection using Cu^{2+}-dependent DNA-cleaving DNAzyme via AuNRs-based fluorescence resonance energy transfer assay. In the absence of Cu^{2+}, the fluorescence of TAMRA was significantly quenched. Upon the presence of Cu^{2+}, the DNA was cleaved at the guanine base site into two parts. The cleaved short TAMRA-labeled DNA fragment has a lower charge density than the Sub-Enz duplex, which weakened the electrostatic interaction between DNA and AuNRs and the fluorescence intensity of the system recovered.

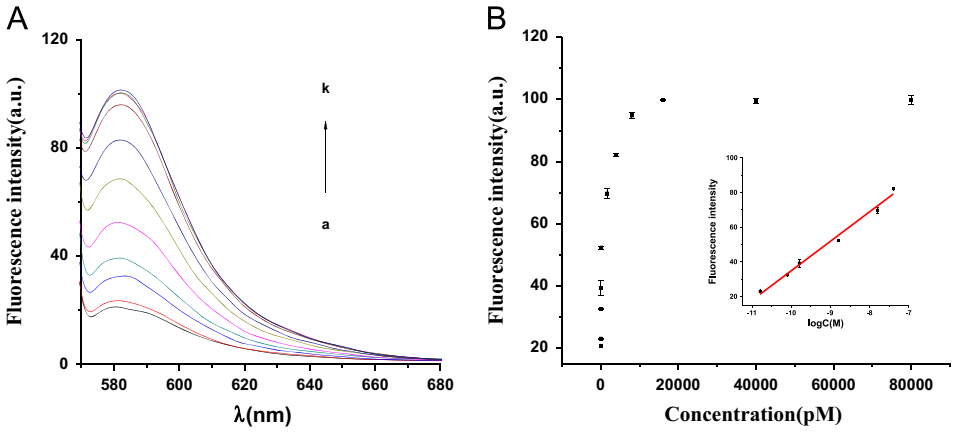


Fig. 1. (A) Fluorescence spectra of Sub-Enz/AuNRs in the absence and presence of different concentrations of Cu^{2+} ions: (a) 0, (b) 16 pM, (c) 80 pM, (d) 160 pM, (e) 1.6 nM, (f) 16 nM, (g) 40 nM, (h) 80 nM, (i) 160 nM, (j) 400 nM, (k) 800 nM. (B) The relationship between the fluorescence intensity and the concentration of Cu^{2+}. The inset shows a linear relationship (R^2=0.988) in the concentration range from 16 pM to 40 nM with a detection limit of 9.83 pM calculated for a signal-to-noise ratio of 3 (S/N=3). The standard deviations are obtained from at least three independent experiments.

spectrum of the acceptor FRET occurs and is sensitive to intra- and intermolecular distance in the range of <10 nM (Willard et al., 2001; Jares-Erijman and Jovin, 2003). From Fig. S2A, the fluorescence intensity of the Sub-Enz duplex solution decreased after mixing with AuNRs, indicating that effective FRET occurred between TAMRA and the AuNRs. On the contrary, the fluorescence intensity increased after addition of Cu^{2+}, which attributed to the specific and irreversible cleavage of the 3′-TAMRA-labeled substrate strand by DNAzyme at the guanine base site. To exclude other possibilities that lead to the fluorescence quench or restoration, we studied the influence of Cu^{2+} on the fluorescence of the 3′-TAMRA-labeled substrate strand. The fluorescence of the 3′-TAMRA-labeled substrate strand in the absence and presence of different concentrations of Cu^{2+} without AuNRs were monitored. As shown in Fig. S2B, the fluorescence emission spectra of the Sub had no fundamental change without AuNRs as the concentration of Cu^{2+} increased. This illustrated that Cu^{2+} could not influence the fluorescence of the Sub and also revealed that the change of fluorescence intensity was due to the specific interaction between DNAzyme and Cu^{2+}.

