Supporting Information

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

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Chemicals and Materials

DNA oligonucleotides labeled with ferrocene (Fc) were synthesized and purified by Sangon Biotech (Shanghai) Co., Ltd. The sequences of employed oligonucleotides are listed as follows.

Anti-ATP aptamer sequence: 5'-ferrocene(Fc)-ACCTGGGGGGAGTATTGCGGAGGAAGGT CAGAACCCAGGT-SH-3'; Its partly-complementary sequence: 5'-ACCTTCCTCCGCAATAC TCCCCCAGGT-3'

ATP, CTP, GTP and UTP were purchased from Sangon Biotech (Shanghai) Co., Ltd. 6-Mercapto-1-hexanol (MCH), tris (hydroxymethyl) aminomethane (Tris), tris (2-carboxyethyl) phosphinehydrochloride (TCEP) and Hemin were obtained from Sigma Aldrich. Hemin (0.2 mM) was diluted in HEPES buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, pH 7.4). Phosphate buffer solution (PBS, pH 7.4) contained NaCl (100 mM), Na₂HPO₄·12H₂O (10 mM) and NaH₂PO₄ (10 mM). All the other chemicals were of analytical grade. All aqueous solutions were prepared with ultrapure water (Milli-Q, Millipore).

Electrode preparation, DNA immobilization and ATP detection

The substrate gold electrodes (2.0 mm in diameter) were first polished with emery paper and further polished with 0.05 μ m alumina powder for 5 min and then successive ultrasonically cleaned in acetone and water for 5 min, respectively. These electrodes were then electrochemically cleaned in 0.5 M H₂SO₄ to remove any remaining impurities. Finally, the electrodes were washed with ultrapure water and dried in a nitrogen stream for further modification.

DNA duplexes (dsDNA) were prepared as follows: First, anti-ATP aptamer mixed with its complementary sequences (1 μ M each), then TCEP was added to reduce the disulfide bond of these two probes in this solution. After that, the mixture was heated to 90°C for 5 min and then slowly cooled to room temperature. After drying with purified nitrogen, the prepared gold electrode was immersed in this solution for 16 h to achieve dsDNA modified electrode. Finally, the modified electrodes were then rinsed with MCH for 1 h to displace non-specifically bound oligonucleotides.

For ATP detection, the dsDNA electrode was incubated in an ATP solution with different concentrations diluted by 20 mM Tris buffer containing 140 mM NaCl, pH 8.0 at 37°C for 3 h. It is well known that anti-ATP aptamer (ATA) can form G-quadruplex structure in the presence of

ATP. As a result, as the ATA structure switched from the duplex to the G-quadruplex, the ferrocene (Fc) moiety switched from the distal state to the proximal, which inducing a change in electrochemical signal. So, we can quantify ATP with a measurable electrochemical signal.

The dsDNA electrodes, after incubated in an ATP solution, can form G-quadruplex DNAzyme upon addition of 0.2 mM hemin solution for 3h. More importantly, the catalytic activity of G-quadruplex DNAzyme can be greatly improved in the presence of ATP. Based on these, H_2O_2 releasing from cells can also be detected.

Differential pulse voltammetric measurements were performed using a model 660C electrochemical workstation (CH Instruments) and a three-electrode system. The Fc-labeled dsDNA modified gold electrode was used as a working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the counter electrode.

Preparation of ATP extracts and H₂O₂ from K562 cells

K562 cells were harvested by trypsinization, and the number was estimated by a Petroff Hausser cell counter (USA) prior to each experiment. Then a suspension of 9.0×10^5 K562 cells (1 mL) were separated from culture medium by 5 min of centrifugation at 1500 rpm and then washed twice with PBS (pH 7.4).to obtain a homogeneous cell suspension. After that, the cells were disrupted by sonication for 20 min at 0 °C and the lysate was centrifuged at 10000 rpm for 10 min in order to remove the homogenate of cell debris. After diluting 4×10^2 and 4×10^5 fold, the cell lysate solutions with 2.25×10^3 /mL and 2.25/mL K562 cells can be employed for the following detection of ATP with the dsDNA modified electrode.

It is reported that ascorbic acid (AA) selectively kills cancer cells by generating $H_2O_2^{-1}$. Therefore, after a steady state background was attained, 4 μ M AA was injected to the cell suspension to motivate cells generation of H_2O_2 .

To achieve the optimal analytical properties of the electrochemical immunoassays, some experimental parameters including pH and ionic strength of the assay solution were studied. Fig.S1A shows the dependence of CV peak currents on pH of PBS. An optimal current was obtained at pH 7.4 PBS. A higher or lower pH resulted in the decrease of catalytic currents. In addition, as shown in Fig.S1B, the CV peak currents increased with the increment of ionic strength of assay solution and tended to level off after 10 mM PBS. Thus, a pH 7.4 PBS (10 mM) was chosen as the supporting electrolyte.



Fig.S1 Signal dependence of the developed electrochemical immunoassays on (A) different pH of PBS from 5.0 to 10.0; (B) different ionic strength of the assay solution for DNAzymes by using 1.0 mM ATP as a model in 1mM, 5 mM, 10 mM, 15 mM and 20 mM PBS solution, respectively.



Fig.S2 CV of modified electrode after treated with the lysate of 2.25/mL K562 cancer cells in 10 mM $\rm H_2O_2$ solution.

The amount of cellular adenosine in 2.25/mL K562 cells was estimated to be 7.47 nM based on the current of $3.59 \ \mu$ A at -0.6 V and curves depicted in Fig.4B. The amount of adenosines is $3.32 \ pmol \ per \ cell$.

Reference

1 Q. Chen, M. G. Espey, M. C. Krishna, J. B. Mitchell, C. P. Corpe , G. R. Buettner, E. Shacter and M. Levine, *P.N.A.S.*, 2005, **102**, 13604.