Enhanced catalytic DNAzyme for label-free colorimetric detection of DNA†

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Received (in Cambridge, UK) 7th August 2007, Accepted 12th September 2007
First published as an Advance Article on the web 28th September 2007
DOI: 10.1039/b712165b

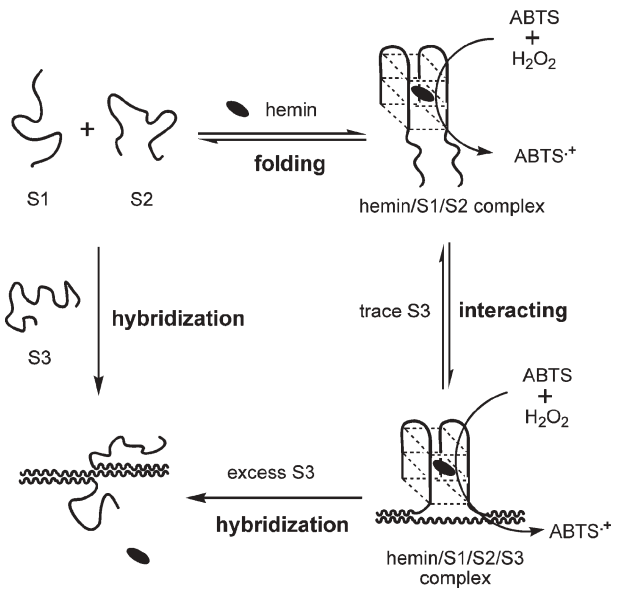
A DNAzyme-based label-free method for the colorimetric detection of DNA is introduced, with a supramolecular hemin–G-quartet complex as the sensing element and a 36-mer single-strand DNA as the analyte that is detected at 10 nM.

Catalytic DNAs are a kind of synthetic deoxyribozyme that has attracted increasing interest for the preparation of new biocatalysts.[1,2] Recently, several DNAzymes have been selected *in vitro* and applied to various biological and chemical reactions, *e.g.*, RNA transesterification,[3–6] DNA cleavage,[7–9] DNA ligation,[10,11] N-glycosylation,[12] phosphorylation,[13] adenylation,[14] and porphyrin metalation.[15] An interesting kind of G-quartet-based DNAzyme formed by hemin and guanine-rich DNA aptamers reveals peroxidase-like activities,[16] catalyzing the H_2O_2-mediated oxidation of 2,2-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) to produce the colored radical cation ($ABTS^{\bullet+}$, $\varepsilon_{414} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$).[17] The DNAzymes have been used as novel catalytic beacons for the colorimetric detection of DNA and telomerase activity[18] or the amplified analysis of small molecules and proteins.[19] And also, the hemin–G-quartet DNAzymes can catalyze the oxidation of luminol by H_2O_2 to generate chemiluminescence (CL) emission, based on which a series of CL aptasensors for DNA analysis have been developed.[20,21] Furthermore, the use of DNAzymes for sensing biomolecules has a significant advantage that any labeling and/or modification step is obsolete, thus enabling the colorimetric or CL detection of the targets through label-free approaches.[19,22]

The G-quartet-based DNAzymes are also formed by hemin and two guanine-rich single-strand DNAs.[23] Willner *et al.* have engineered a supramolecular hemin–G-quartet complex consisting of a guanine-quadruplex unit and two free nucleic acid parts.[23] In their work, this supramolecular DNAzyme was applied to sensing the complementary DNA of its two free parts, based on its catalytic activity for the luminol/H_2O_2 reaction. Here we find this complex can also catalyze the H_2O_2-mediated oxidation of ABTS. More importantly, a surprising phenomenon is observed that the catalytic activity of this DNAzyme is greatly enhanced after it is treated with a low level of the complementary DNA, making it possible to develop a novel label-free colorimetric method for DNA detection.

