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LETTER

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Unimolecular Catalytic DNA Biosensor for Amplified Detection of L-Histidine via an Enzymatic Recycling Cleavage Strategy

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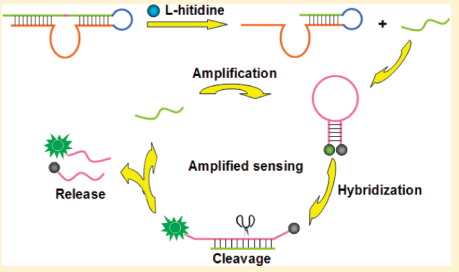
Supporting Information

ABSTRACT: Fluorescence catalytic beacons have emerged as a general platform for sensing applications. However, almost all such sensing systems need covalent modification of the DNAzymes with fluorophore−quencher pairs, which may require elaborate design of the synthetic routes and many heavy and complicated synthetic steps and result in increased cost and lower synthesis yield. Here we report the construction of fluorescent cascadic catalytic beacons. With separation of the molecular recognition module from the signal reporter, this new design both avoids DNAzyme modifications and improves sensitivity through an endonuclease-based cascadic enzymatic signal amplification. This allows detection of L-histidine with high sensitivity (LOD = 200 nM) and excellent specificity. The proposed sensing system has also been used for detection of L-histidine in cellular homogenate with satisfactory results.



DNAzymes are nucleic acids with high catalytic activities toward specific substrates,[1] and they are isolated from combinatorial oligonucleotide libraries by in vitro selection.[2] Similar to protein enzymes, most DNAzymes depend on certain metal ions or neutral molecules as cofactors for expanding their functionalities.[3] Moreover, compared with ribozymes, DNAzymes possess some unique features, such as relatively lower production costs and higher stability to resist hydrolysis, which make DNAzymes an attractive general platform for sensing applications.[4] Therefore, the Lu group and others have converted DNAzymes into highly sensitive and selective biosensors for metal ions based on various signal transduction mechanisms, including fluorescence (known as fluorescent catalytic beacons),[2f,5] colorimetry,[6] and electrochemistry.[7] Among them, fluorescent catalytic beacons have attracted considerable attention during the past decade, particularly since such fluorescence-based sensing systems can provide in situ and real-time information for a variety of applications. To realize the multiple enzymatic turnover property of DNAzymes, molecular beacon structure has also been employed in the design of substrate for DNAzyme based fluorescent sensing systems.[8] However, almost all these sensors require covalent labeling of the DNAzyme (enzyme or substrate strand) with fluorophore−quencher pairs in order to produce an efficient signal output triggered by a target, which may require elaborate design of the synthetic routes and many heavy and complicated synthetic steps, and result in increased cost and lower synthesis yield.[9] Therefore, development of a de novo DNAzyme-based fluorescent sensing system that avoids modifications on either the enzyme strand or the substrate strand should be of general interest.

In addition to avoidance of DNAzyme modifications, it is also critically important to improve sensitivity by counteracting background interference when DNAzyme-based sensors are applied in complex environmental or biological samples. Signal amplification is an efficient way to improve the sensitivity of a biosensor. To accomplish this, several endonuclease/exonuclease-based enzymatic recycling cleavage strategies have been employed for isothermal amplified detection of target DNA or other targets using aptamers as the molecular recognition module.[10] Quite a few DNAzymes that are highly specific for various metal ions or neutral molecules have been isolated using in vitro selection. However, to the best of our knowledge, such an endonuclease-based enzymatic recycling cleavage strategy has not been employed in the design of DNAzyme-based sensors for amplified detection of targets. Herein, we propose a universal design strategy to construct fluorescent cascadic catalytic beacons by separating the molecular recognition module from the signal reporter, an engineering step which can both avoid DNAzyme modifications and improve sensitivity through enzymatic signal amplification. To demonstrate the feasibility of the proposed strategy, an L-histidine-dependent DNAzyme was chosen as a model to construct the cascadic catalytic beacon for neutral molecules for the first time. To date, almost all research has been focused on development of DNAzyme-based biosensors for metal ions, rather than neutral molecules. L-Histidine plays a

