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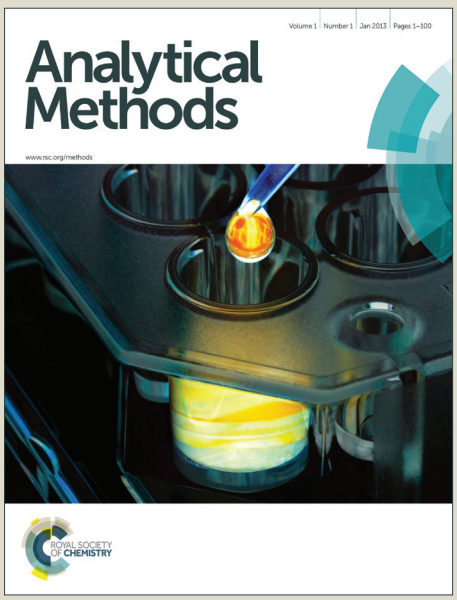

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Colorimetric Detection of ATP with DNAzyme: Design an Activatable Hairpin Probe for Reducing Background-Signal and Improving Selectivity

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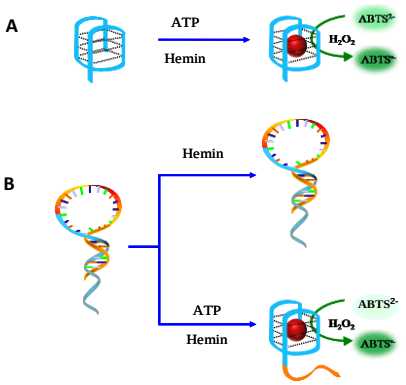
On the basis of the phenomenon that ATP can induce the formation and increase the catalytic activity of the G-quadruplex, an activatable hairpin DNA probe which includes the HRP-mimicking G-quadruplex was designed and utilized for low background and high selective colorimetric detection of ATP.

Adenosine triphosphate (ATP) is the mediator of energy exchanges that occur in all living cells, in both catabolic and anabolic processes and also widely used as an index for biomass determinations in clinical microbiology, food quality control and environmental analyses.[1] Methods based on fluorescent biosensors,[2] electrochemical biosensors,[3] and laser Raman biosensors[4] have been proposed for ATP detection. Though these methods are successful in ATP analysis, they still have the disadvantages of complicated synthesis, laborious and uneconomical label and sophisticated detection devices. Therefore, to develop an alternative method that could conveniently and high sensitively detect ATP is quite necessary.

Due to their high stability, low cost, simple synthesis, and easy modification,[5] DNAzymes, which are isolated through *in vitro* selection, [6] have been used to catalyze a broad range of chemical and biological reactions. The G-quadruplex, as one typical kind of DNAzymes, can interact with hemin to form hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking catalytic DNAzyme, which plays a role in catalyzing the H_2O_2-mediated oxidation of $ABTS^{2-}$ to the colored product $ABTS^{\cdot-}$.[7] Kong *et al*. reported the G-quadruplex can interact with ATP, they proposed a colorimetric ATP detection approach involving "always-on" G-quadruplex DNAzyme (Scheme 1A),[8] the results demonstrated ATP can increase the tendency of catalyzing the H_2O_2-mediated oxidation of $ABTS^{2-}$ to the colored product $ABTS^{\cdot-}$. This might be due to the fact that ATP can form charge transfer complexes with aromatic molecules and participate in free radical redox chemistry.[9] However, because of the high background signals, it is limited to be applied for the detection of ultra-low concentrations.

Herein, a new catalytic sensing approach for low background and selective detection of ATP based on hemin/G-quadruplex-ATP DNAzyme (HGAD) is proposed. ATP plays two roles here in our strategy, firstly, it acts as "trigger" to induce the conformation change of hairpin structure; then, it can be used as an "enhancer" to increase the catalytic activity of the DNAzymes for signal amplification. The simple hairpin structure which included the HRP-mimicking DNAzyme in caged was designed as illustrated in Scheme 1B, the probe with sequence GGGTAGGGCGGGTTGGGTAAGAATCATATAC-TCCCGCC comprises two domins: Domain I (in orange) and Domain II (in blue). In particularly, Domain II, the sequence of G-quadruplex, was



Scheme 1. Schematic analysis of ATP by the "always-on" human G4 (A) and activatable DNAzyme hairpin structure HGAD (B).

designed to partially complement to Domain I for the purpose of low background. To ensure the stable and reasonable hairpin conformation in the solution, the relative stability of the stem duplex H-G-6 (sequence was shown in Table S1, ESI) was designed, ΔG_0= -6.35 kcal· mol^{-1} (http://www.idtdna.com) and T_m=43 °C (Figure S1,

ESI). In the absence of ATP, part of the Domain II was caged in the duplex structure of the stem to form the hairpin structure with the Domain I. Upon hemin addition, weak catalytic reaction was happened, so the background signal is lower than "always-on" human G4. While in the presence of ATP, the conformation of H-G-6 was changed and the formed H-G-6/ATP complexes can act as a HRP-mimicking DNAzyme. This results in the catalytic activity toward the H_2O_2 oxidation of $ABTS^{2-}$ to $ABTS^{-}$. Therefore, the reaction displayed a much deeper green color and strong absorbance at 420 nm upon ATP addition.

