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Sensitive Determination of DNA Based on the Interaction between Norfloxacin−Tb^{3+} Complex and DNA

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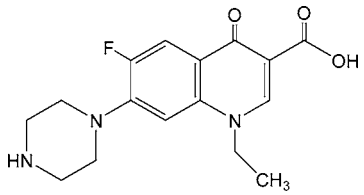
The fluorescence intensity of the norfloxacin (NFX)−Tb^{3+} complex enhanced by DNA was studied. Therefore, a sensitive fluorescence method for the determination of DNA was developed. The optimal conditions of the method were as follows: the hexamethylenamine (HMA)−HCl buffer was adopted for adjusting the pH to 6.5 ± 0.1, the concentrations of NFX and Tb^{3+} were both fixed in 1.0×10^{-6} mol L^{-1}, and the excitation and emission wavelengths were selected at 290 and 545 nm, respectively. Under the optimal conditions, the enhanced fluorescence intensity was in proportion to the concentration of DNA in the same range of 5.0×10^{-9}−1.0×10^{-6} g mL^{-1} for hsDNA and thermally denatured ctDNA. The detection limits (S/N = 3) were 0.9 and 0.6 ng mL^{-1}, respectively. In addition, the interaction between NFX−Tb^{3+} and DNA was discussed in detail. The experimental results from UV absorption spectra, fluorescence spectra, and the salt effect study indicated that the interaction between norfloxacin−Tb^{3+} complex and DNA had at least two different binding modes: the electrostatic binding and the intercalation binding. The mechanism of the fluorescence enhancement effect was also discussed.

KEYWORDS: DNA; NFX−Tb^{3+} complex; fluorimetry; interaction

INTRODUCTION

Determination of DNA is of great importance in biochemistry, biotechnology, pharmacodynamics, and other areas. For this purpose, many methods have been developed such as spectrophotometry (*1*), chemiluminescence (*2*), light scattering (*3*−*5*), electrochemical (*6*, *7*), and chromatography (*8*). However, the fluorometric methods have attracted more attention. It was well-known that the fluorescence intensity of DNA itself was very weak, and the direct use of their fluorescence emission properties to investigate their biological properties had been limited (*9*). Generally, fluorescent probes including organic dyes such as ethidium bromide (EB) (*10*), Hoechst 33258 (*11*), Phosphin 3R (*12*), and metal ions were employed to investigate DNA. Because of the luminescence characteristics of rare earth ions such as narrow spectral width, long luminescence lifetime, large stocks shift, and strong binding with biological molecules, they were widely used as fluorescent probes to study DNA. In particular, attentions have been directed toward two rare earth cations, Tb^{3+} and Eu^{3+}, as their resonance energy levels overlap with ultraviolet light (*13*, *14*). In recent years, the coordination complexes of metal ions especially rare earth ions as a probe to study DNA have attracted much attention (*14*, *15*−*20*). However, many of these fluorescent DNA probes are highly toxic and can be mutagenic, especially if the union to DNA is irreversible; for example, EB is well-regarded as a carcinogenic compound. Therefore, the search for low toxic, selective, and sensitive DNA probes is an active field of research (*29*).

Scheme 1



Norfloxacin (NFX) is one of the third generation members of quinolone antibiotics, which is widely used in the clinic. The structure of NFX is shown as follows (**Scheme 1**).

NFX could form the coordination complex with Tb^{3+}, which emitted the characteristic fluorescence of Tb^{3+} (*21*). However, the fluorescence intensity of the system was greatly increased when DNA was added. On the basis of the above findings, the fluorescence enhancement effect of the NFX−Tb^{3+} complex by DNA was investigated in the paper. It was found that the fluorescence probe of the NFX−Tb^{3+} complex had a higher sensitivity and wider range for determining DNA at nanogram levels than most of the reported probes. In addition, the proposed fluorescence probe has some advantages as compared with organic dyes and other coordination complexes of metal ions as a probe. The method is handy and inexpensive, and the reagents are easily obtainable and environmentally friendly. The interaction mode between DNA and NFX−Tb^{3+} and the mechanism of the fluorescence enhancement effect are also discussed in the paper.

