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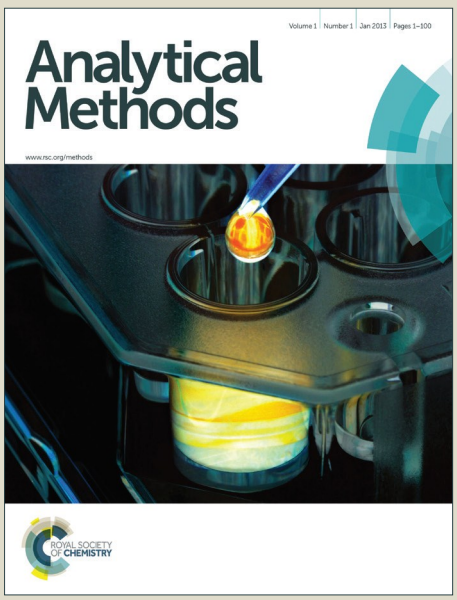

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ARTICLE

Conformational switch of G-quadruplex as a label-free platform for fluorescence detection of Ag^+ and biothiol

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Xiaoli Yang,[a] Wei Wei, Jianhui Jiang*, Guoli Shen and Ruqin Yu*

G-rich DNA sequences could be promote to form G-quadruplexes structures in the presence of water-soluble fluorogenic dye, thioflavin T (ThT), which is weakly fluorescent in the free state, but exhibits obvious fluorescence enhancement once it binds to G-quadruplex structures with high specificity. We developed a novel approach using G-quadruplex-specific fluorescence enhancement of ThT for label-free detection of Ag^+ and biothiol. This approach relies on the coordination of Ag^+ with guanine, which inhibits the formation of the G-quadruplex structure and delivers a quenched fluorescence signal, and the stronger coordination of biothiol with Ag^+, which releases Ag^+ from guanine and restores G-quadruplex with an activated fluorescence. This "turn-off/on" biosensor may provide a label-free, robust, yet sensitive platform for the detection of Ag^+ and GSH.

Introduction

Recent years have witnessed huge effort toward developing DNA-based Ag^+ sensors in scientific research and industrial innovations. Many biosensor approaches have been designed based on interactions between Ag^+ and cytosine–cytosine (C–C)[1], including colorimetric biosensors[2], electrochemical biosensors[3], as well as fluorescent biosensors[4]. Usually, these methods required time-consuming, high cost and complex purification steps, which further limited their applications. Therefore, it remains a challenge to develop a simple, cost-effective and label-free sensor for Ag^+ detection in complex system with high sensitivity and selectivity.

It has been reported that thioflavin T (ThT), a commercially available water-soluble fluorescent dye, could bind to the 22AG human telomeric DNA with high specificity[5]. Commonly, ThT is weakly fluorescent in the free state, but exhibits 2000-fold enhancement in its fluorescence upon interacting with the quadruplex DNA structures. This observation has been extended to several other quadruplexes[6]. Due to its high quadruplex DNA dependence and low background fluorescence, various ThT based label-free fluorescent sensor have been reported[7].

Here, we report a novel lable-free biosesor for Ag^+ and biothiol detection based on G-quadruplex conformational switching that can be sensitively probed by the fluorescence of ThT. Unlike common metal ions such as K^+, Pb^{2+}, Cu^{2+} which stabilize G-quadruplex, Ag^+ was found to destabilize DNA G-quadruplexes[8], because Ag^+ can coordinate with guanine bases in G-quadruplexes and destruct the G-quadruplex structure. The G-rich sequences can fold into quadruplex structures with ThT as inducer, however, In the presence of the Ag^+, G-rich oligonucleotide sequence can not fold into a G-quadruplex structure due to specific interaction between Ag^+ and guanine bases. On the basis of this principle, using Ag^+-induced structure-switching of G-rich sequences, we envision using this strategy to generate a label-free sensor for Ag^+ detection. Furthermore, in light of the stronger interaction between Ag^+ and the biothiol, this Ag^+-sensing system can be extended for the detection of biological thiol.

