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Study on Triplex DNA by Use of Molecular "Light Switch" Complex of $Ru(phen)_2(dppx)^{2+}$

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Abstract: A new method for the study of triplex DNA is established according the fluorescence enhancement of molecular "Light Switch" complex of $Ru(phen)_2(dppx)^{2+}$ when it intercalate into triplex DNA. Because the fluorescence intensity of $Ru(phen)_2(dppx)^{2+}$ bonded to triplex DNA is in the case higher than that bonded to duplex DNA in certain range of DNA concentration, the method is much more sensitive than other methods reported previously.

Keywords: $Ru(phen)_2(dppx)^{2+}$, molecular "Light Switch", triplex DNA.

Studies on the structural diversity of DNA have drawn much attention in recent years. Felsenfeld *et al.*[1] first proposed the structure of triple-helix RNA and successfully synthesized a kind of triplex RNA. Mirkin and coworkers[2] discovered a triplex DNA (H-DNA) in an acid solution of plasmid. In the same year, Dervan *et al.*[3] attached a man-made single-stranded DNA to real gene DNA and locally formed a kind of interstrand triplex. This could be used to incise duplex DNA in a precise manner, showing high potential for applications as a gene controller, man-made restriction endonuclease, and preventing DNA from combining with protein *et al.* Research on three-stranded DNA is becoming an attractive field in biochemistry and molecular biology. It is urgent to establish an efficient and sensitive method to study on triplex DNA. Bai *et al.*[4,5] developed a method according to the increasing fluorescence of EB when bonded to triplex DNA. But this method was limited because the fluorescence of EB bonded to duplex DNA is higher than bonded to triplex DNA. Molecular "Light Switch" is a kind of complex that does not luminescence in water but emits in nonaqueous solvents or in the presence of DNA. In our previous study, "Light Switch" complex of $Ru(phen)_2(dppx)^{2+}$ (phen=1, 10 - phenanthroline, dppx=7, 8-dimethy-ldipyrido [3, 2 - a: 2′, 3′-c] phenanthroline) has been used to the determination of duplex DNA[6,7]. Here we have established a new fluorescence method to study the triplex DNA constituted by $d(A)_{12}$ and $d(T)_{12}$ by use of $Ru(phen)_2(dppx)^{2+}$. Compared with the method of EB, this method has two advantages: higher sensitivity and the fluorescence enhancement of $Ru(phen)_2(dppx)^{2+}$ bonded to triplex DNA is higher than that bonded to

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duplex DNA in certain range of DNA concentration.

Experimental

Double distilled water was used to prepare all solutions. Unless stated, all the chemicals were of analytical-reagent grade or better. Oligodeoxyrinucleotides AAAAAAAAAAAA($d(A)_{12}$), TTTTTTTTTTTT($d(T)_{12}$), were purchased from the GIBCOBRL Co. The molar extinction coefficients are ($d(A)_{12}$) = $1.830 \times 10^5 L \cdot M^{-1} \cdot cm^{-1}$, ($d(T)_{12}$) = 1.116×10^5 $L \cdot M^{-1} \cdot cm^{-1}$, respectively. Concentrations were calculated according to their extinction coefficients. $Ru(phen)_2(dppx)(BF_4)_2 \cdot 3H_2O$ was homemade according to the reference[8,9], which was described previously[10]. The stock solution of $Ru(phen)_2(dppx)^{2+}$ (1.0×10^{-4} $mol \cdot L^{-1}$) was prepared by dissolving 9.4 mg $Ru(phen)_2(dppx)^{2+}(BF_4)_2 \cdot 3H_2O$ in 100 mL water. The buffer solution of pH=7.0 was controlled by 0.2 $mol \cdot L^{-1}$ Tris-HCl buffer.

The fluorescence intensity was measured with a Shimadzu RF - 5301 spectrofluorometer with a quartz cell (1cm×1cm cross-section) equipped with a xenon lamp and dual monochrometer. The pH was measured with a Model pHB-4pH meter (Shanghai Leici Equipment Factory, China). Samples containing appropriate concentrations of $Ru(phen)_2(dppx)^{2+}$ and related oligonucleotides were made up to 10mL in 0.02 $mol \cdot L^{-1}$ Tris-HCl buffer solution (pH=7.0, 50 $mmol \cdot L^{-1}$ NaCl). The fluorescence intensity was measured with the following settings of spectrofluorometer: excitation wavelength (λex), 448 nm; excitation slit(EX), 10 nm; emission wavelength(λem), 604 nm; Emission slit (EM), 10 nm.

Results and Discussion

Oligodeoxyrinucleotides $d(A)_{12}$ and $d(T)_{12}$ were used to form duplex DNA($(dA\text{-}dT)_{12}$) and triplex DNA($dT^*dA\text{-}dT)_{12}$, where *denotes the Hoogsteen base pairing and the dash denotes the Watson-Crick base pairing). The titration of $d(A)_{12}$ with $d(T)_{12}$ in the presence of $Ru(phen)_2(dppx)^{2+}$ is shown in **Figure 1**. The fluorescence intensity increased with the increasing concentration of $d(T)_{12}$, and the fluorescence intensity was linear with the concentration of $d(T)_{12}$ until the ratio of $d(T)_{12}$:$d(A)_{12}$ reached 1:1. The regression equation is $I=6.58+2.035\times10^9C$(r=0.9994), accompanied with the formation of duplex DNA($(dA\text{-}dT)_{12}$). The fluorescence intensity increased continuously with increase of $d(T)_{12}$ before the ratio of $d(T)_{12}$:$d(A)_{12}$ reached 2:1, the new linear regression equation is $I=136.07 +1.157 \times 10^9C$ (r=0.9970), which showed the transformation of duplex DNA→triplex DNA($dT^*dA\text{-}dT)_{12}$). However, the titration of $d(T)_{12}$ with $d(A)_{12}$ in the presence of $Ru(phen)_2(dppx)^{2+}$showed no transformation process(**Figure 2**), and the triplex DNA was formed directly. In the transformation process of duplex DNA($(dA\text{-}dT)_{12}$) →triplex DNA($dT^*dA\text{-}dT)_{12}$), the Hoogsteen $d(T)_{12}$ third strand lies in the major groove of the template duplex DNA, running parallel to the $d(A)_{12}$ strand. Hence, the third strand changed the binding mode or may inhibit the binding of major groove interactive drugs[11]. The fluorescence enhancement in this process shows that $Ru(phen)_2(dppx)^{2+}$ may intercalate in the major groove of DNA, which is in accordance