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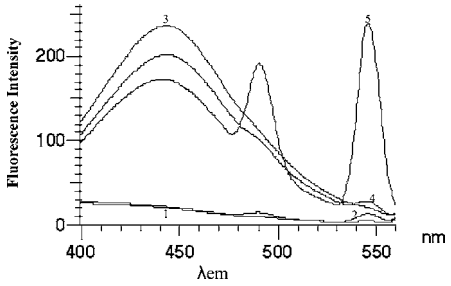

Figure 1. Emission spectra (ex = 290 nm): (1) Tb^{3+}, (2) hsDNA–Tb^{3+}, (3) NFX, (4) NFX–Tb^{3+}, and (5) NFX–Tb^{3+}–hsDNA. Conditions: NFX, 1.0×10^{-6} mol L^{-1}; Tb^{3+}, 1.0×10^{-6} mol L^{-1}; hsDNA, 1.0×10^{-6} g mL^{-1}; and HMA, 0.1 mol L^{-1}; pH 6.5.

MATERIALS AND METHODS

Apparatus. The fluorescence spectra and intensities were measured on a model F-2500 spectrofluorimeter (Hitachi, Japan) with a quartz cell (1×1 cm^2 cross-section) equipped with a xenon lamp and a dual monochromator. All absorption spectra were measured on a UV-2401PC spectrophotometer (Shimadzu, Japan). All pH measurements were made with a MP 220 pH meter (Mettler Toledo, China).

Reagents. All chemicals were of analytical reagent grade, and distilled or deionized water was used. Commercially prepared herring sperm (hs) DNA and calf thymus (ct) DNA (from Sigma) were suspended directly in 0.05 mol L^{-1} sodium chloride solution at a final concentration of 100 μg mL^{-1} as stock solutions. All diluted solutions of DNA were used within 24 h. All of the stock solutions and their diluted solutions were stored in a refrigerator at 4 °C until used. Native forms of DNA were thermally denatured by incubating them at 100 °C boiling water for 10 min, followed by cooling in an ice−water bath. After establishing the absorbance ratio A_{260}/A_{280} in the range of 1.80−1.90 for DNA, the concentration of DNA was determined according to the absorbance at 260 nm by using $\epsilon_{DNA} = 6600$ L mol^{-1} cm^{-1}. A stock standard solution (0.01 mol L^{-1}) of Tb^{3+} was prepared by dissolving the corresponding oxide (Tb_4O_7, 99.99%) in 1:1 (v/v) hydrochloric acid, and the solution was evaporated to near dryness; then, the residue was dissolved in water and diluted to the concentration of 1.0×10^{-2} mol L^{-1}. A stock solution of NFX (1.0×10^{-3} mol L^{-1}) was prepared by dissolving the corresponding NFX in water. Working solutions were prepared by appropriate dilution with water. A 1.0 mol L^{-1} hexamethylenamine (HMA)−HCl buffer solution was prepared by dissolving the corresponding HMA in water and adjusting the pH with hydrochloric acid to give a final total volume of 500 mL.

Methods. To a 10 mL test tube, solutions were added according to the following order: 1 mL of 1.0×10^{-5} mol L^{-1} Tb^{3+}, 1 mL of 1.0 mol L^{-1} HMA−HCl buffer, 1 mL of 1.0×10^{-5} mol L^{-1} NFX, and 1 mL of DNA with appropriate concentration. The mixture was diluted to 10.0 mL with water and allowed to stand for 10 min. The entrance and exit slits for all fluorescence measurements were both maintained at 10 nm. The fluorescence intensity was measured at $\lambda_{ex} = 290$ nm and $\lambda_{em} = 545$ nm. The enhanced fluorescence intensity of NFX−Tb^{3+} by DNA was represented as $\Delta F = F - F_0$. Here, F and F_0 were the fluorescence intensities of the systems with and without DNA, respectively.

