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Potassium-induced G-Quadruplex DNAzyme as a chemiluminescent sensing platform for highly selective detection of K⁺

Hong Wang,^a Dong Mei Wang,^a Ming Xuan Gao,^a Jian Wang,^{* b} Cheng Zhi Huang^{* a,b}

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A simple and highly selective chemiluminescence (CL) detection method for K⁺ was developed based on K⁺-stabilized G-quadruplex DNAzyme which catalyzed luminol-H₂O₂ reaction system. Herein, a G-quadruplex DNAzyme stemming from a common guanine-rich DNA sequence named PS2.M is introduced as a catalyst. Upon the addition of K⁺, PS2.M is promoted to fold into G-quadruplex as a cofactor binding with hemin, effectively catalyzing the redox reaction of luminol-H₂O₂ and generating CL emission and exhibiting good horseradish peroxidase (HRP) mimicking-enzyme activity. The intensity of CL shows a linear dependence on the concentration of K⁺ within a range of 2-120 μM with a limit of detection (3σ) of 1.66 μM, giving a vital clue to quantify K⁺ content in urine samples. This strategy firstly opens up CL as an effective and facile approach to detect K⁺ with high selectivity.

1. Introduction

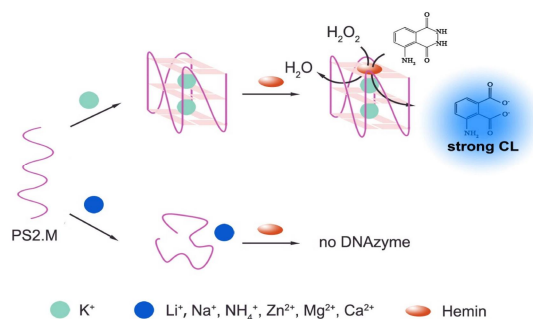
Potassium (K⁺) plays a vital role in all kinds of metastasis of biological organisms, such as transmitting nerve, maintaining extracellular and intracellular osmolality and muscular strong, activating enzyme, regulating the concentration of other ions and balancing pH in living cells.^{1,2} Abnormal concentration of K⁺ in extracellular and intracellular fluids will trigger a series of diseases or make body discomfort, such as disorder heart rhythms.^{3,4} Therefore a simple, sensitive and selective detection method of K⁺ is essential to biomedical clinical diagnosis, and it has been one of the most attractive issues for researchers. Although some prominent progresses have been achieved,¹⁻⁹ selective detection of extracellular K⁺ is still a challenge mainly due to the greatly excessive sodium and other trace metal elements in physiological conditions. Several techniques have been devised for resolving this difficulty in recent years,¹⁰⁻¹⁴ One prototypical example is the conspicuous and promising K⁺ sensors based on G-quadruplex DNAs,^{1-3, 6-12} wherein G-quadruplexes have special secondary structures with four-strand helix derived from G-rich sequences and play an important role in the binding of single-strand aptamers to ligands.^{15,16} Such structures are usually induced by alkali metal ions, especially K⁺,^{8, 17} whose appropriate size exactly make it locate in the cavity between two adjacent G-tetrads of a G-quadruplex, binding to eight carbonyl oxygen atoms from the G-tetrads.⁷ This coordination contributes K⁺ to most efficiently stabilizing G-quadruplexes among all alkali cations,^{7,15} which endows some G-quadruplex DNAs for K⁺ with high selectivity.^{7, 18} It is this selectivity that fluorescent or electrochemical "aptasensors" based on G-quadruplex have been developed recently.^{2,4} Besides the selective stabilization by K⁺, G-quadruplex also can be further balanced through binding to its

ligands. For example, hemin can couple with some G-quadruplex DNAs to form the effective DNAzymes.¹⁹ These studies have inspired our efforts to create a novel DNAzyme-based approach for K⁺ detection in a label-free way.

