Rational design of an optical adenosine sensor by conjugating a DNA aptamer with split DNAzyme halves†

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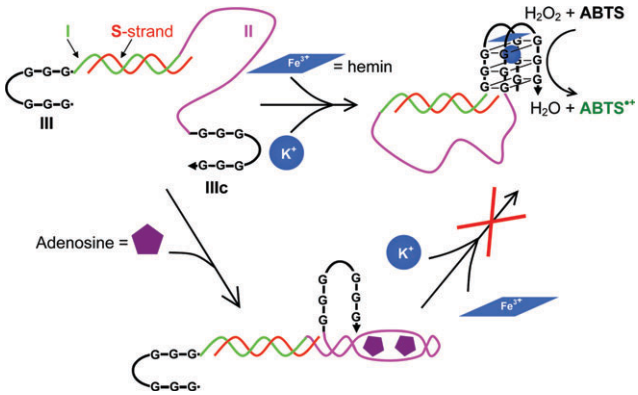
Split halves of a hemin-binding DNAzyme have been assembled with an anti-adenosine aptamer to build a homogeneous allosteric sensor for adenosine with high selectivity and sensitivity.

Aptamers are functional DNA or RNA molecules with special target-binding abilities, which can be obtained through an *in vitro* selection procedure called SELEX (systematic evolution of ligands by exponential enrichment).[1] A variety of aptamers with high affinities and specificities have been selected against various targets, including small molecules,[2,3] proteins,[4] and even whole cells.[5] Aptamer-based sensor assays have emerged to be very attractive toward optical,[6] electrochemical,[7] microgravimetric[8] and other ways[9] of detections for various molecules.

Catalytic nucleic acids, as another class of functional DNA or RNA molecules, have also received great attentions for their analytical and various other applications. DNAzymes can behave as peroxidase[10] or to promote hydrolytic cleavage of DNA or RNA strands.[11] One interesting example of a catalytic DNAzyme that reveals a peroxidase activity is formed by capturing a hemin molecule to a guanine-rich DNA quadruplex.[10,12] In contrast to regular protein-based enzymes, DNAzymes are structurally simple, synthetically easy to produce, thermally stable and relatively robust against chemical or enzymatic degradations, which secures the widespread use and re-engineering of them toward many applications. For example, the G-quadruplex-hemin enzyme has been used to design novel sensors for DNA, proteins, small molecules as well as telomerase activity assaying.[13–15,16a]

Here we report a rationally designed, easy-to-build, colorimetric and homogeneous adenosine assay by incorporating a DNA aptamer[3] and two split DNAzyme[10] halves into a functional chimera structure. Split deoxy-ribozymes (or ribozymes) have been investigated for their use in building sensing assays toward various targets.[13a,15–17] It has been found that split DNAzymes could be properly assembled to achieve a highly sensitive discrimination of single-base-mismatches in DNA targets.[16]

Scheme 1 illustrates the structure and the sensing mechanism of the adenosine sensor we designed. The major part of the sensor is a 58-mer oligonucleotide (termed as L-strand in the following text) that can be subdivided into four domains termed as I, II, III and IIIc according to their different functions as shown in Scheme 1. Region I can basepair with a 15-mer single stranded DNA (termed as S-strand) to form a rigid duplex spacer within the sensor structure. Region II is an anti-adenosine aptamer that serves as an adenosine-recognition domain. Regions III and IIIc are two halves of a split DNAzyme that, once recombined, constitutes a signal-transducing element in the sensor module. Domain II is quite flexible in the absence of an adenosine target and allows regions III and IIIc to freely approach each other and form an intact G-quadruplex upon hemin binding. The regenerated DNA enzyme (a G-quadruplex) can catalyze the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) into a blue-green colored radical product: $ABTS^{\bullet+}$, which is responsible for the absorbance output of the resulting assay. If adenosine is present in the assay solution at the beginning, region II will transform into a special compact secondary structure upon recognizing an adenosine target, which, under the help of the duplex spacer, will be able to keep the two fragmented enzymatic halves (III and IIIc) stay at a distance from each other. Therefore, further addition of hemin in the presence of potassium ions will not lead to the formation of a recombined G-quadruplex, and thus a loss of the peroxidase activity of the sensor assembly will be observed. During this process, the duplex spacer formed between region I and the S-strand serves to define the separation extent between the two enzyme halves. Therefore, in the presence of an adenosine target, the peroxidase activity of the sensor will be suppressed with a reduced colorimetric output, which forms the basis for adenosine sensing.



Scheme 1 Sensing mechanism of a rationally designed DNA-based adenosine assay. The positions of hemin and K^+ are only for schematic purpose.