RESULTS AND DISCUSSION

Fluorescence Spectra. The emission fluorescence spectra of NFX, NFX−Tb^{3+}, DNA−Tb^{3+}, and NFX−Tb^{3+}−DNA systems were shown in **Figure 1**. From **Figure 1**, it could be seen that the weak characteristic fluorescence of Tb^{3+} was observed in the NFX−Tb^{3+} system. While DNA was added, the fluorescence intensity of the NFX−Tb^{3+}−DNA system was greatly enhanced. The emission peaks were at 490 and 545 nm, which corresponded to the transitions from the $^5D_4$ level of Tb^{3+} to the $^7F_6$ and $^7F_5$ levels, respectively. It indicated that the

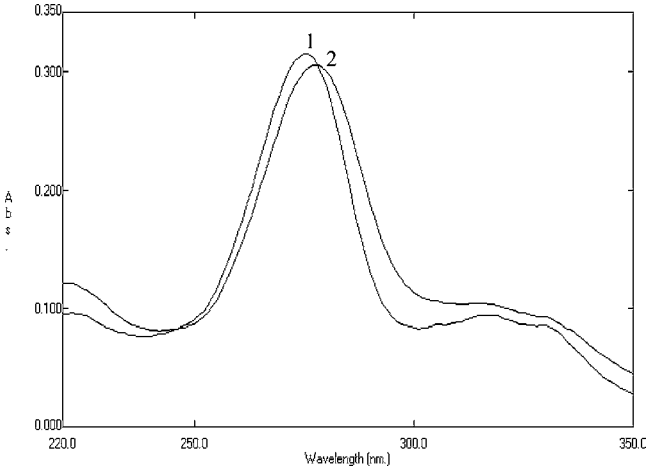

Figure 2. Absorption spectra (all absorption spectra have subtracted the background absorption from all of the reagents by using the reagents as a reference solution except NFX, which was being evaluated): (1) NFX–Tb^{3+} and (2) NFX–Tb^{3+}–hsDNA. Conditions: NFX, 1.0×10^{-5} mol L^{-1}; Tb^{3+}, 1.0×10^{-5} mol L^{-1}; and hsDNA, 10 μg mL^{-1}.

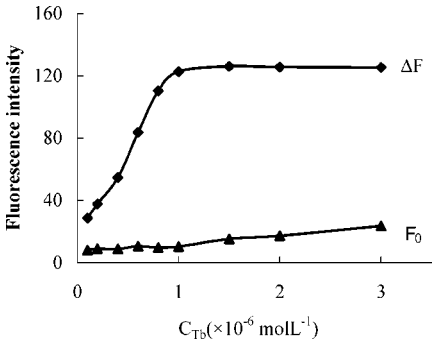

Figure 3. Effect of Tb(III) concentration on the fluorescence intensity. Conditions: NFX, 1.0×10^{-6} mol L^{-1}; hsDNA, 1.0×10^{-6} g mL^{-1}; and HMA, 0.1 mol L^{-1}; pH 6.5.

interaction between the NFX−Tb^{3+} complex and the DNA might occur in the NFX−Tb^{3+}−DNA system. It could also be seen that the fluorescence intensity of the NFX−Tb^{3+}−DNA system was remarkably sensitive than that of Tb^{3+}−DNA. The emission intensity at 545 nm was stronger than that at 490 nm; therefore, the peak at 545 nm was used for fluorescence intensity measurements.

The maximum excitation wavelength of the NFX−Tb^{3+}−DNA system was about 276 nm. Considering the interference of multiple peak (at 552 nm), we selected the excitation wavelength at 290 nm for fluorescence intensity measurements.