The role of ascorbate acid was also investigated to prove the response mechanism of the sensor. According to Y. Lu and co-workers, it should be certificated that ascorbate acid reacted with Cu^{2+} to generate Cu^{+} which then participated in the consequent reaction of cleaving DNA (Liu and Lu, 2007; Zhang et al., 2013). The comparison of the responses in the absence and presence of ascorbate acid was studied and shown in Fig. S3. In the presence of ascorbate acid, the response of fluorescence intensity with Cu^{2+} was more obvious than that of without ascorbate acid. According to the above phenomenon, ascorbate acid was necessary in this reaction strategy. To verify the DNA cleavage, we monitored the reaction using a gel electrophoresis, from which we can confirm that the dsDNA has been cleaved by Cu^{2+} (Fig. S5). Thus, results of gel electrophoresis evidenced that the restoration of fluorescence after incubation with Cu^{2+} was credited to the fact that the Sub-Enz duplex was cleaved by Cu^{2+}.

To further demonstrate the feasible and reasonable of this strategy, more control experiments have been carried out. The sensing mechanism was also studied by fluorescence polarization (FP) assay. FP is an intrinsically powerful technique for the rapid and homogeneous analysis of molecular interactions in biological/chemical systems, such as nucleic acid hybridization and detection of metal ions (Kumke et al., 1997; Ruta et al., 2009). The fluorescence polarization value P is sensitive to changes in the rotational motion of dye-labeled DNA probes, which in turn depend upon their molecular volume (molecular weight) (Zhang et al., 2011). So fluorescence polarization value was recorded under different circumstances to validate the cleavage reaction of the Sub by the DNAzyme strand in the presence of Cu^{2+}. As shown in Fig. S4, in the absence of Cu^{2+}, the FP value of the Sub-Enz duplex is high because the molecule is larger when the Sub-Enz duplex is close to the AuNRs. Then, it is obviously discovered that the FP value of the sensing system obviously decrease in the presence of Cu^{2+}. As mentioned above, the DNAzyme is composed of an Enz and a Sub which can be specifically cleaved by the Enz in the presence of Cu^{2+}. The surface charge density of the short TAMRA-labeled DNA fragment is lower, leading to a decrease in electrostatic interaction with AuNRs. Due to the weaker interaction with AuNRs relative to the Sub-Enz duplex, the freedom of the short TAMRA-labeled DNA fragment improved, resulting in a decrease in FP. The results of the FP measurements further indicated that the Sub can be cleaved by the Enz in the presence of Cu^{2+}. The experimental conditions including the buffer solution, reaction temperature and time, the volume of AuNRs have been optimized and shown in Figs. S6–S9. Then the pH7.4 PBS3 buffer was chosen, the optimal reaction time and temperature was 1.5 h and 37 °C, and the volume of AuNRs was 25 μL.

3.2. Sensitivity

Under the optimal conditions, different concentrations of Cu^{2+} in the range of 0.016 nM–800 nM were added into the Sub-Enz duplex and AuNRs solution, respectively. As expected, a dramatic increase in the fluorescence intensity was observed with the increase of the Cu^{2+} from Fig. 1(A). As shown in the inset of Fig. 1(B), the proposed method obtained a good linear response (R^2=0.988) of recovery rate against the logarithm of the Cu^{2+} ions concentration over the range from 0.016 nM to 40 nM with a detection limit of 9.83 pM calculated for a signal-to-noise ratio of 3 (S/N=3). Meanwhile, our method was sensitive comparison with other reported methods (Table S1).

In addition, the sensitivity of the FP assay was also investigated by using different Cu^{2+} ions concentrations from 8 pM–100 μM. Fluorescence polarization changes of Sub-Enz/AuNRs were sensitive to Cu^{2+} ion in a concentration-dependent manner (Fig. S10). This system shows good performance when the Cu^{2+} concentration lies between 8 pM and 100 μM. The Fig. S10 inset shows a good linear correlation (R^2=0.991) for the concentration of Cu^{2+} over the range of 8 pM to 320 pM. Thus, the current limit of detection for this method is approximately 8.40 pM Cu^{2+} (S/N=3),