Firstly, circular dichroism (CD) spectrum was investigated to confirm whether ATP has the ability to trigger the hairpin probe to form a G-quartet structure. For the sake of the high stability of stem, no obvious peak at 242 nm, 262 nm can be observed initially, as shown in Figure 1 (the green line). However, upon ATP addition, the H-G-6 exhibits obvious peak of G-quartet structure (the pink line), suggesting that ATP triggers the confoumation of H-G-6 and forms a parallel G-quadruplex-ATP complex structure[10].

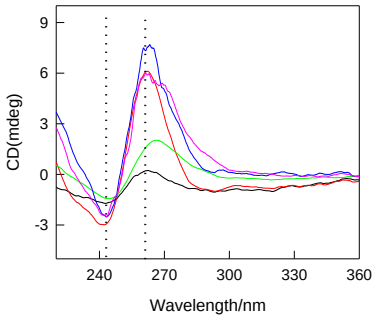


Figure 1. CD spectra of 1 μM random DNA (black), 1 μM G-quadruplexes (red), 1 μM H-G-6 (green), 1 μM H-G-6 upon addition of 2 μM ATP (blue) and 1 μM G-quadruplexes upon addition of 2 μM ATP (pink) in 20 mM HEPES containing 8 mM $MgCl_2$, 10 mM KCl, 200 mM NaCl.

Next, ATP was added into H-G-6 to test the feasibility of the design. As shown in Figure 2A, H-G-6/hemin (curve b) is a low absorbance at 420 nm in solution and slight higher than the hemin (curve a), the color of H-G-6/hemin is very shallow green (b in the inset) which demonstrated the configuration of the probe is still inactive hairpin structure. A remarkable absorbance intensity enhancement was acquired at the assay after binding 2.5 mM ATP (curve c), suggesting ATP can trigger the configuration of H-G-6 change into HGAD and increase the catalytic activity of the DNAzymes, and thus increase the tendency of catalyzing the H_2O_2-mediated oxidation of $ABTS^{2-}$ to the colored product $ABTS^{-}$. The result in a deep green color of the solution proves the feasibility of our design. Meanwhile, we optimized the stem length of the hairpin by monitoring of absorbance changes at 420 nm of probe upon the additions of ATP and hemin (Figure S2, ESI). The background becomes much lower, with the stem length increasing from 4 to 8 bases, and the optimal catalytic ability of the hemin/G-quadruplex-ATP DNAzyme is H-G-6 with the stem length of 6 bases. The optimal reaction buffer contains of 8 mM Mg^{2+}, 10 mM K^{+}, 200 mM Na^{+} at pH=7.0 (Figure S3, ESI). The A/A_0 produced by this approach is significantly enhanced: whereas we observed a 37.5 times signal increase when H-G-6 amplification was employed, the corresponding signal gains with the "always on" human G4 without reducing background signals was only 5.7 (Figure 2B), where A, A_0 are the absorbance of $ABTS^{-}$ at 420 nm catalyzed by the H-G-6/hemin complex with and without ATP, respectively.

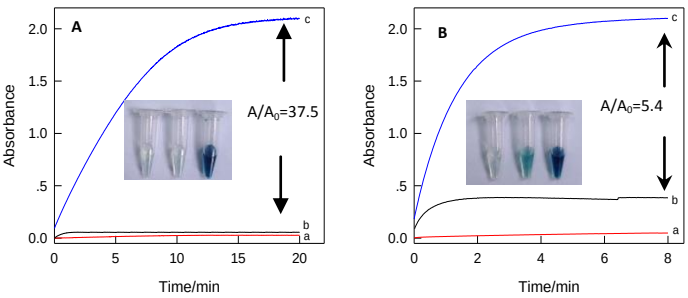


Figure 2. (A) Real-time absorption records at 420 nm of hemin/H-G-6-mediated ABTS-H_2O_2 system. Hemin(a), (a)+H-G-6(b), and (b)+2.5 mM ATP(c). (B) Real-time absorption records at 420 nm of hemin/human G4-mediated ABTS-H_2O_2 system. Hemin(a), (a)+human G4(b), and (b)+2.5 mM ATP(c). The inset photographs showing the colorimetric changes of A and B(The image a, b, c are correspond to the cureve a, b, c, respectively). All measurements were performed in the solution containing 1 μM of hemin, 1 mM ABTS and 2 mM H_2O_2.