Influence Factors on the Fluorescence Intensity of the System. *Effect of Acidity and Buffer.* The acidity of the solution had a great influence on both the formation of the NFX−Tb^{3+} complex and the capability of DNA to bind the complex. Experimental results indicated that the maximum fluorescence intensity of the system was reached at the pH range of 6.3−6.7. So, we selected pH 6.5 for further research. Among HMA−HCl, Tris−HCl, NaAc−HAc, Na_2HPO_4, sodium citrate, hydroxylamine, and hydrogen potassium phthalate, it was found that HMA−HCl was the most suitable buffer. The proper concentration of HMA was 0.1 mol L^{-1} in the solution.

Effect of Tb^{3+} Concentration. The effect of Tb^{3+} concentration on the fluorescence intensity of the NFX−Tb^{3+} and NFX−Tb^{3+}−DNA systems was shown in **Figure 3**. It could be seen from **Figure 3** that the fluorescence intensity of the NFX−Tb^{3+} system (F_0) increased with the increase of the Tb^{3+} concentra-

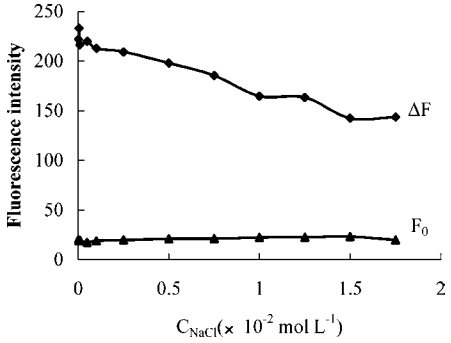

Figure 4. Effect of salt concentration on the fluorescence intensity. Conditions: NFX, 1.0×10^{-6} mol L^{-1}; Tb^{3+}, 1.0×10^{-6} mol L^{-1}; hsDNA, 1.0×10^{-6} g mL^{-1}; and HMA, 0.1 mol L^{-1}; pH 6.5.

tion. It could also be seen that the ΔF of the NFX−Tb^{3+}−DNA system reached the maximum when the molar ratio of NFX to Tb^{3+} was 1:1, which indicated that the composition ratio for NFX to Tb^{3+} in the NFX−Tb^{3+}−DNA system was 1:1. So, we selected the molar ratio 1:1 of NFX to Tb^{3+} for further research. The suitable concentrations of NFX and Tb^{3+} were the same, 1.0×10^{-6} mol L^{-1}, for the fluorescence enhancement effect of the NFX−Tb^{3+} complex by the trace concentration of DNA was limited when the concentrations of NFX and Tb^{3+} were higher.

Effect of Salt Concentration. In the experiments, DNA, which directly dissolved in the water, was used. Sodium chloride was used to study the influence of salt concentration on the fluorescence intensity of the NFX−Tb^{3+} and NFX−Tb^{3+}−DNA systems. The results were shown in **Figure 4**. It could be seen from **Figure 4** that the effect of salt concentration on the fluorescence emission of the NFX−Tb^{3+} system (F_0) was weaker, while the effect on that of the NFX−Tb^{3+}−DNA system (ΔF) was obvious. In the NFX−Tb^{3+} system, the salt concentration only affected the interaction between NFX and Tb^{3+} ions. While in the NFX−Tb^{3+}−DNA system, the electrostatic attraction between the positive charged Na^+ and the chelate reagent of NFX and the phosphate group of DNA blocked the interaction among Tb^{3+} ions, NFX and DNA, which weakened the electrostatic binding, intercalation binding, or groove binding (*22*). Therefore, the effect of salt concentration on the NFX−Tb^{3+}−DNA system was far greater than that of the NFX−Tb^{3+} system. In addition, it could be seen from **Figure 4** that when the concentration of sodium chloride was less than 2.5×10^{-3} mol L^{-1}, the effect of salt concentration on the fluorescence intensity of the NFX−Tb^{3+}−DNA system was very limited. While the concentration of sodium chloride was more than 2.5×10^{-3} mol L^{-1}, the fluorescence intensity of the NFX−Tb^{3+}−DNA system decreased obviously with the concentration of sodium chloride increasing.

