Triplex DNA logic gate based upon switch on/off their structure by

Ag⁺/Cysteine

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Experimental conditions

Chemicals and materials

All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). AgNO₃ was purchased from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China). Cysteine was purchased from BBI Co., Ltd. All chemicals were of analytical reagent grade or better, and were used without further purification. Nanopure water (18.1 M Ω) 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 μ M 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⁻¹cm⁻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.

Results and discussion

Because the ionic strength could have an influence on the stability of triplex DNA, we investigated the effect of NaNO₃ concentration on ΔI . As shown in SFig. 1, ΔI first increased with NaNO₃ concentration, which could be due to more sodium ions could decrease the repulsive force between phosphate backbone and promoted the formation of triplex DNA in the presence of silver ion. However ΔI reached to a platform at NaNO₃ concentration of 30 mM and decreased with increase of NaNO₃ concentration is higher than 40 mM, which was because too much monovalent cations weren't favorable for the formation of triplex DNA.¹ So 30 mM was chose as the optimal NaNO₃ concentration.



SFig. 1 Effect of NaNO₃ on Δ I. 20 mM PBS (pH 7.8), 17.5 nM Oligo 1, 17.5 nM Oligo 2, 20 nM Ag⁺, the incubation time of 30 minutes.

The stability of triplex DNA was influenced by pH value of the solution, so we studied the effect of pH value on ΔI . As shown in SFig. 2, ΔI first increased and then decreased with increasing pH value. The low pH brought about the formation of a small amount of intramolecular triplex DNA without silver ions, but too high pH would be unfavorable for the formation of triplex DNA. So we chose 7.8 as the optimal pH value.



SFig. 2 Effect of pH on Δ I. 20 mM PBS, 30 mM NaNO₃,17.5 nM Oligo 1, 17.5 nM Oligo 2, 20 nM Ag⁺, the incubation time of 30 minutes.

The Oligo 2 participated in the formation of triplex DNA and its concentration could have influence on ΔI , so we investigated the effect of Oligo 2 concentration on ΔI . As shown in SFig.3, ΔI increased with Oligo 2 concentration and reached a platform at Oligo 2 concentration of 17.5 nM. That was because more amount of

triplex DNA can be formed with an increasing number of Oligo 2, but too much Oligo 2 became saturated. So 17.5 nM was chose as the optimal Oligo 2 concentration.



SFig. 3 Effect of Oligo 2 concentration on \triangle I. 20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 20 nM Ag⁺, the incubation time of 30 minutes.

DNA hybridization time influenced the formation of triplex DNA, we investigated the effect of reaction time on ΔI . As shown in SFig. 4, ΔI tended toward stability when the solution was incubated for 30 minutes. So 30 minutes was determined as the incubation time.



SFig. 4 Effect of the incubation time on ΔI. 20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 17.5 nM Oligo 2, 20 nM Ag⁺.



SFig. 5 Melting curve of triplex DNA in the presence of Ag⁺. 20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 17.5 nM Oligo 2, 40 nM Ag⁺



SFig.6 Effect of temperature on the fluorescence of the mixture of Oligo1 and Oligo 2 in the absence of Ag^+ .

20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 17.5 nM Oligo 2



SFig. 7 Selectivity of the method. Experimental conditions: 20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 17.5 nM Oligo 2, the incubation time of 30 minutes, 40 nM Ag⁺ and other metal ions of 1 μM,10 μM EDTA.



SFig. 8 Selectivity of the method. Experimental conditions: 20 mM PBS (pH 7.8), 30 mM NaNO₃, 17.5 nM Oligo 1, 17.5 nM Oligo 2, 50 nM Ag⁺, the incubation time of 3 minutes after the addition of 120 nM cysteine or 1.2μ M other amino acids.

References

1 V. N. Soyfer, V. N. Potaman, Triple-helical nucleic acids, Springer, New York, 1995.