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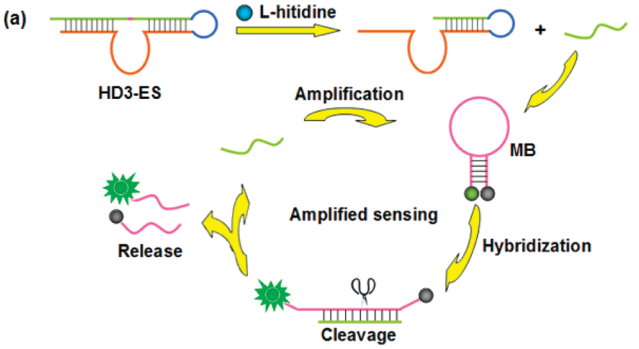
Scheme 1. (a) Schematics of DNAzyme-Based Sensing System for the Detection of L-Histidine by Using Enzymatic Signal Amplification Strategy and (b) Sequences of the HD3-ES and MB Probe



(b) HD3-ES: 5'-CGCTGAGGACACrAGGAAGAGATG$(T)_{10}$CATCT–
CTTAACGGGGCTGTGCGGCTAGGAAGTAGTGTCCTCA-3'
MB: 5'- /FAM/-CCACG AGTCAGTG TCCTCAGCGTGG-/DABCYL/-3'

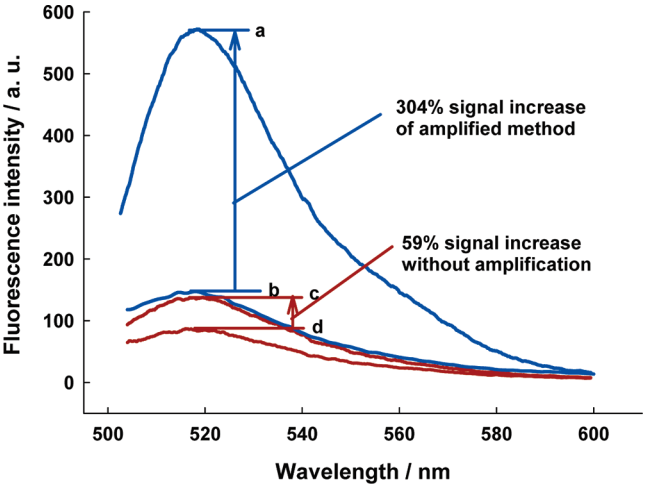


Figure 1. Fluorescence responses of the sensing system under different conditions: (a) HD3-ES + MB + Nt.BbVCI + L-histidine (100 μM), (b) HD3-ES + MB + Nt.BbVCI, (c) HD3-ES + MB + L-histidine (100 μM), and (d) HD3-ES + MB.

significant role in the growth and repair of tissues as well as in controlling the transmission of metal elements in biological bases.[11] The L-histidine-dependent DNAzyme was first selected by Breaker and Roth in 1998.[12] However, no DNAzyme-based efficient fluorescent biosensor has been reported so far, although Elbaz et al have designed a colorimetric biosensor by using an HRP-mimicking DNAzyme as a color reporter.[6d] Unfortunately, the authors used an inappropriate DNAzyme sequence named HD2 with low catalytic activity rather than the optimized sequence HD3 to construct the sensor, resulting in a poor detection limit of 500 μM for L-histidine.

In our new design (Scheme 1), the unmodified DNAzyme HD3-based unimolecular DNA-catalytic system serves as a molecular recognition module for L-histidine (see Table S1 in the Supporting Information). The signal reporter part is composed of a molecular beacon (MB) and a nicking endonuclease, Nt.BbvCI, which can recognize a specific sequence of a hybridized double-stranded DNA but hydrolyzes only one specific strand.[13] As shown in Scheme 1, the molecular recognition element includes three main components. The green and orange portions are the substrate and the enzyme sequence of DNAzyme, respectively. The blue portion is a polyT sequence which links the substrate and the enzyme together as a single molecule. The intramolecular linkage of the two strands can improve their hybridization efficiency to afford high catalytic activity. Such linkage can also eliminate the competing hybridization of the reporter MB with the partial substrate strand in the absence of target, thus lowering the background and, in turn, improving the sensing performance of the cascadic catalytic beacon system.[5e,14] The signal reporter probe MB, which is designed to be complementary to the cleaved product of HD3 (partial substrate strand), contains a recognition site for Nt.BbvCI. In the absence of a target, the entire molecular recognition module is very stable, as a consequence of strong intramolecular hybridization. Because of this high stability, the reporter MB cannot hybridize with the uncleaved partial substrate to form a double-stranded recognition sequence. Since Nt.BbvCI is disabled and cannot cleave the MB, no remarkable fluorescent signal is triggered. However, in the presence of a target, the cleaved partial substrate is released from the DNAzyme and hybridized with the MB, thereby opening its hairpin structure to form the double-stranded recognition site for Nt.BbvCI. Once the MB is cleaved, the cleaved partial substrate is dissociated from the sensing system, and fluorescence is restored. The released partial substrate can then hybridize with another MB and trigger the second cycle of cleavage. Eventually, each cleaved partial substrate can undergo many cycles to trigger the cleavage of many MBs, providing an amplified detection signal for the target.