Effect of Phosphate. Phosphate as a group of DNA, it acts as the framework of DNA. The effect of phosphate on the fluorescence intensity of the NFX−Tb^{3+} and NFX−Tb^{3+}−DNA systems was shown in **Figure 5**. It could be seen from **Figure 5** that the fluorescence intensity of the NFX−Tb^{3+}−DNA system increased with the increase of phosphate concentration at very low concentrations. When the concentration of phosphate was higher than 1.0×10^{-7} mol L^{-1}, the fluorescence intensity of the system decreased with the increase of phosphate concentration. The results indicated that the coordinate water molecules in the NFX−Tb^{3+}−$(H_2O)_n$ complex could be replaced by the phosphate group of DNA and the phosphate at very low concentration, which would result in the decrease of the nonradiative energy loss through O−H vibration of H_2O in the Tb^{3+} complex and the increase of the fluorescence intensity of the system. While at a high concentration of phosphate, the phosphate would strongly compete with the phosphate group of DNA in Tb^{3+} complex, which would make the Tb^{3+} complex leave DNA helix and resulted in the decrease of the fluorescence intensity of the system. Thus, the phosphate will interfere with the determination of DNA.

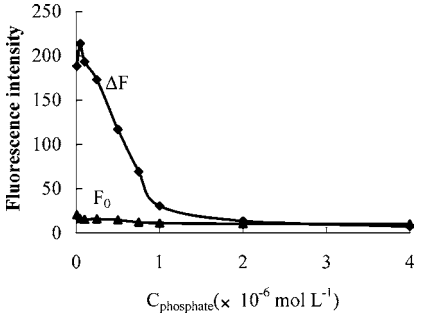

Figure 5. Effect of phosphate on the fluorescence intensity. Conditions: NFX, 1.0×10^{-6} mol L^{-1}; Tb^{3+}, 1.0×10^{-6} mol L^{-1}; hsDNA, 1.0×10^{-6} g mL^{-1}; and HMA, 0.1 mol L^{-1}; pH 6.5.

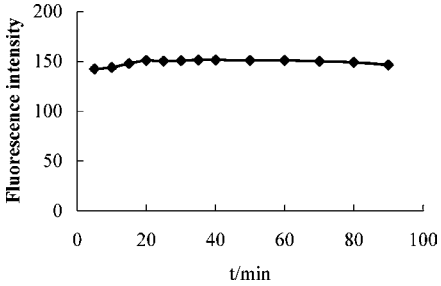

Figure 6. Stability test. Conditions: NFX, 1.0×10^{-6} mol L^{-1}; Tb^{3+}, 1.0×10^{-6} mol L^{-1}; hsDNA, 1.0×10^{-6} g mL^{-1}; and HMA, 0.1 mol L^{-1}; pH 6.5.

Table 1. Linear Ranges and Detection Limits for DNA Determination

species	linear range (μg mL^{-1})	correlation coefficient (r)	detection limit (ng mL^{-1}) (S/N = 3)
hs DNA	0.005–1.0	0.9974	0.9
denatured ct DNA	0.005–1.0	0.9957	0.6

Stability Test. The experiments indicated that at room temperature the fluorescence intensity of the NFX−Tb^{3+}−DNA system reached a maximum in 20 min after all of the reagents had been added and remained stable for at least 1 h (see **Figure 6**).

Analytical Application. *Calibration Curve and Detection Limit.* The experiments indicated that for denatured ctDNA, the optimum conditions were the same as the hsDNA. The calibration graphs for denatured ctDNA and hsDNA were constructed in the optimum conditions. The analytical parameters of this method were listed in **Table 1**. This table showed that there was a good linear relationship between the enhanced fluorescence intensity (ΔF) and the concentration of DNA in the wide range. Their limits of detection all reached at ng mL^{-1} level. A comparison between this probe and other reported probes for DNA in sensitivity was summed up in **Table 2**. It could be seen from **Table 2** that the sensitivity of this method was better than most of the well-known methods including the EB probe. Therefore, this method will become a valuable tool for studying the biological properties of DNA.

