Chemiluminescence thrombin aptasensor using high-activity DNAzyme as catalytic label†

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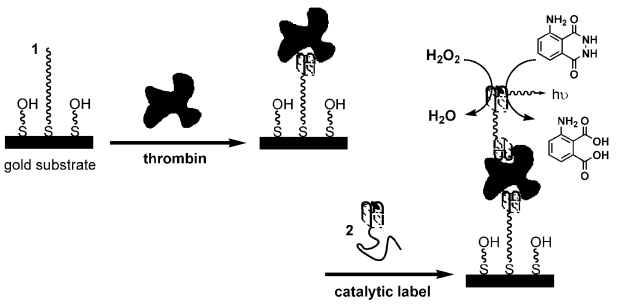
Received (in Cambridge, UK) 2nd June 2008, Accepted 21st July 2008
First published as an Advance Article on the web 23rd September 2008
DOI: 10.1039/b809296f

With a designed high-activity DNAzyme as the catalytic label, an ultrasensitive chemiluminescence thrombin aptasensor is developed, enabling a 10- to 100-fold improvement in the detection sensitivity as compared with previous counterparts.

Several G-quadruplex aptamers have been found to bind hemin to form a kind of peroxidase-like enzyme,[1–3] also called G-quadruplex-based DNAzymes. Such artificial enzymes can effectively catalyze the H_2O_2-mediated oxidation of 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS)[1,2,4] or luminol,[5,6] giving rise to a color change or chemiluminescence (CL) emission. In comparison with protein peroxidases, the DNAzymes are relatively easy to tether to DNA sequences or other targets and serve as the catalytic label, which significantly contributes to their applications to the colorimetric or CL detection of various biomolecules.[4,6–9] Interestingly, a supramolecular DNAzyme formed by two G-rich single-stranded DNAs can be used directly to sense the target DNA,[10,11] owing to its two free nucleic acid parts. As a novel kind of catalytic label, G-quadruplex-based DNAzymes have shown their great potential in bioanalysis.

Thrombin serves as an ideal analyte for testing a new sensing system due to its biological significance. Most importantly, thrombin includes two binding sites for DNA aptamers,[12] which enables the development of sandwich aptasensors for thrombin detection.[4,9,13–19] Two G-quadruplex aptamers[12,20] are found to recognize different exosites of thrombin with high affinity, and so they can serve as the sensing elements together in protein analysis.[18,21,22] By tethering thrombin-binding aptamer (TBA) to an appropriate indicator (*e.g.* enzymes[9,13,18] and nanoparticles[14,16,17,19]), the specific thrombin–TBA interaction allows introduction of these hybrid systems onto TBA-modified substrates to construct various thrombin aptasensors in a sandwich manner. Here we employ G-quadruplex-based DNAzyme as the catalytic label to develop a sandwich system for sensing thrombin. To improve the detection sensitivity, we design a new DNAzyme whose peroxidase-like activity is higher than the previous counterpart.[23] The use of this high-activity DNAzyme as catalytic label enables the sensitivity for analyzing thrombin to be improved by 10- to 100-fold as compared with the reported CL aptasensors.



1 = 5'-HS$(CH_2)_6$ TTTTTTTTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3'
2 = 5'-GTGGGTAGGGCGGGTTGGTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3'

Scheme 1 Procedure of the development of CL sandwich aptasensor for thrombin detection with 15-mer TBA as the sensing element. The DNAzyme G18 tethered to TBA serves as the catalytic label to promote the oxidation of luminol by H_2O_2, giving rise to CL emission.

