J|A|C|S COMMUNICATIONS

Published on Web 09/29/2009

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

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We have developed an effective molecular engineering mechanism that senses metal-ion-controlled DNAzyme catalytic reactions, thus generating a sensitive probe for metal ions. DNAzymes are DNA sequences that catalyze chemical reactions, such as cleavage of 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 Cu^{2+},[2] Zn^{2+},[3] Pb^{2+},[4] Hg^{2+},[5] UO_2^{2+},[6] and Ca^{2+}.[7] The focus on the development of DNAzyme-based probes for metal ions has led to the advance of many different design principles. One strategy utilizes a molecular beacon consisting of two oligomers: DNAzyme and substrate. When the target ion is bound, 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 cleavage of the 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 Pb^{2+} and L-histidine-dependent DNAzymes, yielding HRP-mimicking nucleic acids that enable the colorimetric detection of Pb^{2+} and L-histidine,[8] and they used catalytic nucleic acids as labels to detect DNA and investigate telomerase activity.[9] They also designed an autonomous DNA-based machine to amplify the detection of M13 phage single-stranded DNA[10] (ssDNA) and used it to detect the Hg^{2+} ion.[11] These approaches do offer a general means of DNAzyme-based probe design, but they mostly still involve complicated modifications to the DNAzyme and the hybridization of two oligomers by 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.[12] recently proposed covalently linking the DNAzyme and leaving a substrate fragment with polythymine to create a unimolecular beacon with a strong intramolcular interaction for lead ion monitoring. Herein, we report the development of a novel and versatile allosteric dual-DNAzyme unimolecular probe with a simple, label-free design. As illustrated in Scheme 1, this unimolecular probe is a combination of a DNA-cleaving DNAzyme (D-DNAzyme) and an HRP-mimicking DNAzyme (H-DNAzyme) that includes 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 the target metal ion, these three domains act cooperatively in the DNA-cleaving active state as a result of strong intramolecular interactions, and the resulting structure reveals higher stability than the G-quadruplex structure (active state of H-DNAzyme). Conversely, when the probe meets its target, the cleavage of substrate by D-DNAzyme disturbs 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, which in turn gives a colorimetric signal. Compared with other DNAzyme-based sensor designs, the allosteric dual-DNAzyme unimolecule strategy provides a robust, label-free probe construction by integrating DNAzyme, substrate, and signaling moiety into one molecule. This design utilizes the intramolecular allosteric effect and signal amplification effect of HRP-DNAzyme, theoretically allowing the dual-DNAzyme unimolecule approach to be used for some cleaving DNAzymes with similar structures.

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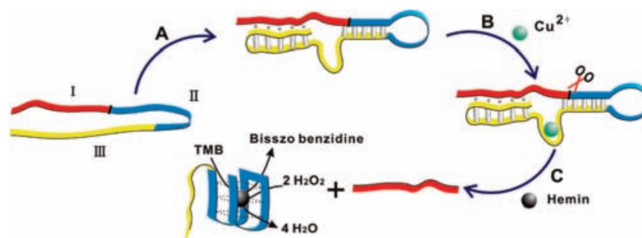
Scheme 1. Schematic Representation of the Colorimetric Detection of Cu^{2+} Ion Using the Dual-DNAzyme Allosteric Unimolecule System[a]



[a] The point of scission is indicated by the black line. The Cu^{2+}-dependent cleavage of substrate (domain I) results in the formation of active HRP-mimicking DNAzyme (domain II).