Table 2. Comparison of the Detection Limits in Different Fluorescent Probes[a]

fluorescence probes	species of DNA	detection limit (S/N = 3) (ng mL^{-1})	refs
EB	nDNA	10	*10*
hoechest 33258	nDNA	10	*11*
La^{3+}–8-hydroxyquinoline	fsDNA/DN ctDNA	68/76	*15*
Al^{3+}–8-hydroxyquinoline	fsDNA/DN ctDNA	130/240	*20*
Al^{3+}–salicylidene-*o*-aminophenol	fsDNA/ctDNA	52/49	*18*
Tb^{3+}–1,10-phenanthroline	fsDNA/DN ctDNA	200/100	*14*
Eu^{3+}–tetracycline	fsDNA/ctDNA	10/10[b]	*16*
Eu^{3+}–oxytetracycline	fsDNA/ctDNA	11.2/15.1	*17*
this probe	hsDNA/DN ctDNA	0.9/0.6	

[a] DN, thermally denatured. [b] S/N = 2.

Table 3. Tolerance Molar Excesses of Foreign Substances (2.5×10^{-6} mol L^{-1} hsDNA)

substances	tolerable molar excess
Na^+	1000
Ca^{2+}	20
Mg^{2+}	20
Zn^{2+}	4
PO_4^{3-}	0.04
L-phenylalanine	>800
L-glycine	>800
L-histidine	>800
L-glutamic acid	100
L-tryptophan	4
L- tyrosine	2
ascorbic acid	10
adenine	2
guanine	2
thymine	20
uracil	2
protein: bovine serum albumin	10[a]

[a] Mass concentration ratio (1 μg mL^{-1} hsDNA).

Selectivity. Under the optimum conditions, various coexisting substances in the organism including common metal ions, amino acids, bases, and proteins were examined for interference. At the hsDNA concentration of 2.5×10^{-6} mol L^{-1}, the highest permissible molar excesses of foreign substances causing a ±5% relative error in the fluorescence intensity were listed in **Table 3**. Most of them except the phosphate did not interfere with the determination; therefore, good selectivity existed in this method.

Determination of DNA in Synthetic Samples. The presented method was applied to the determination of DNA in the synthetic samples based on the tolerance molar excesses of coexisting species. The results were shown in **Table 4**. It could be seen from **Table 4** that the recovery and precision of this method were satisfactory.

Sample Determination by Different Methods. Fish sperm DNA (fsDNA) sample was purchased from Sigma (its purity was about 97%). A sample with some content of fsDNA was prepared by directly dissolving it in 0.05 mol L^{-1} sodium chloride solution and then determined by different methods. The results were shown in **Table 5**. From **Table 5**, it showed that the results determined by the presented method were in accordance with these obtained by the well-known methods of UV−vis method and EB probe (*10*).

Mechanisms of the Interaction and the Fluorescence Enhancement. *Mechanism of the Interaction.* In the interaction mode between the NFX−Tb^{3+} complex and the DNA, there are other interaction modes such as intercalation or groove binding in the NFX−Tb^{3+}−DNA system besides the nonspecific electrostatic binding. Generally, red shift and hypochromic effects are observed in the absorption spectra of small molecules if they intercalate into DNA (*23*). From **Figure 2**, it can be seen that the maximum absorption wavelength of the NFX−Tb^{3+} complex in the NFX−Tb^{3+}−DNA system had undergone a red shift about 2 nm and its absorbance had decreased a little as compared with in the NFX−Tb^{3+} system, which indicated that the binding between NFX−Tb^{3+} and DNA was in the mode of intercalation binding rather than that of groove binding (*27*).

