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An Allosteric Dual-DNAzyme Unimolecular Probe for Colorimetric Detection of Copper(II)

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We have developed an effective molecular engineering mechanism which senses the metal ion controlled DNAzyme catalytic reactions, thus generating a sensitive metal ion sensing probe. DNAzymes are DNA sequences that catalyze chemical reactions, such as cleaving ribonucleic acid targets.¹ Among the DNAzymes attracting most attention are those that are divalent metal ion cofactor-specific. Accordingly, DNAzyme-based sensors have been reported for such metal ions as Cu²⁺,² Zn²⁺,³ Pb²⁺,⁴ Hg²⁺,⁵ UO₂²⁺,⁶ and Ca²⁺.⁷ Focusing on the development of DNAzyme-based probes for metal ions, many different design principles have been advanced. One strategy utilizes a molecular beacon consisting of two oligos: DNAzyme and substrate. Upon target ion binding, the dye-labeled substrates quenched by a quencher-modified DNAzyme are irreversibly cleaved and released to produce a fluorescent signal.^{2,4,6} Another probe design uses the conformation alteration that results from the cleavage of substrate by the DNAzyme. In this case, the horseradish peroxidase (HRP)-mimicking DNAzyme is activated by a cleavage process, thus generating colorimetric or chemiluminescence readout signals. The Willner group has made significant advancements in this field. For example, they employed Pb²⁺ and L-histidine-dependent DNAzymes, yielding HRP-mimicking nucleic acids that enable the colorimetric detection of Pb²⁺ and L-histidine,⁸ or used catalytic nucleic acid as labels to detect DNA and investigate the telomerase activity.⁹ They also designed an autonomous DNA-based machine to amplify the detection of M13 phage single-stranded DNA¹⁰ (ssDNA) and used it to detect the Hg²⁺ ion.¹¹ These approaches do offer a general means of DNAzyme-based probe design, but they mostly still involve complicated modifications to DNAzyme and the hybridization of two oligos: annealing of the DNAzyme and substrate strands. These, however, are limitations that prevent applications such as on-site detection with sensitivity and stability. To address these problems, Wang *et al.* recently proposed covalently linking the DNAzyme and leaving a substrate fragment with polythymine to create a unimolecular beacon with a strong intramolecular interaction for lead ion monitoring.¹² Herein, we report the development of a novel and versatile allosteric dual-DNAzyme unimolecular probe with simple and label-free design. As illustrated in Scheme 1, this unimolecular probe is a combination of a DNA-cleaving DNAzyme (D-DNAzyme) and a HRP-mimicking DNAzyme (H-DNAzyme), including three main components. Domain I is the substrate of DNA-DNAzyme. Domain II includes the sequence of the H-DNAzyme, and Domain III represents the D-DNAzyme. In the absence of target metal ion, these three domains act cooperatively in DNA-cleaving active state due to strong intra-molecular interaction, and the resulting structure reveals higher stability than the G-quadruplex structure (active state of

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H-DNAzyme). Conversely, when the probe meets its target, the cleavage of substrate by D-DNAzyme will disturb the intramolecular DNA conformation, and this event results in an allosteric transformation from the active state of D-DNAzyme to the active state of H-DNAzyme, giving, in turn, a colorimetric signal. Compared with other DNAzyme-based sensor designs, the allosteric dual-DNAzyme unimolecule strategy provides a robust and label-free probe construction by integrating DNAzyme, substrate, and signaling moiety into one molecule. This design utilizes intramolecular allosteric effect and signal amplification effect of HRP-DNAzyme such that, theoretically, the dual-DNAzyme unimolecule approach can be used for some cleaving-DNAzymes with similar structure.

Heavy metal ion contamination has created an important public health concern in our environment and living systems. After iron and zinc, copper is the third most abundant soft transition metal ion in the human body, and it plays an important role in various biological processes under certain amount. However, because of its widespread use, copper(II) (Cu^{2+}) also poses serious environmental problems and is potentially toxic for all the living organisms.¹³ As a result, there is a high demand for the development of sensitive and selective methods to detect Cu^{2+} ions. To demonstrate the feasibility of the dual-DNAzyme unimolecule probe strategy, the design was applied to detect the Cu^{2+} ion using the Cu^{2+} -dependent nucleic acid cleaving-DNAzyme.¹⁴