Catalytic nucleic acids (DNAzymes) have attracted substantial research efforts as amplifying labels for sensing events.²⁰ Different ion-dependent DNAzymes have been implemented as catalysts for the optical detection of metal ions, such as Pb²⁺, UO₂²⁺, and Cu²⁺ through cleavage of substrates modified with a fluorophore-quencher pair,²¹⁻²³ or by catalytic deaggregation of Au nanoparticles.^{21, 22, 24, 25} One of the most extensively studied DNAzyme is an artificial enzyme, the so-called hemin/G-quadruplex HRP-mimicking DNAzyme,^{26, 27} which can effectively catalyze the H₂O₂-mediated oxidation of ABTS²⁻ or TMB to the colored product,²⁷⁻²⁹ and catalyze the oxidation of luminol by H₂O₂ to yield CL.²⁵ This factitious enzyme is comparatively effortless to link with DNA sequences or other targets and acts as an amplifying label compared with protein peroxidases,²⁰ raising the possibility of the application of the DNAzyme in colorimetric or CL detection of various biochemical molecules.^{3, 5, 7, 8, 20-26, 28, 29} Recently, Willner's group and Wang's group^{30, 31} have reported some biosensors about the DNAzyme wherein the DNAzyme was found to greatly enhance CL.

CL has been proved of a significant and potent analytical technique,^{32,33} and has been featured by its low background, high sensitivity and simple instrumentation,²⁰ but there have been few reports concerning the detection of K⁺ through the combination of CL technique with methodology of DNA so far. Therefore, We herein firstly develop an alternative method to detect K⁺ with good selectivity in body fluid such as urine, as a comparison to the traditional detection method of K⁺ through fluorescence spectrophotometry,² electrochemistry,⁴ and colorimetry.⁹

As scheme 1 depicts, a G-rich sequence named PS2.M, which is sensitive to K^+ ,⁹ presents a random coil state under salt-deficient conditions, but folds into G-quadruplex with addition of K^+ , forming hemin-PS2.M DNAzyme. The formed DNAzyme can effectively catalyze the oxidation of luminol by H_2O_2 , giving rise to the signal of CL (see in Fig. 1).



Scheme 1 Schematic illustration by K^+ -induced G-quadruplex DNAzyme, PS2.M is chosen as the sensing element for CL detection of K^+ .

2. Experimental

2.1 Reagents and chemicals

Purified G-rich oligonucleotide (PS2.M: GTGGGTAGGG-CGGGTGG) were supplied by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). Luminol and hemin were commercially purchased from Sigma-Aldrich (St. Louis, MO, USA). 30% H_2O_2 , Triton X-100, and Ethylene Diamine Tetraacetic Acid (EDTA) were obtained from Chengdu Kelong Chemical Reagent (Chengdu, China). Dimethylsulfoxide (DMSO) was provided by from Chongqing Chuandong Chemical Co., Ltd. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Ningbo Dachuan Fine Chemical Co., Ltd (Ningbo, China).

The stock solution of hemin (2.38 mM) was prepared in DMSO, and stored in the dark at $-20\text{ }^\circ\text{C}$. The stock solution of luminol (10 mM) was prepared in 0.1 M NaOH and stored in the dark.²⁰ Other chemicals were of analytical-reagent grade or better. 18.2 M Ω cm at 25 $^\circ\text{C}$ water was used in all experiments.

2.2 Apparatus

The CL spectral measurements were conducted by using a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) with a series of high-energy optical filters of 230, 260, 290, 320, 350, 380, 400, 425, 440, 460, 490, 535, 555, 575, 620, and 640 nm between the CL flow cell and PMT, as described in ref³⁴. Circular dichroism (CD) spectrum was recorded on a model JASCO-810 spectrophotometer (Hitachi, Tokyo, Japan). Absorption spectra were measured on a UV-3010 spectrometer (Hitachi, Tokyo, Japan). All kinds of solutions were mixed through a QL-901 vortex mixer (Haimen, China).