Other strong evidence to support the conclusion was the competing combination tests between the NFX−Tb^{3+} complex and the EB probe (see **Figure 7**). From **Figure 7** (all absorption spectra have subtracted the background absorption from all of the reagents by using the reagents as a reference solution except EB, which was being evaluated), it could be seen that when DNA was added to the EB solution, the UV absorption of the

Table 4. Determination of DNA in Synthetic Samples ($n = 5$)[a]

synthetic samples ($\times 10^{-6}$ mol L^{-1})	species of DNA	added (μg mL^{-1})	found (μg mL^{-1})	recovery (%)	RSD (%)
A (Ca^{2+}, 1.0; Mg^{2+}, 1.0; Zn^{2+}, 1.0; L-glycine, 1.0; L-tryptophan, 1.0; L-phenylalanine, 1.0; L-tyrosine, 1.0)	hs DNA	0.2	0.194	97.0	3.84
		1.0	0.935	93.5	2.69
	DN ct DNA	0.2	0.201	100.5	0.87
		1.0	0.960	96.0	2.26
B (Ca^{2+}, 1.0; Mg^{2+}, 1.0; Zn^{2+}, 1.0; A, 1.0; T, 1.0; U, 1.0; G, 1.0; BSA, 1.0[a])	hs DNA	0.2	0.210	105.0	3.73
		1.0	0.996	99.6	3.91
	DN ct DNA	0.2	0.183	91.5	1.83
		1.0	0.956	95.6	2.63

[a] DN, thermally denatured; a, ($\times 10^{-6}$ g mL^{-1}); A, adenine; T, thymine; U, uracil; and G, guanine.

Table 5. Determination of fsDNA by Different Methods

	found ($\times 10^{-5}$ mol/L)	average ($\times 10^{-5}$ mol/L)	RSD (%)
UV–vis	1.44		
EB probe	1.59, 1.54, 1.59, 1.50, 1.50	1.54	2.92
the presented method	1.58, 1.58, 1.45, 1.53, 1.52	1.53	3.50

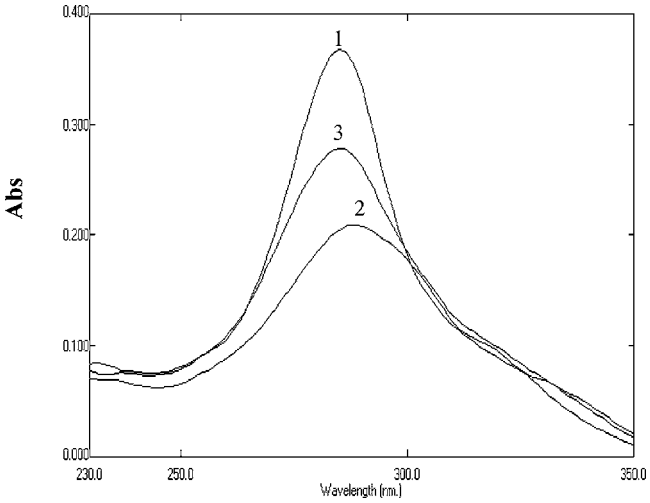

Figure 7. Competition tests between NFX–Tb^{3+} and EB for the binding sites of DNA by UV spectra. Conditions: (1) EB, (2) EB–DNA, and (3) EB–DNA–NFX–Tb^{3+}. c (ctDNA) = 10 μg/mL, c (NFX) = c (Tb^{3+}) = 1.0×10^{-5} mol/L, and c (EB) = 6.0×10^{-6} mol/L.

EB−DNA system decreased remarkably, which indicated that EB molecules (usually used as the intercalation reagent of DNA) intercalated into the stacked base pairs of DNA and resulted in the decrease of the UV absorption of EB because of the shielding effect of the base pairs of DNA. When the NFX−Tb^{3+} complex was added to the EB−DNA solution, the UV absorption of the EB−DNA−NFX−Tb^{3+} system increased remarkably as compared with the EB−DNA system, but it was still lower than that of the EB solution, which showed that some NFX−Tb^{3+} complex molecules intercalated into the stacked base pairs of DNA and displaced part of EB molecules and resulted in the increase of the free EB concentration and increased the UV absorption of EB.

