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Accepted Manuscript

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Journal Name

ARTICLE

Triplex DNA logic gate based upon switch on/off their structure by Ag^+ /Cysteine

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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The formation of intramolecular triplex DNA can be regualated by Ag^+ and Cys (cysteine), which switch off / on the fluorescence of Oligonucleotides, 5'-TAMRA- TTC TCT TCC TCT TCC TTC TGA CGA CAG TTG ACT CTT CCT TCT CCT TCT CTT -BHQ-2-3'(Oligo 1) and 3'- GAA GGA AGA GGA AGA GAA-5'(Oligo 2). Based on this principle, sensors for Ag^+ and Cys are developed. The sensor for Ag^+ has a linear range of 2.5 nM - 40 nM and a detection limit of 1.8 nM, while the sensor for Cys has a linear range of 10.0 nM - 120.0 nM and a detection limit of 8.2 nM. Whatmore, the fluorescence is reversible by adding Ag^+ and Cys alternately, we constructed a DNA logic gate by using Ag^+ and Cys as input, and the fluorescence intensity as the output. The DNA logic gate is simple, fast response, and has a good reversibility.

Introduction

DNA is considered to be an excellent building block for molecular logic gates, which has incomparable advantage over silicon-based devices, and is thought to be the future of computer technologies.[1] DNA logic gate response to different input material[2] such as oligonucleotide,[3] enzyme,[4] protein[5] and metal ion[6] and small molecules.[7] While output can be mainly focused on luminescence,[8] electrochemiluminescence,[9] electrochemical signal[10] and color.[11] Most of these logic gates are based on DNA hybridization,[12] conformation change of G-quadruplex DNA,[13] strand displacement[14] and DNAzyme - catalyzed DNA cleavage. [4]

Triplex DNA plays an vital role in the biological process of gene expression, gene translation, DNA transcription, replication and site-specific DNA cleavage.[15] Therefore, the study of triplex DNA become more and more important, then hole transport,[16] sequence-specific recognition of duplex DNA[17] and other biosensor[18] were reported recently. There are two type of triplex DNA: one is anti-parallel triplex DNA, the homopurine strand bind to the homopurine strand of the duplex in an anti-parallel direction, which is stable under neutral pH conditions; the other type is parallel triplex DNA, the homopyrimidine strand bind to the homopurine strand of the duplex in a parallel direction, acidic pH environment is needed for the formation of parallel triplex DNA because the cytosine in the third strand need to be protonated.[19] Thereby the parallel triplex DNA can be switched on and off by pH environment.[20] For that reasons, Ricci et al.. developed proton - driven logic gate in the last year.[21] Recently, Ihara and coworkers reported that silver ions could specially recognize CGC base triplet and stabilize the parallel triplex DNA under neutral environment.[22] Here we construct a fluorescent DNA logic gate by using Ag^+ and cysteine to regulate the formation of intramolecular parallel triplex DNA.

Experimental

Chemicals and materials. All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). $AgNO_3$ was purchased from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China). Cysteine was purchased from BBI Co., Ltd. All chemicals were of

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† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

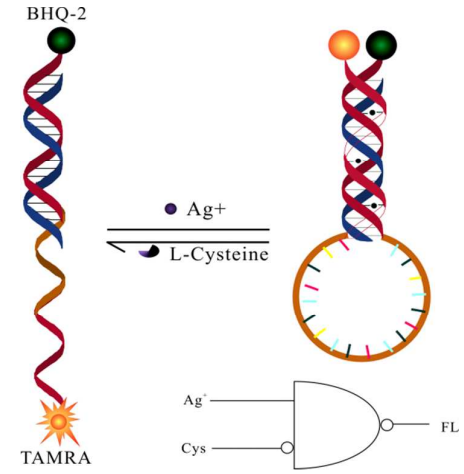
analytical reagent grade or better, and were used without further purification. Nanopure water (18.1 MΩ) was obtained from a 350 Nanopure water system (Guangzhou Crystalline Resource Desalination of Sea Water and Treatment Co. Ltd.). The working solution of 1.75 μM MB was obtained by diluting the stock solution with nanopure water and quantified by using UV-vis absorption spectroscopy according to the following extinction coefficients (ε_{260nm}, $M^{-1}cm^{-1}$): A= 15 400, G= 11 500, C= 7 400, T= 8 700.