Scheme 1 depicts the presented method for sensing target DNAs. When incubated with hemin in the binding buffer, two guanine-rich single-strand DNAs (S1, S2) fold into a G-quartet structure ($K_d = 130\ \mu M^2$)[23] with two free nucleic acid parts (more details see the ESI†). Compared with the hemin–G-quartet structure formed by hemin and an 18-mer DNA aptamer,[16] this supramolecular complex is not so stable due to its ternary structure and two free parts. However, when the two parts are treated with a low level of the complementary DNA (S3), this complex becomes more stable and its catalytic activity increases, which is revealed by a signal increase. When S3 is in excess, the free two parts are entirely hybridized with S3, thus the supramolecular complex is subject to dissociation, resulting in a decrease in the catalytic signal. If S1 and S2 are hybridized together with S3 before incubation with hemin, no DNAzyme is formed throughout, as reported previously.[23] Obviously, a positive readout signal originating from the enhanced catalytic DNAzyme is more beneficial to improving the sensitivity of DNA detection.

Similar to other G-quartet-based DNAzymes,[17,21] the hemin–S1–S2 supramolecular complex can catalyze the H_2O_2-mediated



Scheme 1 Schematic of label-free colorimetric approaches to sensing the target DNA by using a supramolecular hemin–G-quartet complex as the sensing element. This DNAzyme with two free nucleic acid parts is formed by incubating hemin with two guanine-rich single-strand DNAs (S1, S2). The complementary DNA (S3) of its two free parts is used as the analyte.

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† Electronic supplementary information (ESI) available: Materials, instrumentation, preparation and treatment of hemin/G-quartet DNAzyme, and colorimetric measurement. See DOI: 10.1039/b712165b

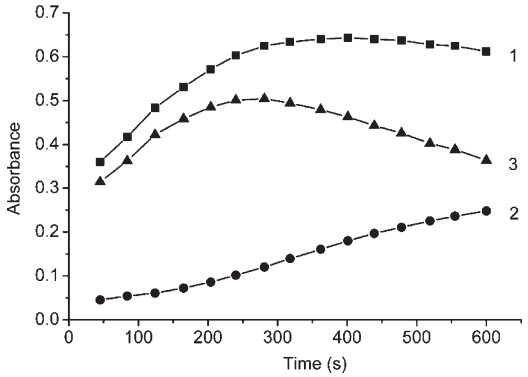

Fig. 1 Time-dependent absorbance changes upon analyzing 1 μM hemin–S1–S2 DNAzyme (curve 1) and 1 μM hemin (curve 2) using the ABTS–H_2O_2 colorimetric measurement. Curve 3 shows the relative signal (A1 − A2) changing with the reaction time.

oxidation of ABTS to cause a color change. In this process, the time-dependent absorbance changes were observed within 10 min when the ABTS–H_2O_2 reactions were monitored by UV-Vis absorption spectroscopy. Fig. 1 shows that, in curve 1, the absorption intensity of $ABTS^{\bullet+}$ (A1) increased to the maximum within 6 min and then decreased gradually due to the slow disproportionation of $ABTS^{\bullet+}$.[24] In curve 2, the absorbance (A2) increased with the reaction time. The relative signal (A1 − A2) increased to the maximum within 4 min (curve 3), which was the optimal time for the ABTS–H_2O_2 colorimetric measurement.

Due to a relatively low stability, the catalytic activity of the hemin–S1–S2 complex was much lower than that of other G-quartet-based DNAzymes formed by hemin and aptamers (data not shown). However, we found its catalytic activity was obviously improved when this DNAzyme was treated with a low level of S3 (Fig. 2A). Here 0.5 μM DNAzyme was used as the sensing element; the absorbance was increased to 178% after incubation overnight with S3 in the binding buffer at room temperature. As the control, 0.5 μM hemin only was used; the addition of S3 resulted in no obvious absorbance change. The surprising phenomenon revealed that treating the hemin–S1–S2 complex with a low level of S3 could not destroy its G-quartet structure but improved greatly its catalytic activity. We assumed that the improved stability of this DNAzyme could be mainly attributed to the formation of the base-pairing duplex motif between S3 and the two free nucleic acid parts flanking the G-quadruplex structure, as reported previously.[25,26] The specific sequence of the duplex contributed little to the stability of the G-quadruplex structure, whereas its length had a decisive effect on the DNAzyme activity. Fig. 2B shows that the catalytic activity of 0.5 μM DNAzyme decreased gradually as the total sequence length of its two free parts (equal to that of the complementary DNA) increased (curve a). After treatment with complementary DNA (0.3 μM) of different sequence length, the DNAzyme activity was improved to a different extent (curve b), and a maximal increase was obtained when the 36-mer complementary DNA was used (curve c). From this graph, it was reasonably concluded that this hemin–G-quartet DNAzyme could serve as a novel sensor for the colorimetric detection of DNA with different sequence, especially the DNA of middle sequence length (*e.g.* 25–50 bases).