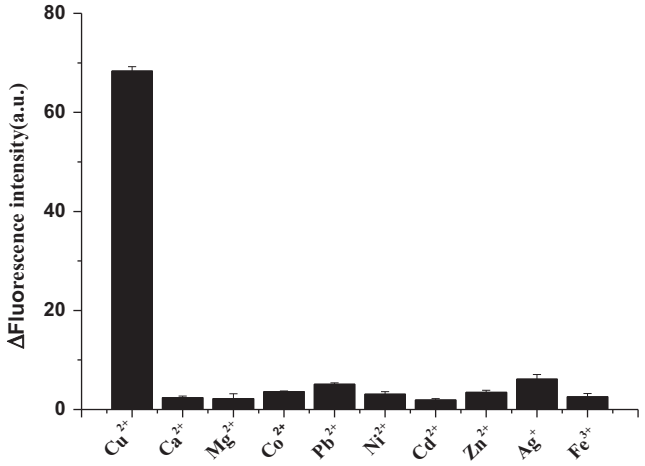


Fig. 2. Responses of the Sub-Enz to different metal ion mixtures. The concentration of Cu^{2+} was 0.016 μM, and the other test metal ions were 2 μM as negative control. The standard deviations are obtained from at least three independent experiments.

which evidences that the designed FP strategy a can also be successfully applied for Cu^{2+} ion detection.

However, a high fluorescent background existed due to the incomplete fluorescence quenching of dye by AuNRs. So we designed another FRET strategy to improve sensitivity. As shown in Scheme 1S, the side of Enz was marked by another quencher BHQ. In the absence of Cu^{2+}, the fluorescence of TAMRA was significantly quenched because of the FRET between TAMRA and two quenchers BHQ and AuNRs after the hybridization of Sub and Enz, which resulted in the decrease of background signal. Upon the presence of Cu^{2+}, TAMRA was released and the fluorescence recovered. Owing to the existence of two quencher the background signal for Cu^{2+} detection decreased significantly, so it achieved a lower detection limit of 5 fM calculated for a signal-to-noise ratio of 3 ($S/N=3$)(Fig. S11).

3.3. *Specificity and reproducibility*

Under the optimal conditions, the fluorescence changes of the complexes caused by other metal ions were recorded. As shown in Fig. 2, all the ions (2 μM) except Cu^{2+} exhibited little changes in the fluorescence recovery. On the contrary, there is an obvious increase in the fluorescence intensity as Cu^{2+} (0.016 μM). Thus, the proposed method meets the selectivity requirements of the Cu^{2+} assay, which is important and helpful in confirming the application of this method for practical samples. At the same time, the precision and reproducibility of the FRET-based DNA sensor were evaluated by using the variation coefficient (CV). The experimental results indicated that the variation coefficient (CV) for 0.2 nM, 5 nM and 10 nM Cu^{2+} was 3.0%, 2.2% and 3.0% ($n=5$), respectively. Hence, the precision and reproducibility of the FRET-based DNA sensor was acceptable. In addition, the standard addition method in tap water was used to assess the practicality of the developed approach. The experimental results were summarized in Table S2 and showed good recovery rates from 99% to 102.7%, with a relative standard deviation (RSD) of 1.43–2.64%, which revealed that it is feasible for Cu^{2+} detection in real tap water samples.

4. Conclusion

In conclusion, we developed a fluorescence method for ultrasensitive detection of Cu^{2+} by AuNRs-based FRET assay using the DNAzyme as a recognition probe. The proposed method exhibits a high sensitivity toward the target with a detection limit of 9.83 pM for Cu^{2+}. Meanwhile, the detection of Cu^{2+} in this strategy can also be carried out by the Fluorescence polarization method. Furthermore, the sensitivity can be further improved according to the introduction another quencher BHQ which achieved a lower detection limit of 5 fM. Overall, this method is a simple, sensitive, selective and convenient fluorescence sensing for homogeneous detection of Cu^{2+}. Importantly, this method can also provide a platform for developing other biosensors for ions and proteins.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.12.032.

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