Experimental

Chemicals and materials

Purified oligonucleotides were synthesized and puried by HPLC from Sangon Biotechnology Co., Ltd. (shanghai, China). Their sequences are listed in Table S1 in ESI. ThT (3,6-dimethyl-2-(4-dimethylaminophenyl)benzo-thiazoliumcation) and reduced L-glutathione, minimum 99%(GSH) were purchased from Sigma-Aldrich Chemical Corporation. The used metal salts $Hg(Ac)_2$, $Pb(Ac)_2$, $FeSO_4$, $Fe_2(SO_4)_3$, $MgSO_4$, $AgNO_3$, $Al(NO_3)_3$, $Ni(Ac)_2$, $MnSO_4$, $Zn(NO_3)_2$, $NaNO_3$, Li_2CO_3, $CuSO_4$ and natural amino acids were purchased from Sinopharm Group Chemical Reagent Co.,Ltd. (Shanghai,China). All reagents were used as received without further purification. All solutions were prepared using ultrapure water, which was obtained through a

[a] State Key Laboratory of Bio/Chemo-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R . China.
Email: jianhuijiang@hnu.edu.cn; rqyu@hnu.edu.cn; Fax: +86-731-88822782; Tel: +86-731-88822577

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: DNA sequences and additional figures. See DOI: 10.1039/x0xx00000x

Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance > 18.25 MΩ. The stock solution of ThT (10 mM) was prepared in ultrapure water, stored in the dark at -20 °C, and diluted to the required concentration with aqueous buffer.

Instrumentation

The absorption spectra were recorded on a Shimadzu model 2450 UV-vis spectrophotometer (Shimadzu, Japan). The fluorescence measurements were performed on an F-7000 fluorescence spectrometer (Hitachi, Japan). The excitation and emission slits were both set as 5 nm. The emission spectra were collected by exciting the samples at 425 nm and scanning the emission from 460 nm to 600 nm. Circular Dichroism (CD) spectra were measured on a MOS-500 Circular Dichroism Chiroptical Spectrometer (Bio-logic, France). Spectra were recorded between 200 nm and 500 nm in 1 cm path length cuvettes. Spectra were averaged from 3 scans, which were recorded at 100 nm/min.

Procedures for the detection of Ag^+ and biothiols

The detection of Ag^+

The probe P1 (22AG human telomeric DNA, 300 nM) was dissolved with 10 mM Tris-HAc buffer (pH=7.0) containing 50 mM KAc. The DNA solution was heated to 95 °C for 5 min and then cooled down slowly to room temperature. Then 3 μM ThT was added to this solution and incubated at 37 °C for 15 min. Subsequently, freshly prepared Ag^+ of different concentrations was added to the mixture, which was incubated at 37 °C for another 20 min. The fluorescence spectra of the mixture were recorded at room temperature in a quartz cuvette.