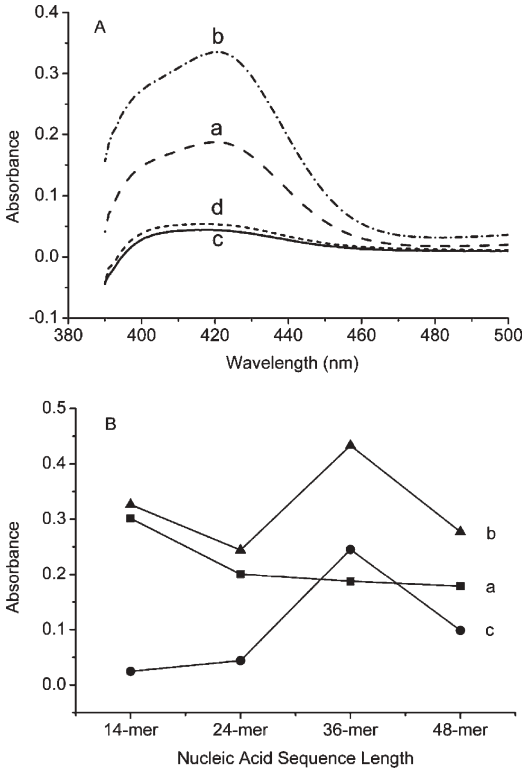

Fig. 2 Effect of complementary DNA on the DNAzyme activity. (A) UV-Vis absorption spectra of the products of 4 min ABTS–H_2O_2 reactions catalyzed by (a) 0.5 μM hemin–S1–S2 complex; (b) 0.5 μM complex plus 0.5 μM S3; (c) 0.5 μM hemin; (d) 0.5 μM hemin plus 0.5 μM S3. (B) Dependence of the DNAzyme activity on the sequence length of the complementary DNAs.

Here the 36-mer complementary DNA (S3) was used as the model to illustrate the application of this G-quartet-based sensor to DNA detection (Fig. 3). As the concentration of S3 increased to 0.3 μM, the absorbance increased gradually. But an absorbance decrease was observed when the S3 concentration was over 0.3 μM. Obviously, high concentration of S3 was not beneficial to enhancing the DNAzyme activity. From Fig. 3A, we found the addition of S3 at a concentration below 10 nM caused no observable absorbance change, indicating a detection limit of 10 nM for S3. Compared with the method described previously,[23] the sensitivity of this method was improved by 60-fold. Fig. 3B (inset) shows a linear relation (r = 0.997) between the absorbance and S3 concentration in the range of 0.01–0.3 μM. The results indicated this approach to sensing DNA was sensitive and effective.

As reported previously,[23] we also found that the hemin–S1–S2 complex was subject to dissociation if S3 was used at a high concentration (Fig. 4). When this DNAzyme (0.5 μM) was investigated, the use of S3 at a concentration of over 1 μM led to an obvious decrease in the DNAzyme activity. We presumed the entire hybridization of excess S3 with the two free parts of this complex was enough to destroy its G-quartet structure, thus the catalytic activity decreased. Through this approach, S3 could be detected at 1 μM. Obviously, the detection sensitivity was much lower than that of the above approach.