Heavy metal ion contamination has created an important public health concern in the 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 below certain amounts. However, because of its widespread use, Cu^{2+} also poses serious environmental problems and is potentially toxic for all 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 Cu^{2+} 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′-AGCTTCTTTCTAATACGGTGGGTAGGGCGGGTTGGGC-TACCCACCTGGGCCTCTTTCTTTTTAAGAAAGAAC-3′). 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) an HRP-mimicking function to give the colorimetric readout signal (domain II). Domain I, as previously noted, is the substrate of the Cu^{2+}-dependent DNAzyme. In the absence of Cu^{2+} ion, the probe is stabilized in triplex, the active state of the Cu^{2+}-specific DNAzyme. Specifically, in the presence of Cu^{2+} ions, the Bi-Enz molecule

 10.1021/ja9062426 CCC: $40.75 © 2009 American Chemical Society

undergoes irreversible self-cleavage at the guanine base site (marked in black in Scheme 1). The cleavage and release of domain I results in spontaneous deformation of the duplex and triplex. The resulting nucleic acid (domain II) can then intercalate hemin, resulting in the formation of the 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 bisszo benzidine compound (Figure S1 in the Supporting Information). The reaction can be halted with 2 M H_2SO_4, resulting in a yellow-colored product, after which the colorimetric readout at $\lambda = 450$ nm is recorded. 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 UV−vis absorption spectra obtained from analysis of different concentrations of Cu^{2+} using the dual-DNAzyme probe. As the concentration of Cu^{2+} increases, the absorbance values at 450 nm increase. However, control experiments in the absence of Cu^{2+} showed a small absorbance signal at 450 nm (Figure 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 the 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 the absorbance of the control samples (shown in Figure S2). Figure 1B shows a good nonlinear correlation ($R^2 = 0.9994$) between ΔAbs and the Cu^{2+} concentration over the range 0.001−1.0 mM.

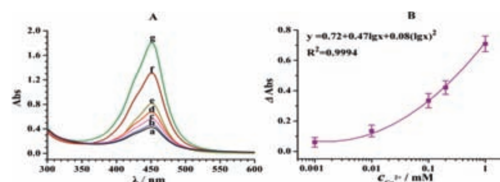


Figure 1. (A) UV−vis absorption spectra (after halting the catalytic reaction by addition of H_2SO_4) for different concentrations of Cu^{2+} in the presence of DNAzyme: (a) 0, (b) 1 μM, (c) 10 μM, (d) 100 μM, (e) 200 μM, (f) 1 mM, and (g) 10 mM. The system included Bi-Enz (6.2×10^{-7} M), TMB (2.4×10^{-4} M), H_2O_2 (7.3×10^{-3} M), and hemin (6.2×10^{-7} M) in 40 μL of reaction buffer. (B) Absorbance changes obtained for Cu^{2+} concentrations of 1 μM, 10 μM, 100 μM, 200 μM, and 1 mM. A polynomial function was used to fit the data. The error bars are relative standard deviations from three repeated experiments.

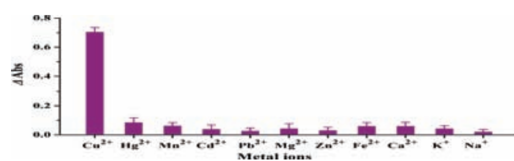


Figure 2. Selectivity of the probe toward Cu^{2+} by the colorimetric method. The concentration of each metal ion was 1 mM. The error bars are relative standard deviations from three repeated experiments.

To evaluate the specificity of sensing Cu^{2+} using 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 heavy transition-metal ions.

To investigate whether the method was applicable to real samples, we tested real and spiked river samples with different Cu^{2+} concentrations. The recoveries of standard addition were 106.1, 112.8, and 108.3% for Cu^{2+} ion concentrations of 1, 5, and 10 μM, respectively, 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+} in aqueous solution at room temperature. The method exhibited a sensitivity of 1 μM (65 ppb) in drinking water, which is much lower than the maximum allowable levels of ~20 μM (1.3 ppm) in the United States, ~30 μM (2.0 ppm) in the European Union, and ~15 μM (1.0 ppm) in Canada. On the basis of 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 to be useful in the future development of other nucleic acid-based probes for toxicological and environmental monitoring.

Acknowledgment. This work was supported by Grant 20627005 from the National Natural Science Foundation, the National Special Fund for SKLBE (2060204), NCET-07-0287 from the Program for New Century Excellent Talents, 09JC1404100 and 06SG32 from the Shanghai Shuguang Program, and U.S. NIH grants.

Supporting Information Available: Additional figures and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA9062426