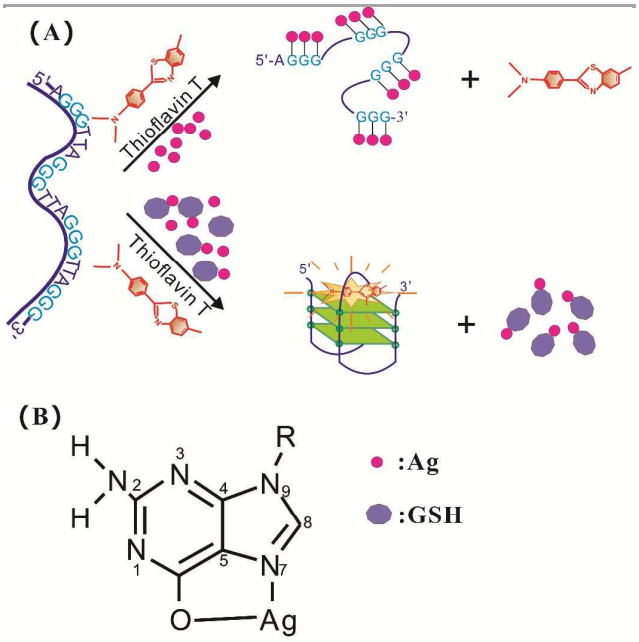
The detection of GSH

P1 (300 nM) was dissolved with 10 mM Tris-HAc buffer (pH=7.0) containing 50 mM KAc. The DNA solution was heated to 95 °C for 5 min and then cooled down slowly to room temperature. Firstly, 3 μM ThT was added to the mixture and incubated at 37 °C for another 15 min. Then 3.5 μM of freshly prepared Ag^+ was added to this DNA solution and freshly prepared GSH of different concentrations was then mixed together. The mixture was allowed to react at 37 °C for 20 min. The fluorescence spectra of the mixture were recorded at room temperature in a quartz cuvette afterwards.

Results and discussion

Sensing mechanism

The sensing mechanism of the proposed biosensor is depicted in Scheme 1. ThT is a water-soluble fluorogenic dye which can selectively bind to G-quadruplex and display very weak interaction with other DNA forms like single/double-stranded DNA and DNA triplex. Moreover, the interaction of ThT with G-quadruplex causes remarkable fluorescence enhancement in the visible region. This G-quadruplex-specific fluorescence enables a label-free platform for probing the conformational switching of G-quadruplex. In the presence of Ag^+, the chelation of Ag^+ with guanine which occurs at the special binding sites (scheme 1B) involved in the formation of G-quadruplex prevents the formation of hydrogen bonds between guanine. As a result, the G-quadruplex structure is coordinated by Ag^+, inducing the release of ThT with a concomitant decrease of the fluorescence signal. The decreased fluorescence signal gives a measure for the quantification of Ag^+. In the assay of biothiol such as glutathione (GSH), the presence of biothiol competes with guanine for Ag^+ because of the stronger interaction of biothiol with Ag^+. This interaction releases Ag^+ from guanine, restoring guanine for the formation of the G-quadruplex structure and re-activating the strong fluorescence of ThT for the quantification of biothiol. Therefore, based on the coordination of Ag^+ with guanine and the stronger coordination of biothiol with Ag^+, we can develop a robust and simple approach for the detection of Ag^+ and biothiols. Because ThT displays about 2000-fold fluorescence enhancement upon interacting with the quadruplex DNA structures, this approach may provide a cost-efficient strategy for highly sensitive and high signal-to-background detection of the conformational switching of G-quadruplex induced by Ag^+ or biothiols.



Scheme 1 (A) Scheme for the the disruption of G-quadruplex structure by Ag^+. (B) the interaction between Ag^+ and guanine.

Optimization of sensing conditions

To verify the feasibility of the experiment, we firstly study the binding efficiency between ThT/Ag^+ and various G-rich probes that can form G-quadruplex structures (Fig. S1). Ultimately, probe P1 (22AG human telomeric DNA) was selected as the optimal probe for further study. Next, the platform for detecting Ag^+ and GSH was studied in 10 mM Tris-HAc buffer including 50 mM KAc. The fluorescent signal of ThT was very weak at 492 nm (purple curve in Fig. 1). When ThT was added into the solution containing P1, the fluorescence intensity enhanced remarkably (black curve in Fig. 1). The above phenomenon well validated that ThT effectively induced the P1 to fold into G-quadruplex structure and caused the obvious fluorescence enhancement. However, when the solution