In conclusion, a DNAzyme-based label-free method for the colorimetric detection of DNA has been introduced, with a supramolecular hemin–G-quartet complex as the sensing element

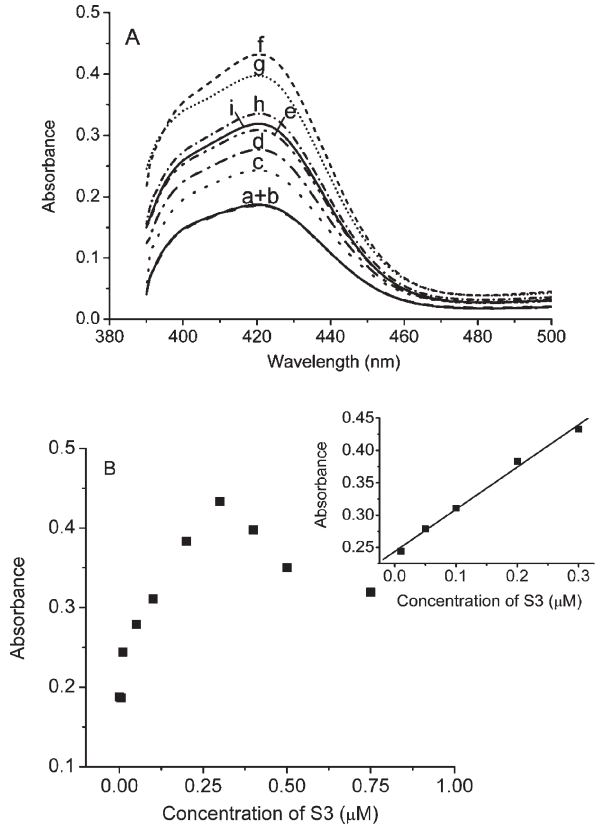

Fig. 3 Investigation of the effect of S3 on the catalytic activity of the hemin–S1–S2 complex using ABTS–H_2O_2 colorimetric measurement. (A) Changes in the absorption spectra after 0.5 μM DNAzyme was incubated with S3 of (a) 0, (b) 0.005, (c) 0.01, (d) 0.05, (e) 0.1, (f) 0.3, (g) 0.4, (h) 0.5, (i) 0.75 μM; (B) Dependence of the absorbance of $ABTS^{\bullet+}$ on the S3 concentration. The inset reveals a linear relation between the absorbance and S3 concentration.

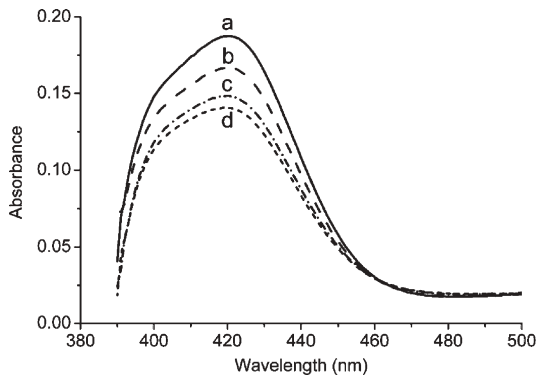

Fig. 4 UV-Vis absorption spectra of the product of 4 min ABTS–H_2O_2 reaction catalyzed by 0.5 μM hemin–S1–S2 DNAzyme after treatment with S3 of (a) 0, (b) 1, (c) 1.5, (d) 2 μM.

and a 36-mer single-strand DNA as the target. When incubated with hemin, two guanine-rich single-strand DNAs folded into the G-quartet structure with two free nucleic acid parts, possessing the peroxidase-like activity that could catalyze the H_2O_2-mediated oxidation of ABTS to cause a color change. An interesting phenomenon was observed that the catalytic activity of this DNAzyme was enhanced when its two free parts were treated with a low level of a complementary DNA sequence, enabling the detection of the target DNA at 10 nM. More significantly, the two free nucleic acid parts of this DNAzyme were optionally tailored for sensing DNAs with different sequences. It indicated this label-free colorimetric method for DNA detection was simple, sensitive, and general.

This work is supported by The National Natural Science Foundation of China with the grants 20427003 and 20675078 and Chinese Academy of Sciences KJCX2.YW.HO9.

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