2.3 Procedure

Formation of K^+ -stabilized hemin/G-quadruplexes. According to ref 19, DNA solution was prepared in the TE buffer (10mM

Tris-HCl, 0.1 mM EDTA, pH 7.4) and heated at 88 $^\circ\text{C}$ for 8 min to dissociate any intermolecules of the DNA, then gradually cooled to room temperature. KCl of different concentrations prepared in diluting buffer (10 mM Tris-HCl, 1% DMSO, 0.05% Triton X-100, pH 7.4) was added into 5 μM DNA twice the volume of KCl to fold for 40 min to form G-quadruplexes at room temperature. After that, an equal volume of 10 μM hemin was incubated with the G-quadruplexes for more than 1h to make the formation of DNAzyme.

Analysis of potassium by CL Method. The CL reaction system of luminol- H_2O_2 was catalyzed by hemin/G-quadruplex DNAzyme in the presence of K^+ at room temperature. Briefly, 40 μL of DNAzyme solution, 50 μL luminol (1 mM) and 160 μL reaction buffer (50 mM Tris-HCl, 1% DMSO, 0.05% Triton X-100, pH 9.0) were added to a cuvette. Then, the measurement was launched and 250 μL H_2O_2 (300 mM) was injected later.

3. Results and discussion

3.1 The enhanced CL of luminol by K^+ -sensitive DNAzyme

The catalysis on luminol- H_2O_2 CL reaction by K^+ -sensitive hemin/G-quadruplex complex is investigated. As Fig. 1 shows, the CL intensity of luminol- H_2O_2 is much weaker compared with that in the presence of hemin or DNAzyme. Nevertheless, hemin itself can serve as a catalyst for the oxidation of luminol by H_2O_2 , but inefficient.²⁵ The CL intensity is fairly weak without K^+ , while in the presence of K^+ , it is significantly enhanced. This distinct results could be understood by the fact that K^+ induced DNA to fold G-quadruplex which can bind with hemin to form a DNAzyme with stronger catalytic activity. It is demonstrated that the CL signal is improved by about 50-fold (Fig. 1 inset) upon the introduction of K^+ .

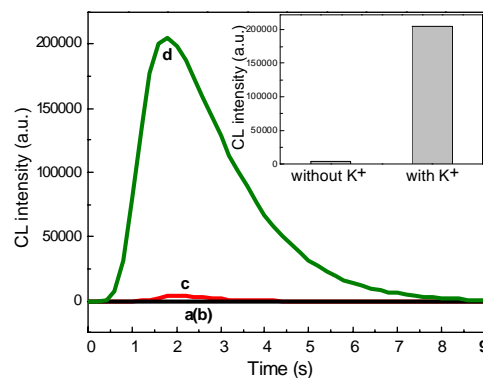


Fig. 1 Kinetic monitoring on luminol- H_2O_2 CL in the presence of (a) DNA; (b) DNA + K^+ ; (c) DNA + hemin; (d) DNA + K^+ + hemin. The inset shows the maximum CL intensities of c and d. Concentrations: luminol, 0.1 mM; H_2O_2 , 150 mM; DNA, 0.2 μM ; hemin, 0.2 μM ; K^+ , 40 μM .

3.2 Optimization of the chemiluminescent assay conditions

Some of crucial experimental conditions including the pH of the reaction buffer and the concentrations of luminol, H_2O_2 and hemin, which affect the chemiluminescent assay, are optimized. It is well known that CL of luminol- H_2O_2 reaction system is pH-dependent. The effect of the reaction buffer pH on the CL intensity of the luminol- H_2O_2 reaction system catalyzed by

PS2.M DNAzyme is investigated firstly. As shown in Fig. 2A, CL intensity is most sensitive in the reaction buffer at pH 9.0. Therefore, pH 9.0 Tris-HCl (50 mM Tris, 1% DMSO, 0.05% Triton X-100) is chosen as the optimal pH for the chemiluminescent assay.