Fig. 1 illustrates typical absorbance-time curves corresponding to different concentrations of adenosine in the assay solutions. For each sample, the absorbance was continuously monitored for 8 min at 418 nm (the absorbance maximum of oxidized ABTS). As can be seen from Fig. 1, following the addition of ABTS and H_2O_2 to an assay solution, oxidation of ABTS started right away,

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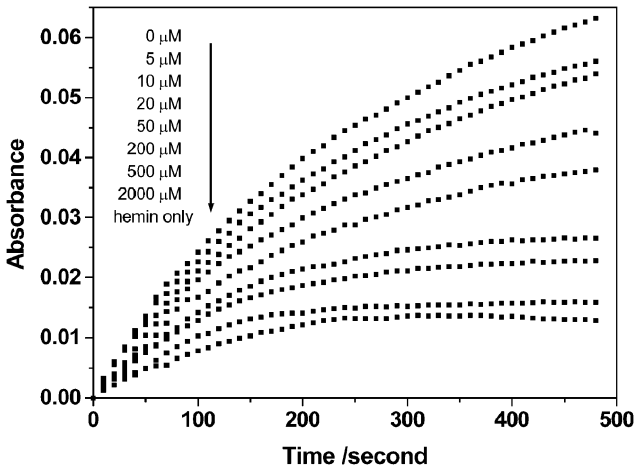

Fig. 1 Kinetic absorbance–time curves for an adenosine sensing assay. Concentrations of adenosine in the assay solutions were indicated in the figure. The bottom curve corresponds to the case with 0.1 μM hemin as the only catalyst (no DNA strands) for the oxidation of ABTS by H_2O_2. Experimental conditions: [L-strand] = 0.1 μM, [S-strand] = 0.1 μM, [hemin] = 0.1 μM, [ABTS] = 0.5 mM, [H_2O_2] = 1.2 mM. The working buffer contains 50 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 5 mM $MgCl_2$ and 10 mM KCl.

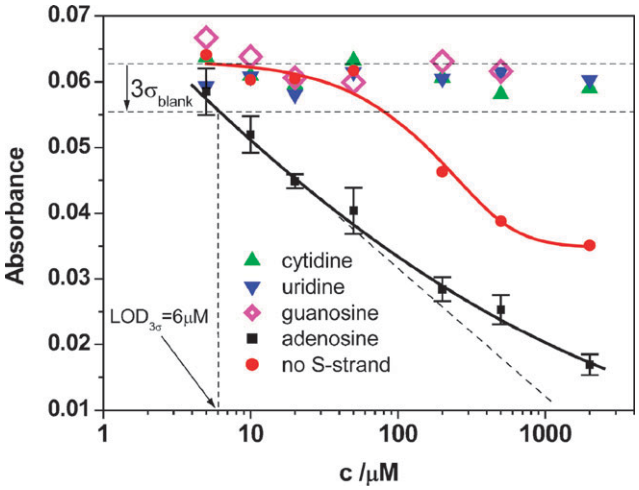

Fig. 2 Absorbance *vs.* concentration curves for an adenosine sensor with absorbance values measured at 418 nm after 8 min reactions in the presence of adenosine (squares) and other three nucleoside analogues including cytidine (up triangles), uridine (down triangles) and guanosine (diamonds). Data for adenosine were obtained from three independent experiments and error bars denote standard deviations. The two horizontal dashed lines indicate a signal decrease corresponding to three-times the standard measurement error (n = 3) below the averaged response of a target-blank assay, based on which a limit of detection (LOD) of 6 μM can be obtained. The red curve (with datapoints shown as red solid circles) represents the response of an S-strand lacking assay.

which caused a gradual accumulation of the colored radical product in the solution, leading to a monotonously increasing absorbance–time curve.

The very top absorbance–time curve in Fig. 1 corresponded to an adenosine-free case with an expected fast increase of optical absorbance with time. The bottom curve was obtained by skipping any DNA strands in the solution with the concentrations of hemin, ABTS and all other chemicals unchanged. Consequently, this control sample exhibited the slowest absorbance increase at 418 nm. These results proved that our designed aptamer–DNAzyme hybrid did have a greatly enhanced peroxidase activity after hemin binding. The big difference between the top and the bottom curves in Fig. 1 thus provided enough space for a suppression-type adenosine detection. After adenosine was introduced to the assay solution, the absorbance–time curves (Fig. 1) showed different levels of suppression, in agreement with the design as shown in Scheme 1.