In order to achieve the system's best sensing performance, the sequence of DNAzyme, the incubation time for the endonuclease-catalyzed cleavage reaction, and the concentration for both DNAzyme and endonuclease were optimized. To construct these fluorescent cascadic catalytic beacons, two different DNAzyme sequences, HD2-ES and HD3-ES, which were both isolated by Breaker and co-workers, were chosen as the target recognition element. By using the HD3-ES enzyme sequence, we observed a (538 $\pm$ 47)% fluorescence enhancement upon the addition of 500 μM of L-histidine, which was obviously higher than that of HD2-ES, which demonstrated only a (51 $\pm$ 6)% signal increase (see the Supporting Information, Figure S1). This result is consistent with the reported catalytic activity of the DNAzymes.[12] Therefore, the HD3-ES-based cascadic catalytic beacon was chosen for further investigation. In the presence of L-histidine with different concentrations, we observed that the fluorescence response nearly reached a plateau after 30 min incubation at 37 °C (see the Supporting Information, Figure S2). Therefore, 30 min of incubation was selected for the further investigation. Experimental results also showed that a concentration of 0.025 U/μL of endonuclease and 10 nM HD3-ES could provide the maximum S/N ratio for the sensing system (see the Supporting Information and Figure S3a,b).

To assess the amplification function of the proposed cascadic sensing system, target-induced fluorescence enhancements in the presence and absence of nicking endonuclease were recorded, respectively. The DNAzyme HD3-ES (10 nM) was first incubated with L-histidine for 20 min at room temperature. After the simultaneous addition of MBs (50 nM) and Nt.BbVCI, the mixture was incubated for another 30 min at 37 °C (see the Supporting Information), after which the fluorescent spectra were recorded. The amplification strategy based on the nicking endonuclease led

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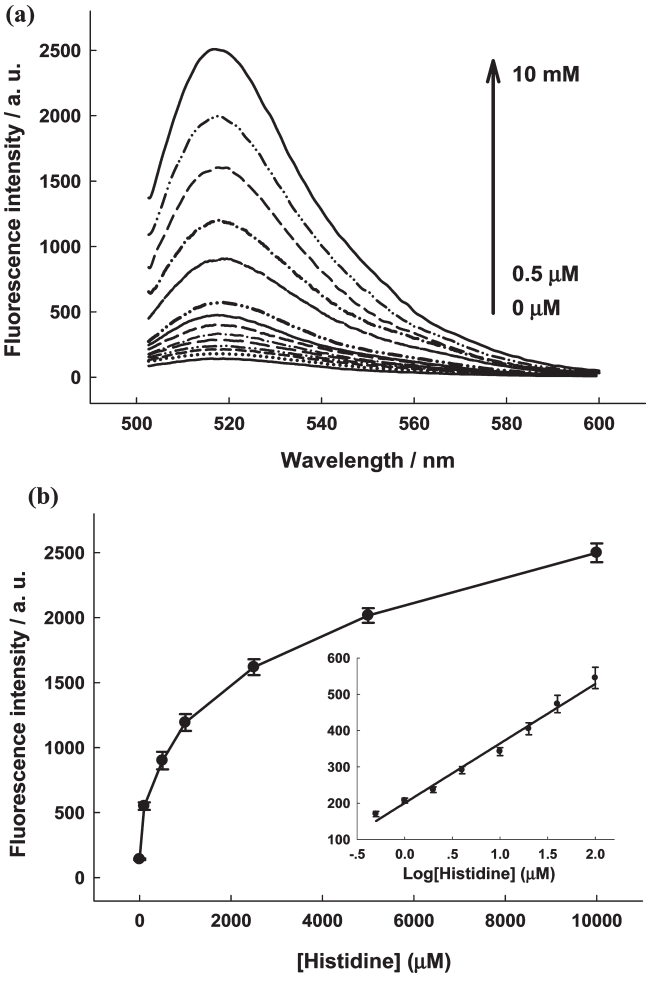