Measurement of fluorescence spectroscopy. The fluorescent emission of solutions were measured with a RF-5301PC spectrofluorimeter (Shimadzu, Japan). Slit widths were both 5.0 nm, the excitation and emission wavelengths were set at 559 and 580 nm, respectively.

Measurement of circular dichroism spectroscopy. The circular dichroism (CD) spectroscopy was obtained with a J-810-150S spectropolarimeter (JASCO International CO. Ltd., Japan) at room temperature. The determination was performed over the wavelength range from 200 to 350 nm in 0.1cm path length cuvettes. The result was obtained by averaging 3 scans at the scanning rate of 100 nm per minute with a response time of 1.0 s and the bandwidth of 1.71nm.

Results and discussion

The scheme for sensing of Ag^+/cysteine based logic gate is demonstrated in Scheme 1, Oligonucleotide 5'-TAMRA- TTC TCT TCC TCT TCC TTC TGA CGA CAG TTG ACT CTT CCT TCT CCT TCT CTT -BHQ-2-3'(Oligo 1) and Oligonucleotide 3'- GAA GGA AGA GGA AGA GAA-5'(Oligo 2) is designed for the logic gate. Hybridization occurred between Oligo 1 and Oligo 2 in the absence of Ag^+, the fluorophore (TAMRA) and quencher (BHQ-2) are far away from each other, the fluorescence signal is strong. While intramolecular triplex DNA can form in the presence of Ag^+ because of its specific recognition to CGC base triplet.[22] However, the fluorescence emission is restored after further addition of cysteine because cysteine can strongly bind to Ag^+. Therefore, the fluorescence can be switched off and on by adding Ag^+ and cysteine alternately, which might be used to construct DNA logic gate.



Scheme 1 Sensing of Ag^+/cysteine and DNA logic gate.

To investigate the feasibility of the scheme, the fluorescence spectroscopy of the Oligo 1 is measured under the different conditions. As shown in Fig. 1, The Oligo 1 has strong fluorescence signal, it has no obvious change upon addition of Oligo 2 or silver ions individually. However, the fluorescence signal of Oligo 1 decreases dramatically when silver ions and Oligo 2 are added together. These results suggest that silver ion promotes the formation of triplex DNA and triggers the quenching of fluorescence.

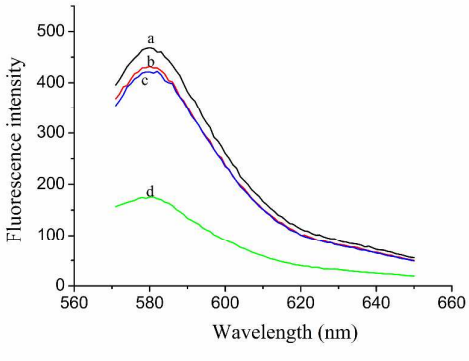


Fig. 1 Fluorescence spectra of Oligo 1 under different conditions.

(a) 17.5 nM Oligo 1; (b) a + 40 nM Ag^+; (c) a + 17.5 nM Oligo 2; (d) a + 17.5 nM Oligo 2 + 40 nM Ag^+, 20 mM PBS (pH 7.8), 30 mM $NaNO_3$, the incubation time of 10 minutes.

The fluorescence quenching may be caused by forming the intramolecular triplex DNA or intermolecular triplex DNA. In order to further investigate the mechanism of the fluorescence quenching, Oligonucleotide 5'-TAMRA-TTC TCT TCC TCT TCC TTC TGA CGA CAG TTG ACT CTT CCT TCT CCT TCT CTT-3'(Oligo 3) and Oligonucleotide 5'-TTC TCT TCC TCT TCC TTC TGA CGA CAG TTG ACT CTT CCT TCT CCT TCT CTT-BHQ-2-3' (Oligo 4), which have the same sequence with that of Oligo 1 and be labeled individually with the fluorophore and the quencher, are designed for the research. As shown in Fig. 2, there has strong fluorescence of the mixture of Oligo 2, Oligo 3 and Oligo 4, no obvious change occurs with further addition of silver ions, which suggests that no intermolecular triplex DNA is formed between Oligo 1 and Oligo 2 in the presence of Ag^+. On the contrary, the fluorescence intensity of the mixture of Oligo 1 and Oligo 2 decreased dramatically upon addition of Ag^+. These results reveal that the decrease of the fluorescence is not due to the formation of intermolecular triplex DNA, but intramolecular triplex DNA.