The CL intensity is also concerned with the concentration of hemin. Either in low or high concentrations, there are almost no discrepancies existing between the catalytic effect of hemin alone and that of DNAzyme according to our results. The background signal is appreciably strong when the concentration of hemin exceeds 0.25 μM and thereby, 0.20 μM is selected as the proper concentration in the chemiluminescent assay.

In order to attain the reliability of data, high sensitivity and good selectivity in the chemiluminescent assay, we carried out the following optimized experiments respectively under the conditions of the optimal pH and concentration of hemin to optimize the concentration of luminol and H_2O_2 . Correspondingly, the optimized concentration of luminol was 0.1 mM, H_2O_2 was 300 mM (Fig. 2B).

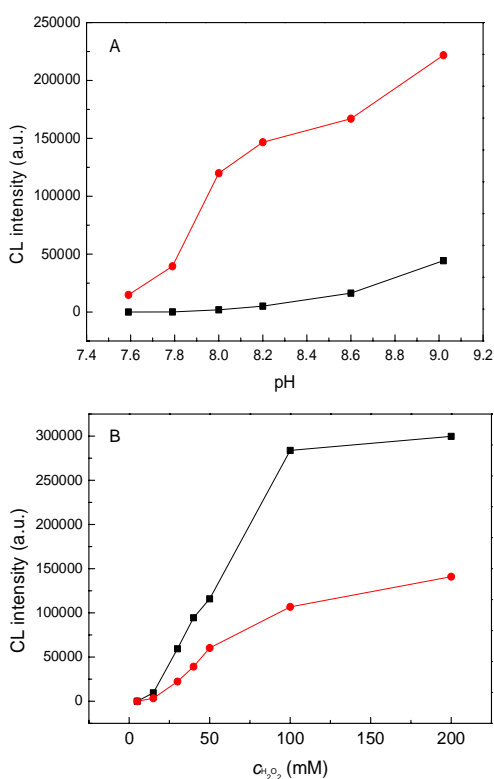


Fig. 2 Optimization of pH and concentration of H_2O_2 (A) CL intensity vs. pH of reaction buffer. Concentrations: DNA, 0.20 μM ; hemin, 0.20 μM ; K^+ , 40 μM ; luminol, 0.1 mM; H_2O_2 , 50 mM. (B) CL intensity vs. the concentration of H_2O_2 . Concentrations: DNA, 0.20 μM ; hemin, 0.20 μM ; K^+ , 40 μM ; luminol, 0.1 mM. pH 9.0. The black symbols refer experimental data, and the red symbols refer control.

3.3 Sensitivity and selectivity for potassium detection

Different concentrations of K^+ solutions were tested to investigate the sensitivity of the proposed strategy. Fig. 2A shows the changes of CL intensity dependent on the amount of K^+ added

into the sensing system. It is apparent that CL intensity is continually enhanced when increasing the concentration of K^+ , and tends to be constant at a certain concentration. Fig. 2B renders the relationship between ΔI (deviations of I and I_0 , wherein I_0 and I are the CL intensity in the absence and presence of K^+ , respectively) and the concentration of K^+ . The diagram illustrates that ΔI is linearly dependent on the concentration from 2 μM to 120 μM . From the inset of Fig. 2B, the equation $\Delta I = 2716 + 2112 c$, $r = 0.986$ is obtained and the detection limit (3σ) is calculated to be 1.66 μM . This suggests that this CL strategy can serve as an excellent knob for detecting the K^+ in human body fluids.

To inspect the specificity of sensing platform for quantifying K^+ , other common positive ions substituting K^+ were adopted to form different hemin/PS2.M complexes catalyzing luminol- H_2O_2 system (see in Fig. 3). The results reveal that only K^+ can remarkably drive PS2.M to form stable G-quadruplex and bind with hemin to enhance the CL of luminol- H_2O_2 reaction while other cations don't have this effect even at a ~ 10 -fold higher concentration than that of K^+ . It's inferred that this sensing platform exhibits dominant specificity for K^+ over other positive ions, and can be generalized to detect K^+ in extracellular environment or urine for its high selectivity.

