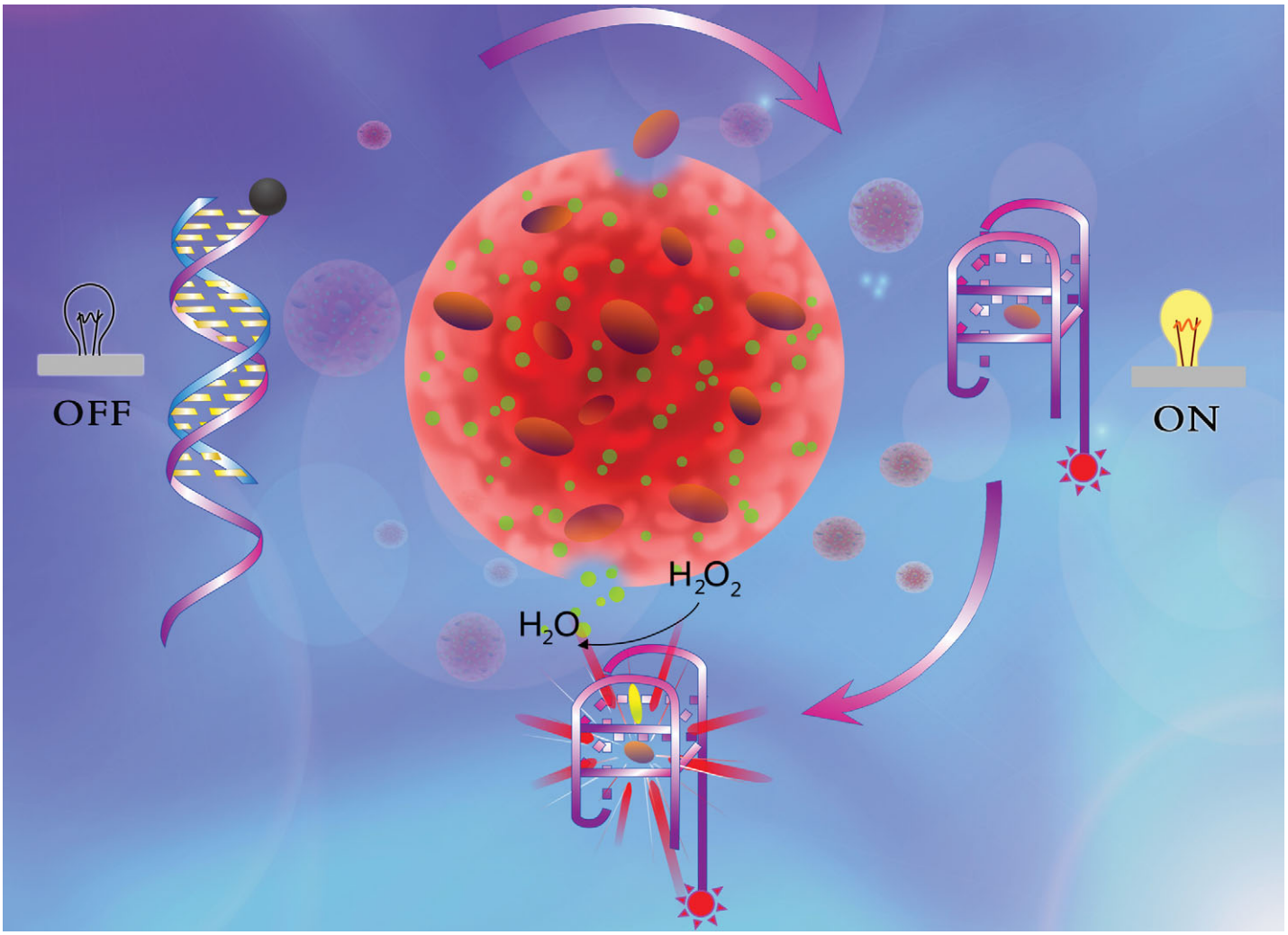

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An improved G-quadruplex DNAzyme for dual-functional electrochemical biosensing of adenosines and hydrogen peroxide from cancer cells†

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A dual-functional electrochemical biosensor for adenosines and hydrogen peroxide from cancer cells was developed based on a traditional switchable electrochemical sensing format and ATP improved G-quadruplex DNAzyme as a biolabel.

Adenosines are endogenous nucleosides which play an important role in biochemical processes as well as in signal transduction.[1] Therefore, it is essential to establish a method to detect adenosines in biochemical studies as well as in clinical diagnosis, especially at lower concentrations because it could report the state of cells with high sensitivity. To date, the reported approaches to detect adenosines, especially adenosine triphosphate (ATP), are mainly based on the binding between the ATP and the anti-ATP aptamer. The assay methods used in these biosensors are varied, ranging from fluorescence,[2] colorimetry,[3] electrochemistry,[4] and electroluminescence,[5] to photoelectrochemistry.[6] Among these methods, those using electrochemical switchable biosensors, which are inherently fast, low-cost and biocompatible, have received considerable attention. And they are quickly becoming attractive methods for the study of biological small molecules, large proteins and even cells.[7] However, the only drawback is that the electrochemical switchable ATP sensors are often confronted with the problem of unsatisfactory sensitivity.