with the results reported previously[12]. It also suggests that $Ru(phen)_2(dppx)^{2+}$not only intercalates in the Watson-Crick base pair, but also intercalates in Hoogsteen base pair.

Figure 1 Fluorescence titration curve of $d(A)_{12}$ (1.52×10^{-7}mol·L^{-1})with $d(T)_{12}$ in the presence of $Ru(phen)_2(dppx)^{2+}$ (1.0×10^{-6} mol·L^{-1}). NaCl:50mmol·L^{-1}

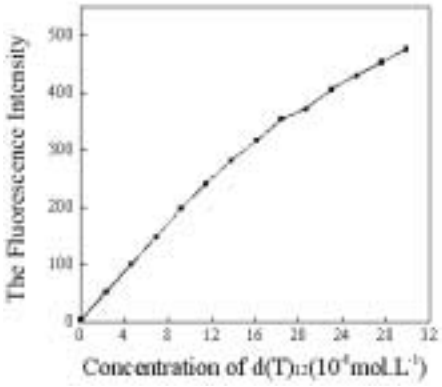


Figure 2 Fluorescence titration curve of $d(T)_{12}$ (2.3×10^{-7}mol·L^{-1}) with $d(A)_{12}$ in the presence of $Ru(phen)_2(dppx)^{2+}$ (1.0×10^{-6} mol·L^{-1}). .NaCl:50mmol·L^{-1}

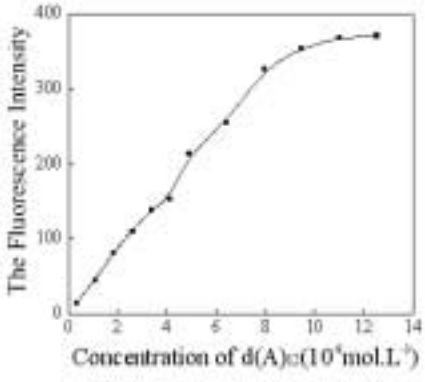
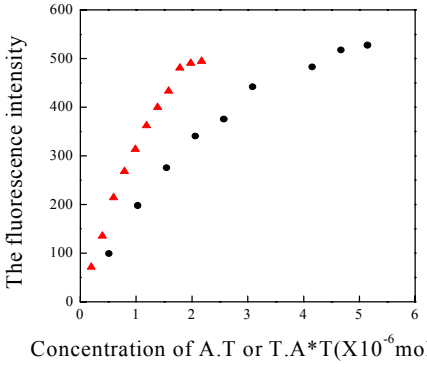


Figure 3 The binding ratios of $Ru(phen)_2(dppx)^{2+}$ binding with triplex DNA(triangle) and duplex DNA(circle)



$Ru(phen)_2(dppx)^{2+}$:1.0×10^{-6}mol·L^{-1}, NaCl:50mmol·L^{-1}
Base pairs/$Ru(phen)_2(dppx)^{2+}$≈4.0, Base triplets/$Ru(phen)_2(dppx)^{2+}$≈2.0

To investigate the reason of the fluorescence enhancement in the transformation process of duplex DNA($(dA\text{-}dT)_{12}$)→triplex DNA($dT^*dA\text{-}dT)_{12}$), the binding ratio of triplet verse $Ru(phen)_2(dppx)^{2+}$ and base pair verse $Ru(phen)_2(dppx)^{2+}$ was studied. It is demonstrated in **Figure 3** that the binding ratio of base pair verse $Ru(phen)_2(dppx)^{2+}$is about 4.0, while that of triplet verse $Ru(phen)_2(dppx)^{2+}$ is about 2.0, which means that the binding site increased in the duplex DNA($(dA\text{-}dT)_{12}$)→triplex DNA($dT^*dA\text{-}dT)_{12}$)

transformation process. And it suggests that the minor groove of Hoogsteen base pair provides new binding sites for $Ru(phen)_2(dppx)^{2+}$, which is consistent with the fluorescence enhancement in this transformation process. The effect of $Fe(CN)_6^{4-}$ on the fluorescence intensity of $Ru(phen)_2(dppx)^{2+}$- triplex DNA indicates that Ru complex indeed binds with the triplex DNA.

In conclusion, when $Ru(phen)_2(dppx)^{2+}$ binds to triplex DNA, it not only intercalates in the Watson-Crick base pair, but also intercalates in the Hoogsteen base pair. Thus, the fluorescence intensity of $Ru(phen)_2(dppx)^{2+}$ bonded to triplex DNA is higher than bonded to duplex DNA in certain range of DNA concentration. $Ru(phen)_2(dppx)^{2+}$ is better for the study of triplex DNA.

Acknowledgments

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