Scheme 1 depicts the development of a CL thrombin aptasensor in a sandwich manner, with the known DNAzyme[1] (herein referred to as G18) formed by an 18-mer hemin-binding aptamer (HBA) as the catalytic label. First, the thiolated aptamer **1** is immobilized on the gold disk electrode, and different concentrations of thrombin are then bound to the surface. The nucleic acid **2**, consisting of 18-mer HBA,[1] 15-mer TBA,[20] and a polyT spacer, is incubated with hemin under appropriate conditions, allowing the formation of DNAzyme G18. This hybrid system is then associated to the second binding site of thrombin, and thus a DNAzyme monolayer is introduced onto the gold substrate. The whole self-assembly process is monitored by cyclic voltammetry and the CL method (see ESI†).

Fig. 1 shows the CL analysis of DNAzyme-modified substrates in the luminol-H_2O_2 system, reflecting the thrombin loading by CL readout. As the concentration of thrombin increases, the integrated CL signal is enhanced, indicating that the introduction of more DNAzyme onto the substrate directed by thrombin. From Fig. 1 it can be concluded that, by using this sensing system with G18 as the catalytic label, a detection limit of 1 nM for analyzing thrombin is achieved. It is comparable to (or better than) those of reported CL sandwich aptasensors.[9,14]

To improve the sensitivity of thrombin aptasensor, we attempt to design a new catalytic label with higher

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† Electronic supplementary information (ESI) available: Materials, instrumentation, preparation of G-quadruplex-based DNAzymes, fabrication of sandwich thrombin aptasensors, chemiluminescence analysis, and characterization of DNA modification and protein loading. See DOI: 10.1039/b809296f

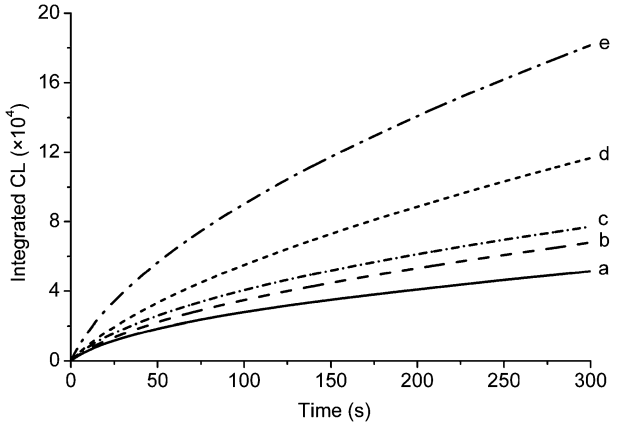


Fig. 1 Time-dependent changes of integrated CL upon analyzing the DNAzyme-modified substrates assembled by different concentrations of thrombin: (a) 0 M, (b) 1×10^{-9} M, (c) 1×10^{-8} M, (d) 1×10^{-7} M, (e) 1×10^{-6} M. Experimental conditions: [luminol] = 0.45 mM, [H_2O_2] = 27 mM, in 25 mM HEPES buffer (pH = 8.0) containing 20 mM KCl and 200 mM NaCl. The voltage of photomultiplier tube is set at 300 V.

peroxidase-like activity. In our previous study,[11] we have found that the activity of G-quadruplex-based DNAzyme can be improved by the formation of base pairs flanking the G-quadruplex core. Here we introduce a base-pairing duplex motif into an existing HBA to produce a new 31-mer HBA.[23] This designed aptamer is found to bind hemin to form a G-quadruplex-based DNAzyme (herein referred to as G31). Under the same conditions, G31 exhibits 1.8-fold higher peroxidase-like activity than G18 (Fig. 2). The excellent enzyme activity of G31 gives this DNAzyme great potential for the sensitive analysis of biomolecules. To demonstrate it, we employ G31 as the catalytic label to develop another CL sandwich aptasensor for thrombin detection (Scheme 2). Here two TBAs are employed as the sensing elements to recognize different exosites of thrombin. The DNA sequence **3**, consisting of 31-mer HBA[23] and 27-mer TBA,[12] is firstly incubated