Figure 2. Sensitivity of the amplified fluorescence sensing system for target L-histidine. (a) Fluorescence emission spectra in the presence of different concentrations of L-histidine, ranging from 0 to 10 mM. (b) The relationship of the fluorescence enhancement with the target concentration. Inset shows the responses of sensing system to L-histidine at low concentration.

to a dramatic increase in the final fluorescence intensity upon the addition of the target L-histidine. As shown in Figure 1, although the introduction of endonuclease resulted in an increased background fluorescence (Figure 1, curve b) compared with that in the absence of endonuclease (Figure 1, curve d), fortunately, it also resulted in a much larger signal to background ratio, assuring a higher sensitivity for detection of target. By introducing endonuclease amplification, we observed a (304 ± 21)% signal increase. In contrast, in the absence of endonuclease, only a (59 ± 7)% increase in the signal was observed (in both cases, an L-histidine concentration of 100 μM was used). Therefore, the signal amplification of the cascadic catalytic beacon is obvious.

The proposed amplified sensing system is highly sensitive and specific to L-histidine. Figure 2a shows the fluorescence-emission spectra of the sensing system upon the addition of L-histidine at different concentrations. A dramatic increase in FAM fluorescence intensity was observed as the L-histidine concentration increased from 0.5 μM to 10 mM. Figure 2b depicts the relationship between the fluorescence intensity and the different concentrations of L-histidine. It shows a very low detection limit of 200 nM for L-histidine (based on 3δ/slope). The detection limit

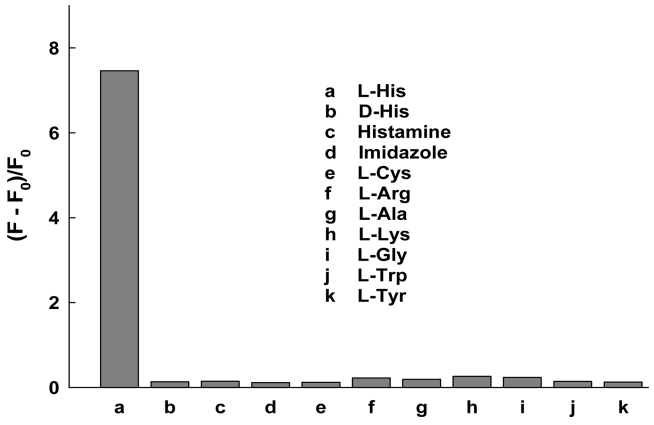

Figure 3. Selectivity of the enzymatic amplified DNAzyme-based assay for L-histidine over other potential interferences (L-histidine at concentration of 1 mM and all other compounds at concentration of 10 mM). F_0 and F are the fluorescence intensity of the sensor in the absence and presence of target amino acids, respectively.

is at least 3 orders of magnitude lower than that of the HRP-mimicking DNAzyme-based sensor.[6d] In contrast, control experiments with L-histidine at various concentrations in the absence of endonuclease were also carried out (see the Supporting Information, Figure S4), and a detection limit of only 8 μM was obtained. These results clearly demonstrate that the endonuclease does indeed play an important role in the signal amplification.

The proposed cascadic catalytic beacon also shows high specificity for L-histidine. We investigated the response with several potential interferences. Significantly higher fluorescence was observed with L-histidine at 1 mM than the other compounds at 10 mM (Figure 3). These results indicate the high specificity of our proposed sensing system.

We also used gel electrophoresis to investigate the viability of our strategy. The free HD3-ES was not cleaved upon incubation for 2 h with the endonuclease Nt.BbVCI (Figure 4, lane 3). The HD3-ES was cleaved in the presence of L-histidine (Figure 5, lane 4). In the absence of Nt.BbVCI, the MB was not cleaved when added into the mixture solution containing HD3-ES and L-histidine during the incubation time (Figure 4, lane 5). However, there was a clearly observed cleavage of MBs in the presence of Nt.BbVCI (Figure 4, lane 6). When L-glycine was used instead of L-histidine, no obvious cleavage product was observed (Figure 4, lane 7). These results demonstrated that the HD3-ES was cleaved by L-histidine but not the endonuclease. Moreover, in the presence of the target L-histidine and Nt.BbVCI, the cleaved partial substrate of HD3-ES could hybridize with the MBs and trigger the cleavage of MBs by the endonuclease.