The recently reported G-quadruplex DNAzyme is an effective catalytic tool for the amplified detection of analytes.[8] The G-quadruplex DNAzyme is an artificial DNAzyme that is composed of hemin and G-quadruplex DNA. It has been widely used in numerous biochemical reactions for its peroxidase activity.[9] In comparison with protein peroxidases, the G-quadruplex DNAzyme with advantages such as high chemical stability, low cost, simple synthesis, and easy modification has attracted increasing interest in recent years. Therefore, it is expected that the problem of unsatisfactory sensitivity in the current electrochemical switchable ATP sensing format can be solved by employing a G-quadruplex DNAzyme with catalytic activity. Fortunately, the anti-ATP aptamer (ATA) can form a G-quadruplex DNAzyme upon addition of hemin. More importantly, the catalytic activity of the G-quadruplex DNAzyme can be greatly improved in the presence of ATP.[10] For instance, when the guanine-rich ATA and its partly-complementary sequence form a DNA duplex on the electrode, G-quadruplex DNAzyme is hard to form with only hemin. However in the presence of ATP, DNAzyme is easy to form due to the binding of ATP–ATA which promotes the formation of ATP–ATA–hemin supramolecular DNAzyme. Based on this, instead of current aptamer-binding mode, here we provide a facile electrochemical switchable approach to detect ATP using ATP improved G-quadruplex DNAzyme with high catalytic activity. The reported approach provided all the superiorities of switchable electrochemical sensing, and also solved the key problem of unsatisfactory sensitivity *via* the improved G-quadruplex DNAzyme as the biolabel. Thus, the developed sensor showed its dual-signal ability which could provide more robust target recognition and potentially limit false positive results commonly observed in biosensing. As is expected, due to the amplifying action of G-quadruplex DNAzyme, a detection limit as low as 0.1 nM for ATP was obtained, which is much lower than that based on ferrocene (Fc). Based on the enhanced catalytic activity of G-quadruplex DNAzyme, we can also detect H_2O_2 release from cells. Thus a dual-signal and dual-functional electrochemical sensor for ATP and H_2O_2 was successfully constructed.

The detailed principle of ATP and H_2O_2 detection is illustrated in Scheme 1. In the first part, the anti-ATP aptamer conformation changed upon its binding to ATP. Then the anti-ATP aptamer formed a stable G-quadruplex structure upon addition of ATP due to its guanine-rich nucleic acid sequence. In the second part, with the addition of hemin, a peroxidase-like G-quadruplex configuration was formed as a DNAzyme. In the first part, the anti-ATP aptamer was first immobilized on gold electrodes in the duplex form with its partly-complementary sequence through thiol-gold chemistry. Here, only a weak signal appeared because Fc molecule at relative distal state cannot efficiently exchange electrons with the electrode for the long distance (OFF state, curve a in Fig. 1A). After reaction with

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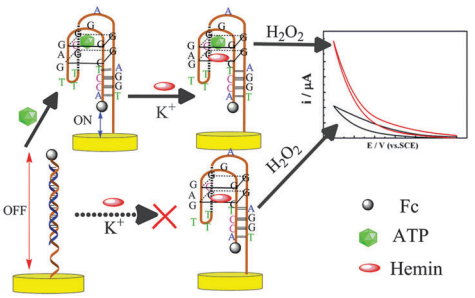

Scheme 1 The assay principle of the developed dual-functional electrochemical biosensor for detection of ATP and H_2O_2.

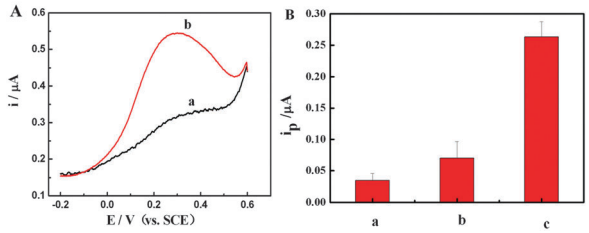

Fig. 1 (A) DPVs for the dsDNA modified electrode after reaction without (a) and with (b) 1.0 mM ATP; (B) DPV responses without (a) and with (c) 1.0 mM ATP and a mixture of 1.0 mM ATP analogues (CTP, GTP, UTP) (b).