To obtain a calibration curve for the adenosine assay, the absorbance at 418 nm for each sample after an 8 min reaction was plotted as a function of adenosine concentration, and the result was shown in Fig. 2. The calibration curve (absorbance *vs.* logarithm of adenosine concentration) was close to linear for adenosine concentrations lower than 50 μM. Considering the interference from random measurement errors, a limit of detection (LOD) of 6 μM could be obtained for this adenosine assay, assuming a signal suppression greater than three-times that of the measurement standard deviation (3σ) of a target-blank solution represents a statistically significant difference from the background (see Fig. 2).

In order to further test the selectivity of this assay, the other three nucleoside analogues including guanosine, cytidine and uridine were chosen as possible interferences. As shown in Fig. 2, none of the three nucleosides produced statistically significant absorbance suppressions of the assay solutions even for concentrations as high as 2 mM (the highest concentration tested for guanosine was 500 μM that was mainly limited by its solubility in the detection buffer). The data in Fig. 2 unambiguously reveal that the assay we designed in this work is highly specific toward the sensing of adenosine.

Compared to other reported homogeneous sensors, our assay has several obvious advantages: (1) it does not require synthesis and DNA conjugations of gold nanoparticles, and also the DNA strands employed do not need to be tagged with any signalling chemical groups; (2) this DNA based assay is more resistive to cations (especially divalent or polyvalent cations) than gold nanoparticle based assays and thus is easier to operate; and (3) building of such an adenosine sensing assay is very easy and cost-effective, which could be beneficial for routine uses. The current assay is much more sensitive than a previously reported assay based on adenosine-induced disruptions of gold nanoparticle aggregates[6c] and is comparable to or better than other reported homogeneous adenosine or ATP assays.[6c,f–h,9,13d] We believe further lowering the detection limit of our assay is still possible if an automatic multi-channel pipettor and a micro-plate reader are employed in order to minimize errors due to multi-step hand operations. Using lowered DNA concentrations should be another choice toward an even more sensitive assay, but a better spectrophotometer has to be used in order to guarantee measurement accuracy. After all these conditions are optimized, the final detection limit of this assay might only be restricted by the relatively high dissociation constant of the adenosine aptamer (K_d = 6 ± 3 μM)[3] and some possible imperfectness in the current version of the sensor structure, the latter deserves a further systematic investigation in our future work. Compared to other detection strategies in literature, the use of split

enzymes increases modularity in sensor designs. In addition, the easily tuneable interaction between the quadruplex halves, as investigated before,[18] offers extra chances to further optimize the performance of the as-constructed adenosine sensor.

Besides the sensitivity and selectivity issues as investigated and discussed above, we were also curious to know if the short S-strand that formed a duplex spacer with domain I in the L-strand really helped the assay to correctly respond to a target. In order to get an answer to this question, we intentionally removed the duplex forming S-strand from the sensor structure and then performed the same tests with this defective assay. Interestingly, the corresponding absorbance–concentration curve in Fig. 2 (marked with red solid circles) showed that such an incomplete sensor structure also responded to adenosine, but the absorbance change is only about half of that of a normal assay (see Fig. 2). The detection limit of this S-strand lacking assay was estimated to be about 80 μM based on the 3σ criterion, much higher than the normal assay in the presence of the S-strand. To provide a rationale for the response of the S-strand lacking assay, we assume that region II of the sensor may have a slight tendency to bind a hemin molecule since its sequence has a very typical G-rich feature similar to the hemin-binding DNAzyme. If this is true, the catalytic activity of the sensor structure might have two sources: one is from the normally formed G-quadruplex and the other from the aptamer sequence after binding a hemin molecule. Although the lack of a rigid duplex spacer from the DNA construct could make the G-quadruplex fail to dissociate in the presence of adenosine, a competition for the aptamer sequence between adenosine and hemin could still lead to a partial loss of the catalytic power of the assay. On the other hand, since the aptamer sequence is in close vicinity of one G-quadruplex half, there exists another possibility for the response of the S-strand lacking assay: any mechanical tension or steric hindrance raised from the three-dimensional folding action of the aptamer structure after binding a target might prevent the formation of a well-defined G-quadruplex. While the real reasons as discussed above are still unknown and should deserve a detailed investigation in the future, it was already clear based on our data that the S-strand did play a key role during the sensing process as depicted in Scheme 1. Because the assay in the absence of the spacer-forming S-strand had a much higher (>13 times) detection limit compared to a normal assay, the S-strand is definitely necessary for a detection process.

In conclusion, we have successfully built a purely DNA structured, homogeneous and colorimetric adenosine sensor, which offers a new dimension for designing aptamer-based sensors with a split G-quadruplex enzyme as a signal-transducing module. The simplicity, high selectivity and sensitivity of the current adenosine sensor should be very desirable for routine analytic applications. This sensing assay also holds a promise for parallel, easy and cheap detections of more molecular targets after further revisions and elaborations, which is currently under our consideration.

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