To test the feasibility of the practical application of the sensing system, we further conducted its L-histidine detection in cellular homogenate, a realistically complex media containing a variety of proteins and other contaminants. In order to avoid the interference of background fluorescence signal of cellular homogenate around 520 nm, varying amounts of L-histidine were added to the diluted cellular homogenate samples (1.0×10^5 cells/mL). As shown in Figure 5, the L-histidine titration curve in the cellular homogenate was similar to that in the buffer solution, with a linear concentration range from 2 to 100 μM for L-histidine, which confirmed that the proposed sensing system was applicable for practical L-histidine detection in real samples with other potentially competing species coexisting.

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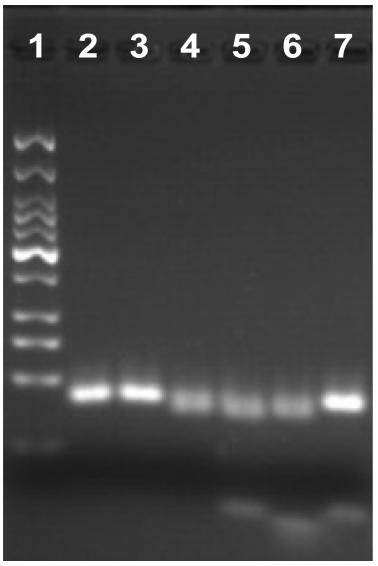

Figure 4. Gel electrophoresis of the sensing system under different conditions: lane 1, DNA marker; lane 2, HD3-ES only; lane 3, HD3-ES treated with Nt.BbVCI for 2 h; lane 4, HD3-ES incubated with L-histidine for 2 h; lane 5, HD3-ES and MB incubated with L-histidine for 2 h; lanes 6 and 7, HD3-ES and MB incubated with L-histidine and L-glycine for 2 h in the presence of Nt.BbVCI, respectively.

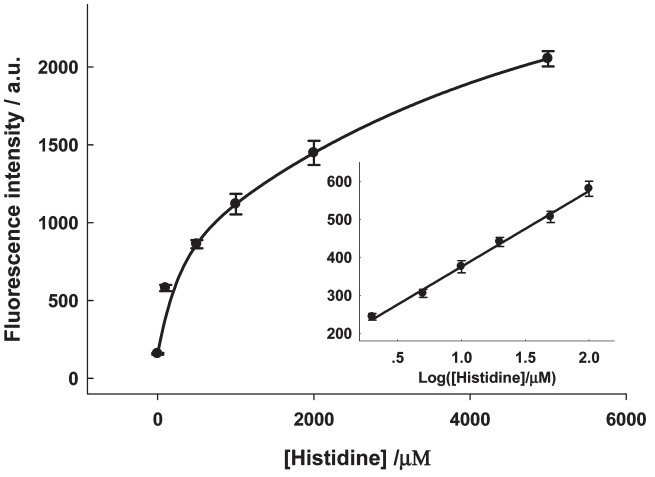

Figure 5. Relationship of the fluorescence enhancement with the L-histidine concentration in cellular homogenate samples. The inset shows the linearity of the fluorescence responses for the sensing system against the logarithm of L-histidine concentrations.

In conclusion, we have developed a novel cascadic catalytic beacon system by employing an endonuclease-based enzymatic recycling cleavage strategy in the DNAzyme-based sensing platform, resulting in ultrahigh sensitivity. By separating the molecular recognition element and signal reporter element, we have avoided any modification of the DNAzyme, a situation which usually leads to a relatively complex optimization process and complicated synthetic steps and results in increased cost and lower synthesis yield. This enzymatic signal amplification strategy affords the new DNAzyme-based sensing system a very high sensitivity, resulting in a cascadic catalytic beacon sensor for L-histidine with a detection limit of 200 nM, much lower than that of previously reported DNAzyme-based L-histidine sensors. It has also been applied for practical L-histidine detection in cellular homogenate with sensitivity similar to that in buffer solution. This novel sensing system is simple in design and can be easily carried out by simple mixing and incubation. Moreover, this strategy is universal, since aptazymes, which are generated by appending aptamers to DNAzymes,[15] can in principle be converted into such cascadic catalytic beacon for amplified detection of a variety of targets. Therefore, with its simplicity, sensitivity, and specificity, the proposed strategy holds great promise in providing a universal sensing platform for the detection of various targets and could find wide applications in the environmental and biomedical fields.

■ ASSOCIATED CONTENT

Supporting Information. Apparatus and experimental procedures and supplementary spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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