ARTICLE

containing P1 was added with freshly prepared Ag^+, followed by the addition of ThT, we could see that the fluorescence signal decreased dramatically on the account of the destruction of the G-quadruplex structure caused by Ag^+ (red curve in Fig. 1). On the basis of this principle, the detection of Ag^+ with high sensitivity and excellent selectivity will be realized. However, when Ag^+ and GSH were added into the solution containing G-quadruplex, followed by the addition of ThT, the fluorescence signal recovered due to the strong binding interaction between Ag^+ and GSH (blue curve in Fig. 1). This indicated that a simple, sensitive method for biothiols detection would be developed. The aforementioned experiments were carried out in buffer solutions containing potassium acetate. We also examined experimental phenomena in the same buffer without K^+ (Fig. S2 in ESI). Compared with the buffer containing K^+, the signal to background ratio was significantly lower in Fig. S2B. The ThT dye (~3 μM) showed its characteristic absorption peak with a maximum at 412 nm in 10 mM Tris–HAc buffer (pH 7.0, containing 50 mM KAc). Along with addition of P1 (up to ~10 μM) to the ThT solution the absorption peak of ThT shifted to 433 nm and 427 nm corresponding to in the absence and the

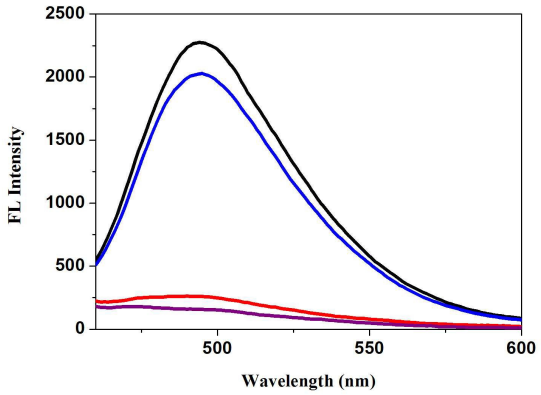


Fig. 1 Fluorescence emission spectra of ThT under different conditions: ThT (purple line); P1 + ThT (black line); P1 + ThT + Ag^+ (red line); P1 + ThT + Ag^+ + GSH (blue line), (ThT 3 μM, P1 300 nM, Ag^+ 3.5 μM, GSH 3.6 μM in 10 mM Tris-HAc buffer (pH=7.0) containing 50 mM KAc)

presence of K^+ respectively, indicating the strong interaction of ThT with P1. Then, the UV Spectrum displayed that the characteristic absorption peak respectively shifted to 412 nm and 433/427 nm corresponding to binding of Ag^+ and GSH.

Circular Dichroism (CD) Study.

In order to validate the conjecture, using CD measurements, we could observe the conformational change of DNA strands in different experimental conditions. It was observed that P1 displayed a characteristic CD peak at 292 nm in 10 mM Tris-HAc buffer (pH 7.0, containing 50 mM KAc) (purple curve in Fig. 2), the phenomena described above indicated that K^+ can promote the formation of (parallel or antiparallel) G-quadruplex (Fig. S3A of ESI). However, a parallel G-quadruplex was formed with the initial additions of ThT with the absorption peak at 450nm in CD spectrum (blue curve in Fig. 2). Compared with the 10 mM Tris-HAc buffer without K^+ (pH 7.0), a characteristic CD band of parallel G-quadruplex appeared at

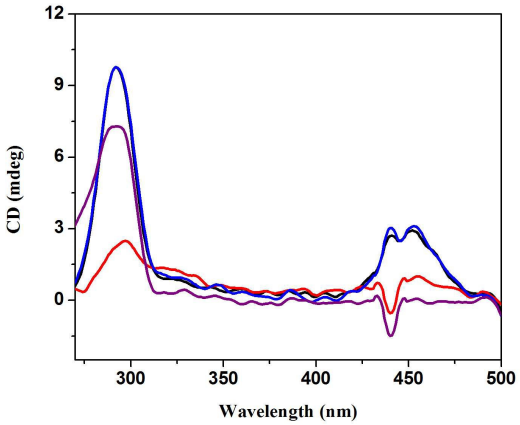


Fig. 2 CD spectra of P1 under different conditions: P1 (black line); P1 + ThT (blue line); P1 + ThT + Ag^+ (red line); P1 + ThT + Ag^+ + GSH (purple line), (ThT 100 μM, P1 10 μM, Ag^+ 116.7 μM, GSH 120 μM)

292 nm and 450 nm with the addition of ThT, but the peak intensity was significantly weaker than the system containing K^+(Fig. S3B of ESI). Then, the CD spectrum showed that the parallel G-quadruplex was destroyed with the addition of Ag+ and then restored by GSH regardless of the kind of buffer systems (red and black curve in Fig. 2 and black and blue curve Fig. S3B in ESI).