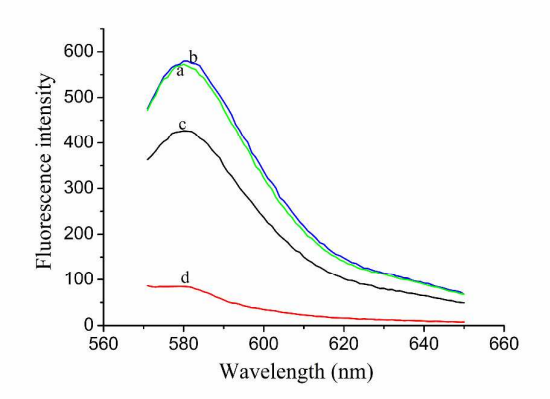


Fig. 2 Fluorescence spectra of the mixture under different conditions.

(a) 17.5 nM Oligo 3 + 17.5 nM Oligo 4 + 35 nM Oligo 2; (b) a + 50 nM Ag^+; (c) 17.5 nM Oligo 1 + 17.5 nM Oligo 2; (d) c + 50 nM Ag^+, 30 mM $NaNO_3$, 20 mM PBS (pH 7.8), 10 minutes of the incubation time.

To obtain the optimal experiment effect, the experiment conditions such as the concentration of $NaNO_3$, pH value, the concentration of Oligo 2 and DNA hybridization time are optimized. The optimal conditions is selected by obtaining the maximum change of fluorescence intensity (ΔI) in the addition of the same amount of silver ions. ΔI was defined as $\Delta I = I_{f\,(no\ Ag+)} - I_{f\,(Ag+)}$, $I_{f\,(Ag+)}$ represents the fluorescence intensity of the solution of Oligo 1 and Oligo 2 in the presence of silver ions, while $I_{f\,(no\ Ag+)}$ represents the fluorescence intensity of the solution in the absence of silver ions. After careful investigation (See ESI), 30 mM $NaNO_3$, pH 7.8, 17.5 nM Oligo 2 and 10 minutes of DNA hybridization time are selected as optimum conditions and used for the research.

Measuring of Ag^+ and Cysteine

Under optimum conditions, we measured the fluorescence intensity of the solution at 580 nm of different concentrations of silver ions. As shown in Fig. 3, there was a good linear relationship between the concentration of silver ion in the range of 2.5 nM - 40 nM and the fluorescence intensity in a solution. The linear regression equation was: $I_f = 408.7 - 6.8\ C_{Ag+}$ (C_{Ag+} : nM), the correlation coefficient r = 0.9943, and the detection limit (3δ / Slope) was 1.8 nM.

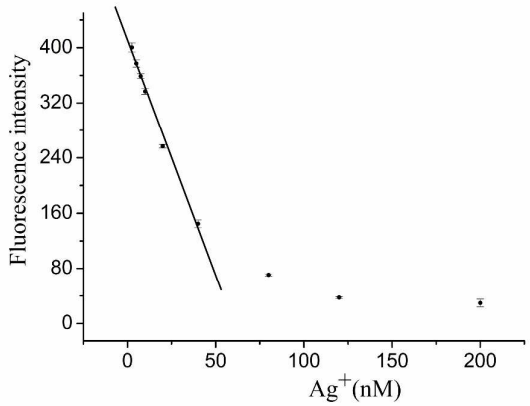


Fig. 3 Plot of fluorescence intensity of solutions versus Ag^+ concentration. Experimental conditions: 20 mM PBS (pH 7.8), 30 mM $NaNO_3$, 17.5 nM Oligo 1, 17.5 nM Oligo 2, the incubation time of 30 minutes.

In order to evaluate the selectivity of the method for silver ions, we made a comparison with the fluorescence intensity of solutions containing 40 nM Ag^+ or other eleven kinds of 1.0 μM metal ions. Under the same conditions, the fluorescence intensity of solutions containing 40 nM Ag^+ was significantly lower than the blank sample, while 25 times of Ba^{2+}, Sr^{2+}, Ca^{2+}, Al^{3+}, Zn^{2+}, Cr^{3+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Mg^{2+} , Pb^{2+}, Fe^{3+} and Cu^{2+} have no effect on the fluorescence intensity. Although the fluorescence intensity decreased dramatically in the presence of 1.0μM Hg^{2+}, the interference of Hg^{2+} can be removed by the addition of EDTA (See ESI). These results suggest that the method has good selectivity for sensing Ag^+.