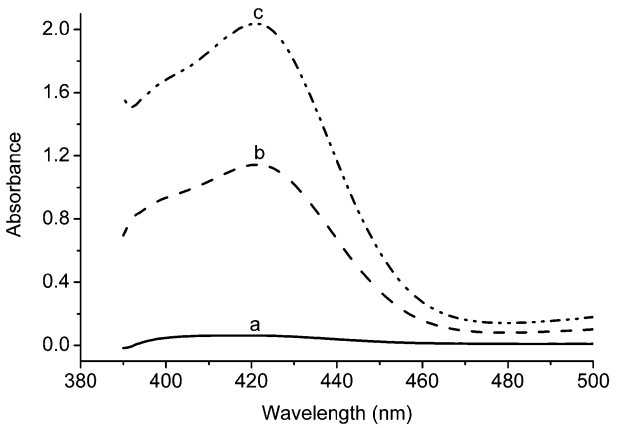
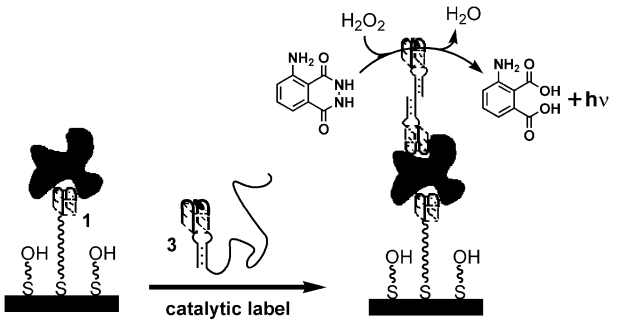


Fig. 2 Comparison between the enzyme activities of G31 and G18 by using UV-Vis absorption spectroscopy. (a) 0.5 μM hemin; (b) 0.5 μM G18; (c) 0.5 μM G31. Experimental conditions: [ABTS] = 5.9 mM, [H_2O_2] = 0.6 mM, in 25 mM HEPES buffer (pH = 8.0) containing 20 mM KCl, 200 mM NaCl, 0.05% (w/v) Triton X-100, and 1% (v/v) DMSO.



1 = 5'-HS(CH_2)$_6$ TTTTTTTTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3'
3 = 5'-AGTCCGTGGGTAGGGCGGGTTGGGGGTGACTGTCCGTGG TAGGGCAGGTTGGGGTGAC-3'

Scheme 2 Construction of another CL thrombin aptasensor by using the DNAzyme G31 tethered to 27-mer TBA as the catalytic label.

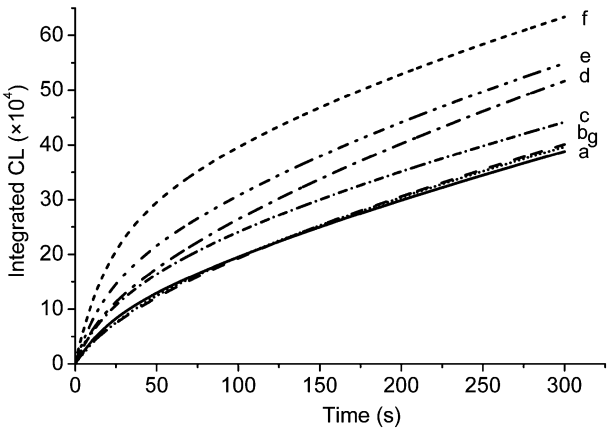


Fig. 3 Time-dependent changes of integrated CL upon analyzing different concentrations of thrombin: (a) 0 M, (b) 1×10^{-11} M, (c) 1×10^{-10} M, (d) 5×10^{-10} M, (e) 1×10^{-9} M, (f) 1×10^{-8} M. Curve g is corresponding to the analysis of 1×10^{-8} M BSA. The voltage of the photomultiplier tube is set at 600 V. Other experimental conditions are identical with those in Fig. 1.

with hemin to form the DNAzyme G31. Then, this hybrid system is introduced onto the thrombin-TBA assembled substrate. Finally, the constructed sandwich system is characterized by using the luminol–H_2O_2 CL method.