Sensitivity and Selectivity of the Ag^+ -Sensing System

To demonstrate the feasibility of this method for the quantitative determination of Ag^+, the fluorescence signals of the sensor as a function of Ag^+ concentration was measured. Under the optimal conditions (Fig. S4 and S5 in ESI), all the measurements were performed three times. As shown in Fig. 3A, the fluorescence responses of the system gradually decreased with increasing concentrations of Ag^+ in the range from 0 to 4 μM, which was attributed to the Ag^+-mediated disruption of G-quadruples structures and the resulting release of ThT. From Fig. 3B, there is a good linear correlation between the peak intensity and the Ag^+ concentration in the range from 50 nM to 3500 nM. The detection limit was estimated to be 2 nM according to the 3σ rule. Compared to several sensors for the detection of Ag^+[9], the result demonstrated that the proposed label-free biosensor had excellent detection performance, which made it favorable for drinking water monitoring. Then, we also examined the content of Ag^+ in tap water and Xiang River (Fig. S7 in ESI). To further test the selectivity of the Ag^+-sensing system, other metal ions including Ni^{2+}, Na^+, Li^+, Al^{3+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Cu^{2+} and Hg^{2+}, were detected under the same conditions (see Fig. S6 in ESI). In contrast, none of the detected metal ions excepted Ag^+ caused a significant reduction of the fluorescence signal. This result demonstrated that the excellent selectivity of the proposed sensing system toward Ag^+. In addition, a kinetic experiment has been provided to verify the superiority of this biosensor (see Fig. S12 in ESI).

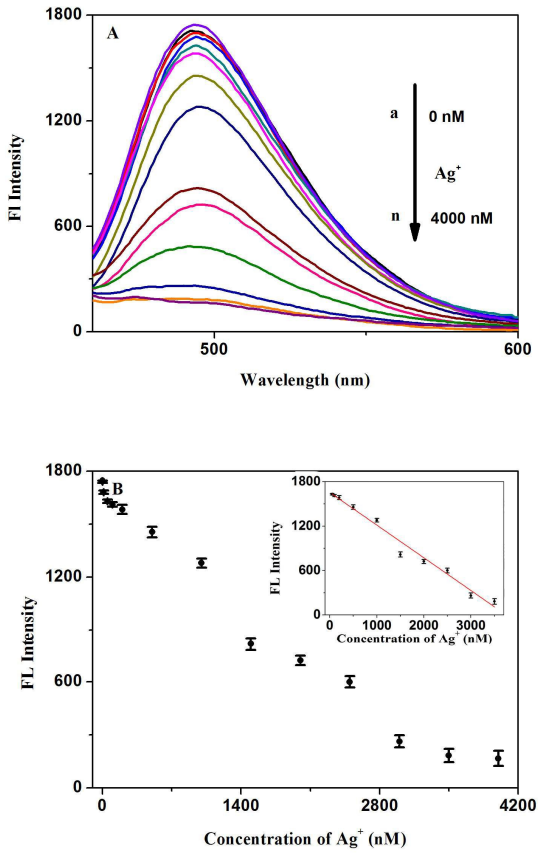


Fig. 3 (A) Fluorescence emission spectra of ThT in the presence of increasing amounts of Ag^+, the intensity decreased as the Ag^+ concentration increased (ThT 3 μM, P1 300 nM) (B) Fluorescence spectral response of the proposed platform at 495 nm versus Ag^+ concentration . Inset: dependence of fluorescence intensity on the Ag^+ concentration .

