

Sequence-specific recognition of double-stranded DNA with molecular beacon with the aid of Ag⁺ under neutral pH environment

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Chemicals and materials

MB and all other oligonucleotides were obtained from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). All chemicals were analytical reagent grade and 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.) and was used for all experiments. MB stock solutions (100 μM) were prepared by solving MB in nanopure water and stored in the dark at -20°C. The working solution of 5.0 μM MB was obtained by diluting the stock solution with 20 mM PBS buffer (pH 7.4) and quantified by using UV-vis absorption spectroscopy according to the following extinction coefficients ($\epsilon_{260\text{nm}}$, M⁻¹cm⁻¹): A= 15400, G= 11500, C= 7400, T= 8700. The target dsDNA and control dsDNA were prepared by annealing equi-molar concentrations of the corresponding complementary single-stranded DNA.

Measurement of fluorescence spectrum

10 μL of 5.0 μM MB were mixed with 490 μL PBS buffer solution that contained different amounts of target dsDNA, after 1.5 hours of incubation (25°C), the

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fluorescence signal was measured with a RF-5301PC spectrofluorimeter (Shimadzu, Japan). Slit widths were both 5.0 nm, the excitation and emission wavelengths were set at 495 and 518 nm, respectively.

Measurement of CD spectroscopy

The circular dichroism (CD) spectroscopy was obtained with a J-810-150S spectropolarimeter (JASCO International CO. Ltd., Japan) at room temperature. The measurement was performed over the wavelength range from 200 to 350 nm in 0.1 cm 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 1.71 nm.

Results

The recognition of dsDNA depended on the concentration of spermine and Ag^+ , pH environment and incubation time. Therefore, the effect of these factors were investigated, it was estimated with the change of fluorescence intensity (ΔI), which was defined as $\Delta I = I_{\text{target dsDNA}} - I_{\text{no target dsDNA}}$ (here $I_{\text{target dsDNA}}$ denotes the fluorescence intensity of the MB in the presence of target dsDNA, and $I_{\text{no target dsDNA}}$ represents the signal in the absence of target dsDNA).

The fluorescence emission of MB fluorophore and formation of triplex DNA were both influenced by the pH environment, so the effect of pH value on ΔI was investigated. The ΔI increased with the pH value over the range of 6.0-7.4 and reached the maximum at pH 7.4. Although the acidic condition was favourable to the triplex formation, but it was unfavorable to the fluorescence of the FAM fluorophore, the fluorescence intensity of FAM increased with the pH value. However, the ΔI decreased dramatically when the pH value was higher than 7.4, which might be due to the reason that triplex DNA could not formed under the higher pH environment.

Hence, pH 7.4 was used for the research. The sensor works at neutral pH environment makes it possible to be applied in the measurement of DNA in cell in the future.

Due to electrostatic repulsion between dsDNA and MB, thereby multivalent cations such as Mg^{2+} and polyamines were usually used to neutralize the negative charges of DNA for triplex formation. Here we selected spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) as the stabilizer, and the effect of its concentration was investigated (See ESI). The ΔI increased with the spermine concentration over the range from 0.01 to 0.30 mM, which was attributed to the stabilization property of spermine for triplex formation^{1,2}. However, the ΔI decreased gradually when the concentration of spermine was higher than 0.30 mM, which might be explained that excess spermine could induce DNA condensation and precipitation³. So 0.30 mM of spermine was used in this research.

Fluorescence restoration of MB depends on the hybridization between MB and target dsDNA, so the fluorescence intensity might be affected by the hybridization time, and the effect of hybridization time was investigated. The ΔI increased with the hybridization time over the range of 0-90 minutes, and reached a platform after 90 minutes. Therefore, 90 minutes was selected for the hybridization time.

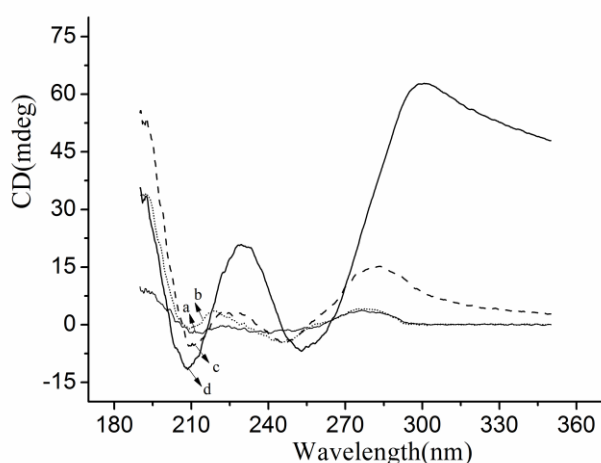


Figure S1 Circular dichroism spectroscopy of MB, target dsDNA and their mixture in

20 mM PBS buffer (pH 7.4) containing 0.30 mM spermine. 45mins of incubation time

- a. 7.5 μM of unlabeled MB; b. 7.5 μM of target dsDNA;
c. a + b ; d. c + 4.0 μM Ag^+ .