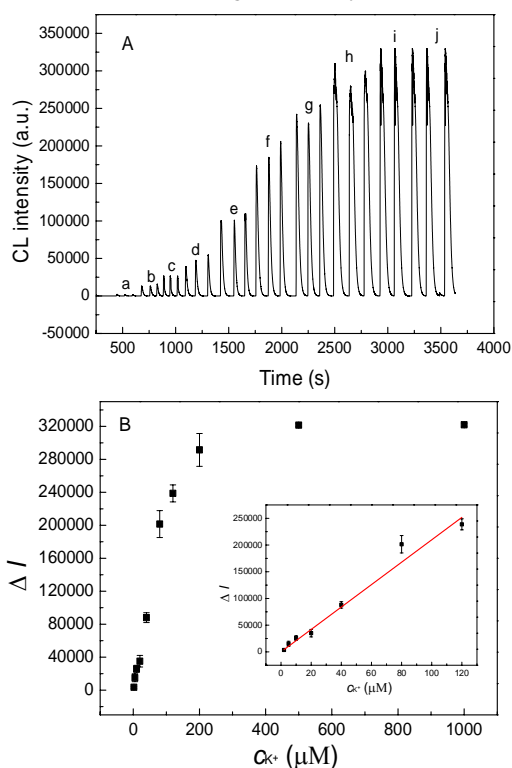


Fig. 3 Investigation of sensitivity of K^+ (A) Kinetic monitoring on the CL of luminol- H_2O_2 system upon addition of different concentrations of K^+ . (B) Plots of CL changes as a function of K^+ concentration. Concentrations: a–j, 0, 2, 5, 10, 20, 40, 80, 120, 200, 500 μM ; luminol, 0.1 mM; H_2O_2 , 150 mM; DNA, 200 nM; hemin, 200 nM; working buffer, pH 9.0.

In order to test the practical applicability of the proposed protocol, we detected K^+ in urine samples by using hemin-PS2.M

DNAzyme induced by K^+ in urine directly to catalyze luminol- H_2O_2 system. The results (see in Table 1) agreed with those obtained from hospital utilizing ion selective electrode, indicating that our sensing platform can be applied to real samples.

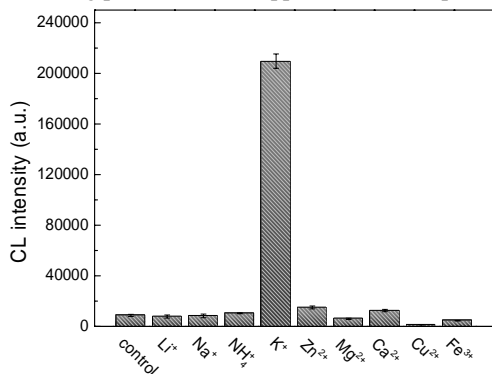


Fig. 4 Selectivity assays based on the catalytic to luminol- H_2O_2 system. Concentrations: luminol, 0.1 mM; H_2O_2 , 150 mM; DNA, 200 nM; hemin, 200 nM; K^+ , 40 μ M; other metal ions, 500 μ M. working buffer, pH 9.0.

Table 1 Analytical results of K^+ in urine

urine sample	content (mM, $n = 3$)	RSD(%)	ISE (mM)
1	13.48 ± 0.76	5.64	13.45
2	22.02 ± 0.43	1.95	22.01
3	18.74 ± 0.32	1.71	18.39
4	46.49 ± 1.74	3.74	47.44
5	48.14 ± 2.81	5.84	47.79
6	31.25 ± 1.03	3.30	31.69

presence of K^+ to bind with hemin to form a DNAzyme, which even though hemin itself has catalytic activity. K^+ can be detected