Sensitivity and Selectivity of the GSH-Sensing System

In order to study the feasibility of this method for GSH quantitation, the addition of 3.5 μM Ag^+ completely coordinate the G-quadruplex structures in reaction solution, then freshly prepared GSH of different concentrations was added to the mixture and the fluorescence intensity was monitored. As shown in Fig. 4A, the fluorescence intensity increased gradually as the concentration of GSH increased. When the concentration of GSH reached 3.6 μM, the fluorescence intensity reached a plateau. There is a good linear relationship between the peak fluorescence intensity and the concentration of GSH from 50 nM to 3600 nM (Fig. 4B). The detection limit of GSH was estimated to be 16 nM according to the 3σ rule. In addition, the limit of detection was better than those for many previous reported approaches[10]. To define the selectivity of the GSH-sensing system, we also examined the effects of several other amino acids and GSSG under the same conditions. As shown in Fig. S8 in ESI, in the absence of GSH, none of the detected amino acids excepted cysteine caused a significant growth in the fluorescence signal. Moreover, we also examined the feasibility of the method for the detection of cysteine (see Fig. S9-S11 in ESI). This result suggested an excellent selectivity of this sensing system for biothiols, which was due to high binding specificity of Ag^+ with biothiols.

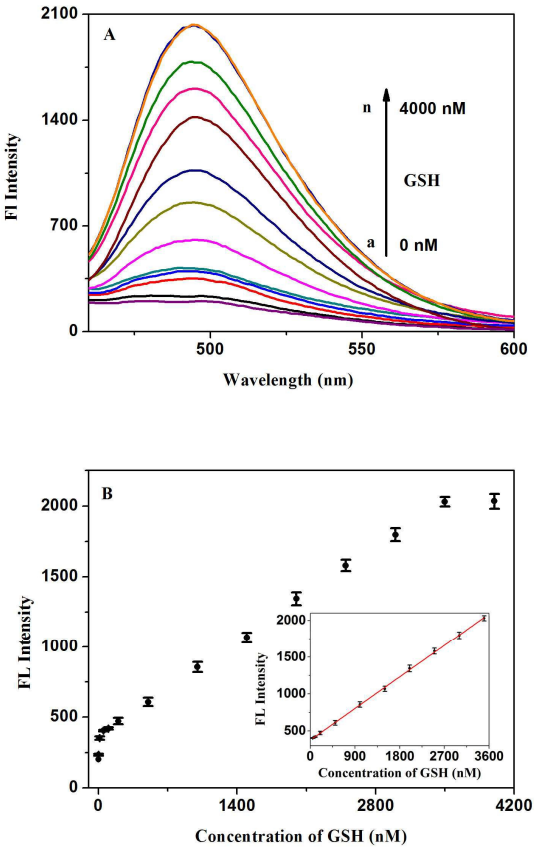


Fig. 4 (A) Fluorescence emission spectra of ThT in the presence of increasing amounts of GSH, the arrow indicating the signal changes with increase in GSH (ThT 3 μM, P1 300 nM, Ag^+ 3.5 μM) (B) Fluorescence spectral response of the proposed platform at 495 nm versus GSH concentration . Inset: dependence of fluorescence intensity on the GSH concentration .

Conclusions

In summary, we have demonstrated a novel label-free biosensor with high sensitivity and excellent selectivity for the detection of Ag^+ and biothiols based on the conformation switching of G-quadruplexs. This approach relies on the fact that Ag^+ could coordinate G-quadruplex structures by interacting with guanine bases, resulting in a sharp decrease in the fluorescence intensity. However, biothiols compete with quadruplex-forming G-rich oligonucleotide, which could make the fluorescence signal recover. Several important features are reflected in this assay. Firstly, the approach proposed herein is simple in design and provided a convenient "mix-and-detect" protocol for rapid detection of Ag^+ and biothiols in a homogeneous environment. It enables us to accomplish the analysis and detect rapidly (60 min) in one-step operation. Secondly, this sensor reveals high sensitivity and selectivity

towards Ag^+ and biothiols. The interference of other metal ions and natural amino acids would be ignored effectively.

Acknowledgements

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