We engineered the allosteric dual-DNAzyme unimolecular probe composed of Cu^{2+} -dependent D-DNAzyme and H-DNAzyme: (Bi-Enz, 5' AGCTTCTTTCTAATACGGTGGGTAGGGCGGGTTGGGCTACCCACCTGGGCCTCTT TCTTTTAAAGAAAGAAC3'). This probe simultaneously employs two catalytic functions: 1) a Cu^{2+} -dependent self-cleavage catalytic activity to detect Cu^{2+} ions (Domain III) and 2) a horseradish peroxidase (HRP)-mimicking function to give colorimetric readout signal (Domain II). Domain I, as previously noted, is the substrate of Cu^{2+} -dependent DNAzyme. In the absence of Cu^{2+} ion, the probe is stabilized in triplex, the active state of Cu^{2+} specific DNAzyme. Specifically, in the presence of Cu^{2+} ions, the Bi-Enz molecule undergoes irreversible self-cleavage at the guanine base site (marked in black). The cleavage and release of Domain I results in spontaneous deformation of duplex and triplex. The resulting nucleic acid (Domain II) can then intercalate hemin, and resulting in the formation of HRP-mimicking DNAzyme under G-quadruplex self-assembly. The H-DNAzyme transduces the sensing events through the catalyzed H_2O_2 -mediated oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) to the blue product, a bisazo benzidine compound (Fig. S1). The reaction can be halted with 2 M H_2SO_4 , resulting in a yellow-colored product, followed by the colorimetric readout at $\lambda=450$ nm. Because the cleavage of Domain I is dependent on the concentration of the Cu^{2+} ions, the activity of the resulting H-DNAzyme provides a quantitative measure.

Figure 1A shows the UV-Vis absorption spectra upon analyzing different concentrations of Cu^{2+} ions by the dual-DNAzyme probe. As the concentration of Cu^{2+} ions increases, the absorbance values at 450 nm augments. However, in the absence of Cu^{2+} ions, control experiments showed a small absorbance signal at 450 nm (Fig. 1A, curve a), indicating that oxidation of TMB by H_2O_2 can also occur in the presence of Bi-Enz without Cu^{2+} ions. This background absorbance results from the minute folding of intact Bi-Enz molecules to G-quadruplex structure. In the presence of hemin, these folded molecules catalyze the oxidation of TMB by H_2O_2 . As shown in Figure 1A, we observed a monotonically increasing absorbance with increasing Cu^{2+} concentration (0, 1 μM , 10 μM , 100 μM , 200 μM , 1 mM, and 10 mM). The absorbance changes (ΔAbs) were obtained by subtracting control samples (shown in Fig. S2). The Fig. 1B showed a good nonlinear correlation ($R^2=0.9994$) between the ΔAbs and Cu^{2+} concentration over the range of 0.001–1.0 mM.

To evaluate the specificity of sensing Cu^{2+} by this dual-DNAzyme probe, other environmentally relevant metal ions in aqueous solutions, including Hg^{2+} , Mn^{2+} , Cd^{2+} , Pb^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Ca^{2+} , K^{+} and Na^{+} , were evaluated. Figure 2 shows the absorbance changes for the dual-DNAzyme probe upon interaction with these metal ions. The minimal changes indicate good selectivity over alkali, alkaline earth, and transition heavy metal ions.

To investigate if the method was applicable to real samples, we tested real and spiked river samples with different Cu^{2+} ion concentrations. The recoveries of standard addition were 106.1%, 112.8% and 108.3% for Cu^{2+} ion concentrations of 1 μM , 5 μM and 10 μM , respectively, when using the colorimetric method.

To conclude, we have developed a label-free allosteric dual-DNAzyme unimolecular probe design based on the allosteric effect of unimolecules from the active state of cleaving-DNAzyme to the active state of HRP-mimicking DNAzyme by self-cleavage. As a proof-of-concept experiment, the design was applied to the rapid and selective colorimetric detection of Cu^{2+} ion in aqueous media at room temperature. The method exhibits a sensitivity of 1 μM (65 ppb) in drinking water, which is much lower than the MAL (maximum allowable level) of $\sim 20 \mu\text{M}$ (1.3 ppm) in the USA, $\sim 30 \mu\text{M}$ (2.0 ppm) in the European Union and $\sim 15 \mu\text{M}$ (1.0 ppm) in Canada. Based on our results, this method opens up new possibilities for the generalized rapid and easy detection of toxic metal ions in environmental samples. Indeed, to test the practical application of this dual-DNAzyme probe, preliminary experiments were performed on real and spiked river water samples. The results reveal good recoveries. We believe that this molecular engineering design may prove useful in the future development of other nucleic acid-based probes for toxicological and environmental monitoring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) UV-Vis absorption spectra (halting the catalytic reaction by adding H₂SO₄) analyzing different concentrations of Cu²⁺: a) 0, b) 1 μM, c) 10 μM, d) 100 μM, e) 200 μM, f) 1 mM g) 10 mM, in the presence of DNazyme. The system included Bi-Enz (6.2×10^{-7} M), TMB (2.4×10^{-4} M), H₂O₂ (7.3×10^{-3} M), and hemin (6.2×10^{-7} M) in 40 μL reaction buffer. (B) Absorbance changes obtained upon the concentrations of Cu²⁺: 1 μM, 10 μM, 100 μM, 200 μM, 1 mM. Polynomial function was used to fit the data. The error bars are relative standard deviations from three repeated experiments.

Figure 2. Selectivity of the probe towards Cu^{2+} by colorimetric method. The concentration of all the metal ions was 1 mM. The error bars are relative standard deviations from three repeated experiments.

Scheme 1.

Schematic representation of the colorimetric detection of Cu^{2+} ion using the dual-DNAzyme allosteric unimolecule system. The point of scission is indicated by a black arrow. The Cu^{2+} -dependent cleavage of substrate (Domain I) results in the formation of active HRP-mimicking DNAzyme (Domain II).