The conclusion was also supported by the data of salt effect on the fluorescence intensity of the NFX−Tb^{3+}−DNA system. In the mode of intercalation binding, the small molecule was intercalated into the space of two neighboring DNA base pairs. Because of the shielding effect of the base pairs, the properties of the small molecule intercalated are not sensitive to the environmental change of the solution in comparison with the molecule of groove binding, in which the small molecule binds to the groove of DNA double helix (*24*). Because of the minor salt effect on the fluorescence intensity of the NFX−Tb^{3+}−DNA system when the concentration of NaCl was lower than 2.5×10^{-3} mol L^{-1} (see **Figure 4**), the binding mode of NFX−Tb^{3+} to DNA should be the intercalation binding rather than the groove binding mode, for the salt effect on the fluorescence intensity of the system was remarkable in the groove binding mode such as the OTc−Eu−DNA system (*17*). When the concentration of Na^+ exceeded to an extent, the electrostatic attraction blocked the interaction among Tb^{3+} ions, NFX, and DNA; thus, the fluorescence intensity of the NFX−Tb^{3+}−DNA system decreased. The above conclusion was in accordance with the other results reported on the interaction between NFX and DNA, in which it was proven that NFX−Mg^{2+} was intercalated between the stacked base pairs of native DNA by an electrochemical method (*25*). In another study on the characterization of NFX binding to the linear single- and double-stranded DNA, the effect of NFX on the change in intrinsic viscosity strongly suggested that NFX had the properties of an intercalative binder (*26*). Therefore, in the NFX−Tb^{3+}−DNA system, the binding mode of NFX−Tb^{3+} with DNA included the electrostatic binding between Tb^{3+} and phosphate group of DNA and the intercalation binding.

Mechanisms of the Fluorescence Enhancement. From **Figure 1**, it can be seen that in the NFX−Tb^{3+} system, the Tb^{3+} ion complexed with the NFX, then emitted the characteristic fluorescence of Tb^{3+} by the energy transfer from NFX to Tb^{3+}, and at the same time, the native fluorescence of NFX at 435 nm was decreased obviously. While DNA was added to the NFX−Tb^{3+} complex system, the absorbance of the NFX−Tb^{3+} complex was decreased, but the fluorescence enhancement of the NFX−Tb^{3+} complex by DNA was remarkable, indicating that there was the interaction between DNA and NFX−Tb^{3+} complex. The detailed explanation is as follows. The coordination number of Tb^{3+} in its complex is 6−8, but the molar ratio of Tb^{3+} ion to NFX in this study shows 1:1; therefore, the coordination number of Tb^{3+} cannot be satisfied. Tb^{3+} will coordinate with H_2O and form a NFX−Tb−$(H_2O)_n$ complex. Tb^{3+} in the NFX−Tb−$(H_2O)_n$ complex will be left one positive charge after binding with NFX, which will easily bind to a phosphate anion group (or other groups) of DNA and be accompanied with the release of H_2O molecule in NFX−Tb−$(H_2O)_n$. Thus, the nonradiative energy loss through O−H vibration of H_2O molecule in the NFX−Tb^{3+} complex will be decreased and the fluorescence of the system will be enhanced by DNA. In addition, Tb^{3+} may complex with the two-site binding of O-6 and N-7 of the guanine base in single-stranded DNA, while other bases such as adenine, cytosine, thymine, and uracil, some of them cannot complex with Tb^{3+}, and some of them even can complex with Tb^{3+}, but its fluorescence intensities are weak (*28*). Therefore, it is possible that unsatisfied Tb^{3+} complexes with O-6 and N-7 of the guanine bases in DNA helix, which exists in the intermolecular energy transfer between DNA and Tb^{3+} in the Tb^{3+}−NFX complex and causes the fluorescence enhancement of the system.

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