To demonstrate the application of the proposed strategy in terms of sensitive label-free visual detection of ATP, Figure S4A shows the changes in the absorbance intensities of the probe H-G-6 upon detecting different concentrations of ATP by catalytic reaction. In the absence of ATP, Domain II of the probe H-G-6 was caged which is catalytically inactive, absorbance of such a mixture was very weak due to the weak interaction between the Domain II and hemin. Along with the increase of the ATP concentration from 100 nM to 3 mM, the absorbance at 420 nm gradually increased. In the case of human G4, no significant absorbance intensity change was found in the target concentration ranging 0-100 μM (Figure S4B, ESI). Figure 3 illustrates the signal-to-background ($S/B=A-A_{hemin}/A_0-A_{hemin}$) of H-G-6 and human G4 upon addition of different concentrations of ATP. In the ATP concentration ranging from 100 nM to 3 mM, a dramatic increase of S/B is observed. When the concentration of ATP was increased to 2.5 mM, the S/B reached the maximum value of 281 (curve a, Figure 3), whereas the max S/B of human G4 is only 6.26(curve b, Figure 3). The limit of Tmb detection, based on 3 times of the signal-to-noise level, was estimated to be ~ 67 nM, which is nearly 3 orders of magnitude lower than other previously reported colorimetric ATP detection systems based on catalytic DNAzyme. [8]

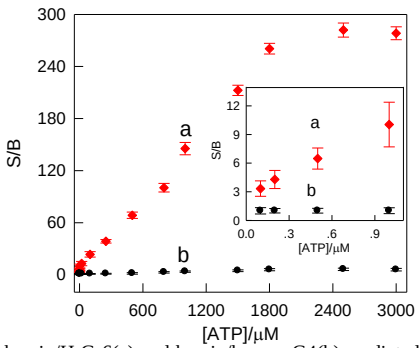


Figure 3. S/B of hemin/H-G-6(a) and hemin/human G4(b) mediated ABTS-H_2O_2 system as a function of ATP concentrations. The inset shows S/B in the ATP concentration ranging 0-1.0 μM.

The high specificity of H-G-6 was tested by introduction of ATP, ATP analogues (ATP, CTP, GTP, UTP dATP, dTTP, dCTP and dGTP), mixture of ATP and ATP analogues . When they were added respectively to interact with H-G-6, the absorbance signal upon ATP or the mixture of ATP and ATP analogues addition is much higher than that of ATP analogues (Figure S5, ESI), which lead to dramatic color changes (Figure 4A). On the contrary, as for the human G4 probe, no obvious color distinction could be observed upon ATP and ATP analogues addition, which was due to the high background of the probe itself (Figure 4B, Figure S5, ESI). This indicates that this approach has excellent selectivity than the trational human G4.

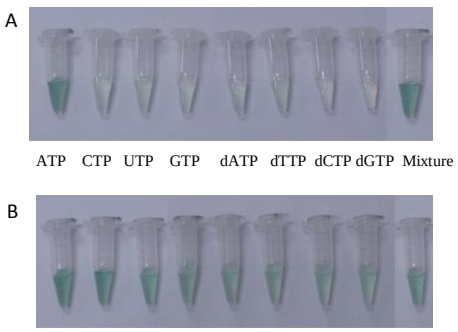


Figure 4. The colorimetric changes of hemin/H-G-6(A) and hemin/human G4(B) mediated ABTS-H_2O_2 system upon addition of 500 μM ATP, the mixture of ATP and ATP analogues , 500 μM the ATP analogues: CTP, UTP, GTP, dATP, dTTP, dCTP, dGTP and mixture .The concentration of H-G-6 and human G4 are 200 nM.

With high sensitivity and selectivity for ATP in aqueous solution, our sensor was further tested with complex samples. One can see from Figure S6 that 200 nM H-G-6 in complex biological sample itself has a low background signal at 420 nm, this background signal is slight higher than that in pure buffer, probably due to protein and enzyme has slight effect on the activity of catalysing the H_2O_2-mediated oxidation of $ABTS^{2-}$ to the coloured product $ABTS^{-}$. Upon addition of various concentrations of ATP (Figure S6, ESI), significant enhancement of absorbance signal was observed. The result can achieve the reported human ATP concentrations detection, which is about 1 μM.[11] In addition, we used this probe to detect ATP directly in cell lysis and the result was shown in Figure S7, significant enhancement of absorbance signal was observed. It can be concluded that the proposed approach can overcome the possible interferences resulting from protein, DNA, and can directly quantify ATP in complex biological fluids efficiently.

Conclusions

In conclusion, we have developed a simple and cost-effective method for colorimetric detection of ATP. Besides the inherent advantages of hemin/G-quadruplex-ATP DNAzyme assays, such as simplicity and high sensitivity, this method offers three extra benefits: (1) the use of hairpin probe that includes G-quadruplex sequence in caged as a ATP acceptor avoids high background signal; (2) ATP, as "trigger" and "enhancer", is conductive to HGAD amplification, and (3) the selectivity of this assay is comparable to that of the previously reported fluorescence-based assay and higher than that of a colorimetric assay based on G-quadruplex.[8,12]

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Notes and references

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TOC

Text: An activatable hairpin DNA probe which includes the HRP-mimicking G-quadruplex was designed and utilized for colorimetric detection of ATP.