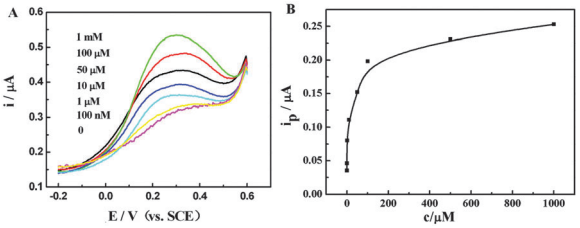

Fig. 2 (A) Representative DPVs for the dsDNA modified electrode after reaction with various concentrations of ATP. (B) The relationship between the peak current and the concentration of ATP.

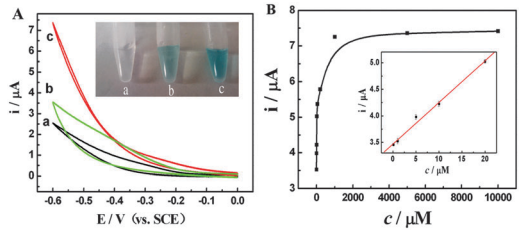

Fig. 3 (A) Voltammetric responses at dsDNA-modified electrode after reaction with only 1.0 mM ATP (a, b) or both 1.0 mM ATP and 0.2 mM hemin (c) in the absence (a) and presence (b, c) of 10 mM H_2O_2. Inset shows the photograph of the TMB substrate after treatment with modified electrodes for (a) 0 min, (b) 5 min, (c) 15 min; (B) the current responses at −0.6 V of the DNAzyme modified electrode to various concentrations of H_2O_2. Inset shows the calibration plots at a low concentration range.

1 mM ATP for 3 h at 37 °C, the double helixes were apt to open and then the complementary sequence was released into the solution. Therewith, the Fc-labeled ATA formed the G-quadruplex conformation, making the attached Fc approach the electrode. A well-defined DPV peak could be observed corresponding to the redox reaction of Fc (ON state, curve b in Fig. 1A). Following this, the selectivity of the aptasensor was investigated using ATP analogues, including cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) (all are 1 mM). After 3 h incubation, the signal of the analogue mixture increased slightly (Fig. 1B, state b) compared with that in the absence of ATP (state a). However upon the addition of ATP (state c), the signal was significantly stronger than the two former states, which confirmed that the prepared electrochemical sensor showed an excellent selectivity for ATP molecules.

Based on the measurable electrochemical signal changes from Fc, ATP could be quantified. As shown in Fig. 2A, the DPV signal gradually increased along with the increase of ATP concentration from 100 nM to 1.0 mM. The relationship between the current and the concentration of ATP is expressed as follows: i (μA) = $-0.149 \times \exp(-c/90.73) - 0.0545 \times \exp(-c/0.796) + 0.243$, $R^2 = 0.9924$.

In order to solve the problem of unsatisfactory sensitivity in the above electrochemical switchable ATP sensing, the second part was actualized. In this part, the formed G-quadruplex structure can combine with hemin resulting in the hemin/G-quadruplex formation and acts as a HRP-mimicking DNAzyme with catalytic activity. To evaluate the possibility of the biosensor, the voltammetric response of the formed G-quadruplex DNAzyme toward the reduction of H_2O_2 was studied. In the absence of H_2O_2, only a weak current was found (Fig. 3A, curve a). However with addition of 10 mM H_2O_2, the shape of CV curves changed significantly with a large cathodic current (Fig. 3A, curve c). It indicated that the formation of G-quadruplex DNAzyme has high catalytic activity toward the reduction of H_2O_2. This conclusion can be further supported by chromogenic reaction of 3,3′,5,5′-tetramethylbenzidine sulfate (TMB). We can easily observe the catalytic process from colorless to blue with the naked eye (inset in Fig. 3A).

More interestingly, the presence of ATP has positive effects on the catalytic activity of the G-quadruplex DNAzyme.[10] It was supported by the following electrochemical signal toward the reduction of 10 mM H_2O_2 under two conditions. In the absence of ATP, the current was quite weak (Fig. 3A, curve b), indicating G-quadruplex DNAzyme is hard to form on the DNA duplex with only hemin. However, largely improved catalytic activity was obtained with the addition of 1 mM ATP (Fig. 3A, curve c). This is because the presence of ATP could bind to ATA and formed the ATP–ATA–hemin supramolecular complex, giving rise to an increase in the catalytic activity. This interesting phenomenon proved the feasibility of the presented strategy for ATP detection.