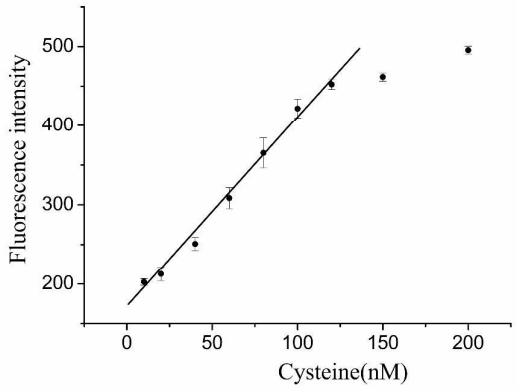


Fig. 4 Plot of fluorescence intensity of solutions versus the concentration of cysteine.

Experimental conditions: 20 mM PBS (pH 7.8), 30 mM $NaNO_3$, 17.5 nM Oligo 1, 17.5 nM Oligo 2, 50 nM Ag^+, the incubation time of 3 minutes after the addition of cysteine.

Cysteine (Cys) could strongly interact with silver ions.[23] Therefore, the addition of Cys could consume Ag^+, and restored the fluorescence signal. As shown in Fig. 4, the fluorescence intensity increased with the concentration of Cys, there had a good linear relationship between fluorescence intensity and Cys concentration over the range of 10.0 nM - 120.0 nM. The linear regression equation was I_f = 166.9 + 2.4 C_{Cys} (C_{Cys}: nM), the correlation coefficient (r) was 0.9902, and the detection limit (3δ / Slope) for Cys was 8.2 nM. Moreover, we explored the selectivity of the method, common amino acids didn't interfere with the detection of cysteine (See ESI).

In order to investigate the mechanism of fluorescence enhancement caused by the interaction of Cys and Ag^+, the circular dichroism spectroscopy of mixture of Oligonucleotides are investigated. Oligonucleotide 5'- TTC TCT TCC TCT TCC TTC TGA CGA CAG TTG ACT CTT CCT TCT CCT TCT CTT-3'(Oligo 5), which is label free and has the same sequence with that of Oligo 1, is designed for investigating the CD spectroscopy. As shown in As shown in Fig. 5, there is no negative peak at 210nm for the mixture of Oligo 2 and Oligo 5, which is the marker for triplex DNA.[24] This result indicates that no triplex DNA formed between Oligo 2 and Oligo 5 in the absence of Ag^+. However, there appears a negative peak at 210nm upon addition of 60 μM Ag^+ into the mixture of Oligo 2 and Olgio 5, which reveals the formation of triplex DNA between Oligo 2 and Oligo 5 in the presence of Ag^+. While the negative peak at 210nm disappears with further addition of 60 μM Cys. These results indicate that no triplex DNA is formed between Oligo 2 and Oligo 5 in the presence of equal concentration

of Ag^+ and Cys. Therefore, the fluorescence signal of Oligo 1 is controlled by whether the formation of triplex DNA between Oligo 1and Oligo 2 occurs or not. The Ag^+ can turn on the triplex DNA and switch off the fluorescence signal, while Cys can consume Ag^+ and switch on the fluorescence.

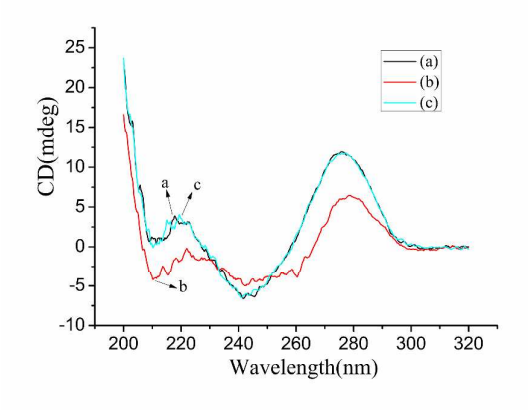
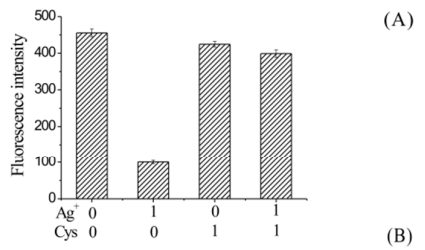


Fig. 5 CD spectroscopy of Oligonucleotides under different conditions. (a) 7.5 μM Oligo 5 + 7.5 μM Oligo 2; (b) a + 60 μM Ag^+; (c) b + 60μM Cysteine. Experimental conditions: 0.1 M PBS (pH 7.8), the incubation time: 4 minutes.