The CL analysis (Fig. 3) shows time-dependent changes of integrated CL upon analyzing different concentrations of thrombin. As the thrombin concentration increases above 1×10^{-10} M, the CL signal becomes higher. But a low concentration (1×10^{-11} M) of thrombin induces no obvious increase in the CL readout (curve b). Further control experiments reveal the addition of foreign protein (1×10^{-8} M BSA) has little influence on the CL signal (curve g), indicating high specificity for sensing thrombin by this DNAzyme-based system.

Fig. 4 outlines the relationship between the thrombin concentration and integrated CL intensity (after 300 s). From this figure it can be concluded that a detection limit of 1×10^{-10} M for analyzing thrombin is achieved with this CL aptasensor. In comparison with other counterparts reported previously[9,14] and described above in this work, a 10- to 100-fold

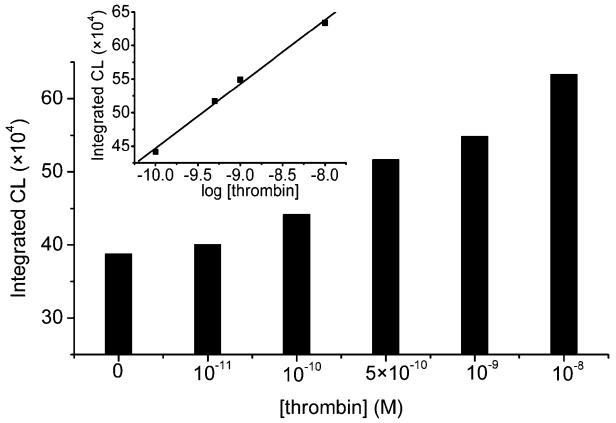


Fig. 4 Dependence of integrated CL intensity (after 300 s) on the concentration of thrombin. The inset reveals a linear relationship ($r = 0.997$) between the readout signal and the logarithm of thrombin concentration in the range of 1×10^{-10}–1×10^{-8} M.

improvement in the detection sensitivity is realized by using the high-activity DNAzyme G31 as catalytic label. Furthermore, the strong intermolecular interactions[12,20] between thrombin and **1** and **3** facilitate the formation of a **1**–thrombin–**3** sandwich system, significantly contributing to the high sensitivity of this CL aptasensor. The inset in Fig. 4 reveals that the CL intensity is linearly dependent on the logarithm of thrombin concentration in the range from 1×10^{-10} to 1×10^{-8} M. This quasi-linear relationship between the readout signal and analyte concentration has also been observed in a previous report.[8]

Besides thrombin, other proteins such as platelet-derived growth factors (PDGF)[24] and avidin[25] have been found to possess at least two binding sites for aptamers or ligands. Replacing TBA with PDGF aptamer[24] or biotin, the high-activity DNAzyme can also serve as the catalytic label for PDGF or avidin detection in a sandwich manner. That is, more CL protein sensors will be developed as described here.

In conclusion, we have developed an ultrasensitive CL sandwich aptasensor for thrombin detection, with our designed DNAzyme G31 as the catalytic label. The DNAzyme is tethered to TBA, which serves as the sensing element to recognize target protein, and is then introduced onto the thrombin-TBA assembled gold substrate. This sandwich system is analyzed by using the luminol–H_2O_2 CL method, indicating a detection limit of 100 pM for thrombin. In comparison with previous CL aptasensors, the sensitivity for analyzing thrombin is improved by 10- to 100-fold due to the use of this high-activity DNAzyme. Changing the sensing element, this sensing system is also applicable to the sensitive detection of more proteins (*e.g.* PDGF and avidin) with two or more sites for ligand binding.

This work is supported by the National Natural Science Foundation of China with the Grants 20675078 and 20735003, 973 project 2007CB714500, and the Chinese Academy of Sciences KJCX2.YW.H11.

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