To achieve the optimal analytical properties of the sensing system, pH and ionic strength of assay solution on the sensitivity of the sensor were investigated (Fig. S1, ESI†). According to the results, 10 mM PBS (pH 7.4) was chosen as the supporting electrolyte. Then, under the optimal conditions, we employed a given amount of ATP (1.0 mM) and hemin (0.2 mM) to observe the catalytic activity of the obtained DNAzyme modified electrodes towards various concentrations of H_2O_2 (Fig. 3B). The current increased gradually with an increasing concentration of H_2O_2. When the concentration of H_2O_2 exceeded 1.0 mM, the current response became steady. The current displays a good linear increase with H_2O_2 in the range 0.10–20 μM, and the calibration

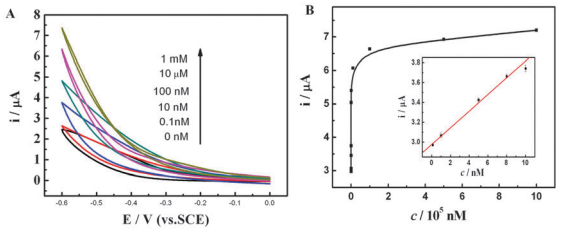

Fig. 4 (A) CVs at the DNAzyme modified electrode prepared in different ATP concentrations in 10 mM H_2O_2 solution; (B) the current responses at −0.6 V to various concentrations of ATP. Inset shows a linear relationship between the current and the ATP concentration.

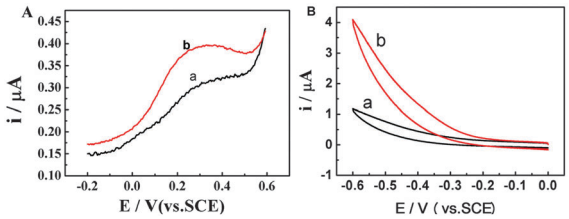

Fig. 5 (A) DPVs of dsDNA modified electrode before (a) and after treatment with the lysate of 2.25 × 10^3 mL^{-1} cancer cells. (B) CVs obtained at the DNAzyme modified electrodes in 4 μM AA without (a) and with 2.25 × 10^6 mL^{-1} K562 cells (b).

equation between the current (i) and the H_2O_2 concentration (c) is expressed as follows: i (μA) = 3.465 + 0.0783c (μM), R^2 = 0.9975.

The effect of ATP concentration on the catalytic reaction was also studied (Fig. 4A). As the concentration of ATP increased, the current increased gradually, consistent with the higher content of DNAzyme units generated. This result indicated that ATP indeed had a positive effect on the catalytic activity of G-quadruplex DNAzyme. When the concentration of ATP reached close to 10 μM, the current increased slowly and tended to become stable. The calibration plot in the range from 0.10 to 10 nM is presented in the inset of Fig. 4B. The linear equation is i (μA) = 2.977 + 0.082c (nM) (R^2 = 0.9963), where i is the current intensity at −0.6 V, and c is the ATP concentration. The limit of detection (LOD) was 0.1 nM (a signal-to-noise ratio of 3).

Finally, as reported, adenosines shared the same aptamer, and ascorbic acid (AA) as the stimulus could motivate cells to generate H_2O_2;[11] then the biosensor was suitable for determining the amount of cellular adenosines and H_2O_2 from human cancer cells. The K562 cell was chosen as a model. Fig. 5A shows the DPVs of the dsDNA modified electrode before and after immersing in the cancer cell lysate for 3 h. Based on the signal changes from Fc, we can estimate the total amount of adenosines in 2.25 × 10^3 mL^{-1} K562 cells to be 6.92 μM according to the curve depicted in Fig. 2B and the concentration of adenosines is 3.08 pmol per cell, which is in line with the results obtained using electroluminescence biosensors.[5a] For the detection of low concentration of adenosines, the signal changes from 10 mM H_2O_2 solution were investigated (Fig. S2, ESI†). 7.47 nM adenosines was estimated in the 2.25 mL^{-1} cell lysate based on the curve in Fig. 4B with *ca.* 3.32 pmol adenosines per cell, which is approximately equal to that obtained from the signal changes of Fc.

The developed electrode can also be used for measuring the flux of H_2O_2 releasing from cancer cells after forming the DNAzyme in hemin for 3 h. Fig. 5B depicts the current toward H_2O_2 releasing from cancer cells. It shows the current was quite low in 4 μM AA solution (curve a, Fig. 5B). However, if AA and cell lysate were added to the PBS, the current response grew rapidly (curve b, Fig. 5B). Therefore, the developed electrode can be used for studying the flux of H_2O_2 released from cells. The current is about 4.01 μA, which corresponds to 6.97 μM H_2O_2 calculated based on the curve depicted in Fig. 3B.

In summary, a novel electrochemical sensing strategy based on the G-quadruplex DNAzyme for detection of both adenosines and H_2O_2 was developed. The method employed the amplification of the ATP improved G-quadruplex DNAzyme and smart electrochemical switch; a detection limit as low as 0.1 nM for ATP was obtained. Based on the enhanced catalytic activity, we can detect the flux of H_2O_2 released from cells with high sensitivity.

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