For above-mentioned reasons, a logic gate can be constructed with Oligo 1, Oligo 2, Ag^+ and Cys, here we define Ag^+ and Cys as input, and the fluorescence intensity at 580 nm as output. For input, we define the presence of Ag^+ and Cys is "1", and the absence is defined as "0"; for output, we define the fluorescence intensity more than 350 as "1", and less than 200 as "0." As shown in As shown in Fig. 6, when there is no input or only input of Cys, there is no factor to stabilize the triplex DNA and the fluorescence emission is strong(more than 350), so the output is "1". When there is only Ag^+ input, the fluorophore and quencher are in close proximity because it turning on the triplex DNA, the fluorescence is quenched and become very weak(less than 200), the output is "0". When Ag^+ and Cys are added as input together, silver ions would bind with Cys and don't stabilize the triplex DNA, the fluorescence emission is strong(more than 350), the output is "1".



Input		Output
Ag^+	Cys	Fluorescence
0	0	1
1	0	0
0	1	1
1	1	1

Fig. 6 (A) Fluorescence intensity of the logic gate in the presence of different inputs, (B) The truth table of the logic gate.

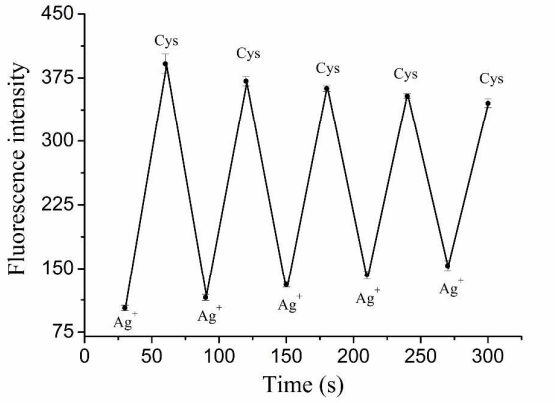


Fig. 7 Reversible fluorescence signal of the logic gate by adding 200 nM Ag^+ and 200 nM Cys alternately.

Reversible operation is an important performance of logic gate. Thus the reversibility of the logic gate is investigated, as shown in As shown in Fig. 7, Although the fluorescence intensity has dropped

slightly after adding Ag^+ and Cys circularly, it is obvious that the fluorescence can be switched off/on by adding Ag^+ and Cys alternately. When Ag^+ act as input, it can induce the formation of triplex DNA and trigger the quenching of the fluorescence, result in the output of "0". With further use Cys as input, it can bind strongly with Ag^+, consume Ag^+ and destroy the structure of triplex DNA, and then the fluorescence is restored, so far the output is "1." As demonstrated in As shown in Fig. 8, the logic gate has good reversibility. Meanwhile, the advantage of the logic gate is that the response time is very short, the output value can be measured in three minutes after addition of input. The proposed DNA logic gate is in accordance with reversible two-input IMPLICATION logic gate.[25] The aforementioned DNA IMPLICATION gates is based upon the formation of G-quadruplex DNA, while the proposed logic gate is based upon the turn on/off the triplex DNA.

Conclusion

In conclusion, triplex DNA can be turned on / off by Ag^+ and Cys, which switch off / on the fluorescence of Oligo 1. Based upon which, two fluorescent sensors for Ag^+ and Cys are developed. The sensor for Ag^+ has a linear range of 2.5 nM - 40 nM and a detection limit of 1.8 nM. While the sensor for Cys has a linear range of 10.0 nM - 120.0 nM and a detection limit of 8.2 nM. Because the fluorescence is reversible by adding Ag^+ and Cys alternately, we construct a DNA logic gate by using Ag^+ and Cys as input, and the fluorescence intensity as the output. The DNA logic gate is simple, fast response, and has a good reversibility.

Acknowledgements

The work was supported by National Natural Science Foundation of China (No: 21375153), the Fundamental Research Funds for the Central Universities (No: 13lgzd05), open project of Beijing National Laboratory for Molecular Sciences and The Science and Technology Foundation of Guizhou Province(Qian Ke He J[2013]NO.2263).

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