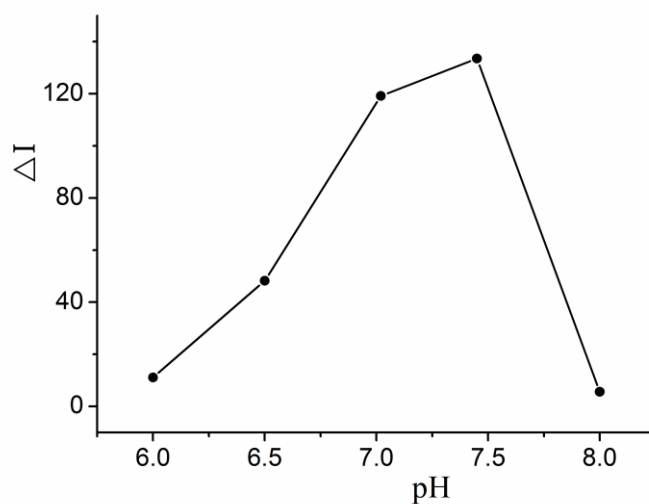


Figure S2 Effect of pH on ΔI . Experimental conditions: 100 nM of MB, 50 nM of target dsDNA, 15.0 mM of NaNO_3 , 0.30 mM of spermine, 0.40 μM of Ag^+ , 1.5 hours of incubation time.

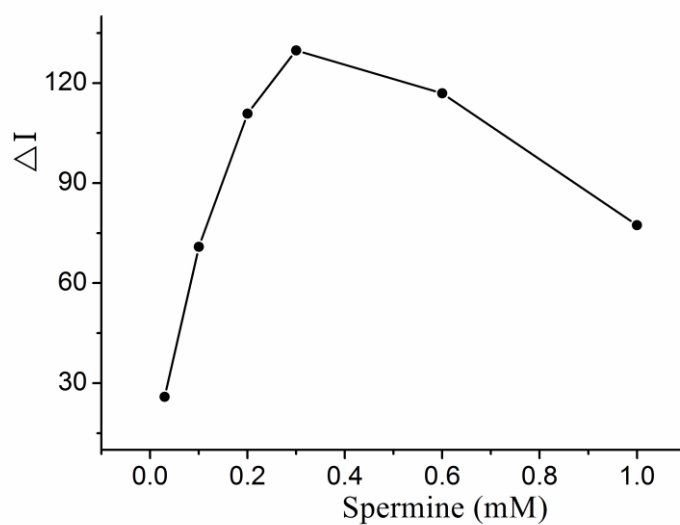


Figure S3 The effect of spermine concentration on the ΔI .

20 mM of PBS (pH 7.4), 100 nM of MB, 50 nM of target dsDNA,
15.0 mM of NaNO_3 , 0.40 μM of Ag^+ , 1.5 hours of incubation time.

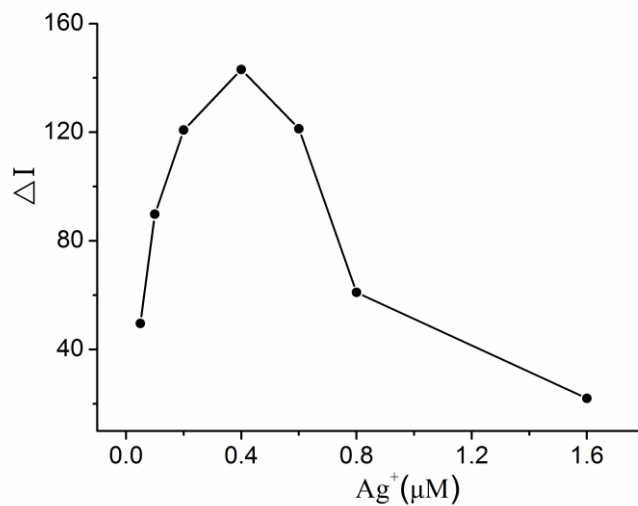


Figure S4 Effect of the concentration of Ag^+ on the ΔI .

20 mM of PBS (pH 7.4), 100 nM of MB, 50 nM of target dsDNA, 15.0 mM of
 NaNO_3 , 0.30 mM of spermine, 1.5 hours of incubation time.

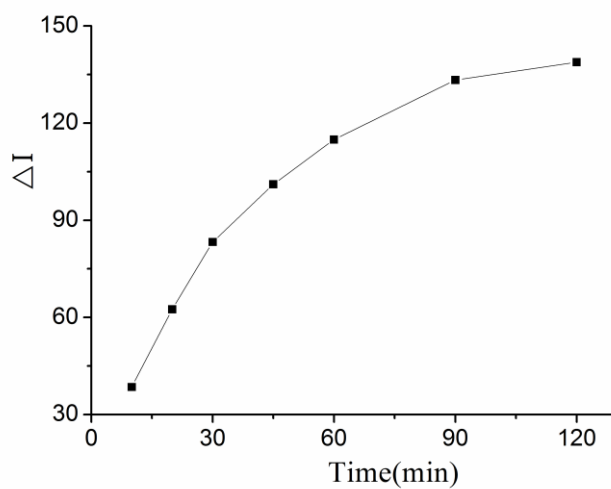


Figure S5 Effect of incubation time on the ΔI .

20 mM of PBS (pH 7.4), 100 nM of MB, 50 nM of target dsDNA, 15.0 mM of
NaNO₃, 0.30 mM of spermine, 0.40 μ M of Ag⁺.

Reference

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