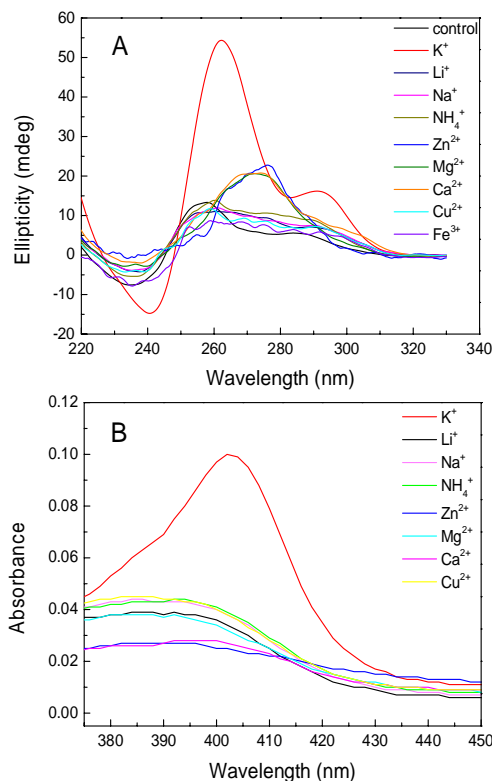


Fig. 5 Characterization of hemin/G-quadruplex (A) CD spectra of PS2.M induced by different metal ions. (B) UV-vis absorption spectra of the complexes of hemin and PS2.M in different metal ions condition. Concentrations: PS2.M, 10 μ M; K^+ , 5mM; other metal ions, 10 mM for CD. PS2.M, 2.5 μ M; hemin, 2.5 μ M; K^+ , 500 μ M; other cations, 5 mM for UV-vis. Tris-HCl buffer, pH 7.4.

Experimental conditions: concentrations, luminol, 0.1 mM; H_2O_2 , 150 mM; DNA, 200 nM; hemin, 200 nM; working buffer, pH 9.0.

3.4 Characterization of hemin/G-quadruplex DNAzyme

CD spectra and UV-vis spectra experiments were performed. The CD spectrum of PS2.M without any metal ions (Fig. 4A, black curve) presents a negative peak around 240 nm and a positive peak near 260 nm which coincides with the characteristic peaks of “parallel” G-quadruplex structure.² Both the peaks of the CD spectrum correspondingly increased after adding K^+ , (Fig. 4A) and the Soret band around at 402 nm of hemin displays a sharply hyperchromic effect (Fig. 4B) which are consistent with previews,⁹ suggesting that K^+ can induce and stabilize G-quadruplex, while other cations including Li^+ , Na^+ , NH_4^+ , Zn^{2+} , Mg^{2+} , Ca^{2+} , Cu^{2+} , Fe^{3+} don't have the effect on folding PS2.M into parallel G-quadruplex conformation from a random coil.

4. Conclusion

In summary, we have introduced a highly selective chemiluminescent method for the detection of K^+ in urine samples based on hemin/PS2.M-G-quadruplex DNAzyme used to catalyze luminol- H_2O_2 reaction system and acting as a label-free sensing element. PS2.M can fold into G-quadruplex in the

as low as 1.66 μ M, and the interference of other positive ions can be almost ignored owing to ultrahigh selective mechanism. In comparison with other reported assays, this study has important characteristics: (1) it broadens the application of CL for the easily functionalized DNAzyme; (2) its cost is greatly reduced and simplicity is achieved for the two roles the DNAzyme plays, sensing element and catalyst; (3) it can be readily applied to detect K^+ in real samples.

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Notes and references

- ^a Education Ministry Key Laboratory on Luminescence and Real-Time Analytical Chemistry, College of Chemistry and Chemical Engineering, Southwest University, Chongqing, PR China .
- ^b College of Pharmaceutical Sciences, Southwest University, Chongqing, PR China. Fax: +86-23-68254659; Tel: +86-23-68367257; E-mail: chengzhi@swu.edu.cn

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