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Study of the Reaction Between the Nucleic Acid and Y-BPMPHD-CTMAB Complex and Its Analytical Application

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The fluorescence quenching of the Y-BPMPHD-CTMAB by nucleic acids is reported. It is considered that the Y-BPMPHD-CTMAB can form a large complex with nucleic acid through the electrostatic attraction in the pH range of 4.2–6.8. Under optimal conditions, the difference of fluorescence intensity between the system without and with nucleic acids is proportional to the concentration of nucleic acids over the range of 4.5×10^{-8}–1.2×10^{-5} g/mL for fsDNA and 3.2×10^{-8}–3.0×10^{-5} g/mL for yRNA, respectively. The detection limits are 14.0 ng/mL for fsDNA and 21.0 ng/mL for yRNA. The method is applied for the determination of nucleic acids in actual sample, and the result obtained is satisfactory.

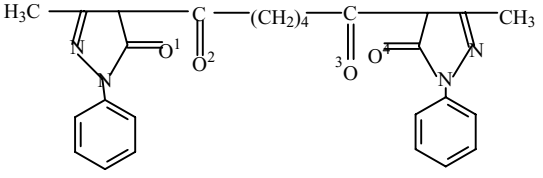
KEY WORDS: Nucleic acids; fluorescence quenching; Y-BPMPHD-CTMAB complex.

INTRODUCTION

Nucleic acids have an important function in life process, so study on them has become an important research field of life science. It is well known that the fluorescence intensity of nucleic acid itself is very weak, the direct use of their fluorescence emission properties has been limited [1], and an extrinsic fluorescence probe is usually used. Generally, fluorescence probes including organic dyes [2–3], metal ions [4,5], and metal complex [6–11] are employed to investigate nucleic acid.

The study of the interaction between nucleic acid and metal complex is an important area in nucleic acid research. Recently, the use of coordination complex of rare-earth ions as a probe to study nucleic acids has attracted much attention. Tetracycline [6,11], phenanthroline [12], adriamycin and pyridine [13,14] are often chosen as chelating reagents. The signal from the interaction between the metal complexes and nucleic acids may provide some evidence for the interaction mechanism of some drugs in living systems. In this paper, the β-diketone derivative 1,6-bi(1′-phenyl-3′-methyl-5′-pyrazolone-4′-)hexane–dione (BPMPHD) is used as chelating reagent, its structure is shown in the following:

It is found that the fluorescence of Y^{3+}-BPMPHD–CTMAB [15] can be quenched by nucleic acids, which can be used as a sensitive probe for the determination of nucleic acids.



EXPERIMENTAL

Chemicals

Stock solutions of nucleic acids (100 μg/mL) were prepared by dissolving commercial fish sperm

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DNA(fsDNA) (Sigma) and yeast RNA(yRNA) (Beijing Baitai Co., China) in 0.05 M sodium chloride solution. A stock solution of ytterium (III) (1.0×10^{-2} M) was prepared by dissolving ytterium oxide (Y_2O_3, 99.9%) in hydrochloric acid and diluting with distilled water. BPMPHD solution (1.0×10^{-3} M) was prepared by adding the appropriate amount of BPMPHD into 95% ethanol, and adding 1:1 NH_3 H_2O until BPMPHD was dissolved completely. Aqueous CTMAB solution (1.0×10^{-2} M) was used. A 0.05 M Tris-HCl buffer solution was prepared by dissolving 3.03 g of Tris in 500 mL deionized water, and then the pH was adjusted to 5.5 by using HCl. All the chemicals used were of analytical grade and doubly deionized water was used throughout.

Apparatus

All fluorescence measurements were made with a Hitachi 850 spectrofluorimeter. All pH measurements were made with a pHS-2 acidity meter (leici, Shanghai). DXD-II video microscopic electrophoresis apparatus (yixing, Jiangsu). And all absorption spectra were made with a UV-Visible recording spectrophotometer (UV-240).

Procedure

To a 25 mL test-tube, solutions are added in the following order: Y^{3+}, CTMAB, BPMPHD, DNA (or RNA) and buffer solution. The mixture is diluted to 10 mL with water and allowed to stand for 25 min. The fluorescence intensity is measured in a l cm quartz cell and the excitation and emission slits are both 10 nm, respectively. The quenched fluorescence intensity of the Y-BPMPHD-CTMAB by nucleic acid is represented as $\Delta F = F_0 - F_1$, here F_0 and F_1 are the intensities of the systems without and with nucleic acid.

RESULTS AND DISCUSSION

Fluorescence Spectrum

The excitation and emission spectra of Y-BPMPHD-CTMAB (1), Y- BPMPHD-CTMAB- fsDNA (2), and Y-BPMPHD-CTMAB-yRNA (3) are shown in Fig. 1. It can be seen that the excitation and emission peaks are located at 300 nm and 446 nm respectively. The fluorescence intensity of Y-BPMPHD-CTMAB system can be quenched by fsDNA and yRNA. This indicates an interaction between nucleic acid and Y-BPMPHD-CTMAB complex. From Fig. 1 (c)—the effect of excitation wavelength on fluorescence emission $\Delta F / F_0$, 300 nm is the most suitable excitation wavelength.

Optimization of the General Procedure

Effects of pH and Buffer Solutions

The effects of pH on the quenching effects are investigated in the pH range of 4.2–6.8, the results are shown in Fig. 2. It can be seen that the ΔF reached a maximum in the pH range of 5.1–5.8. Hiring pH = 5.5, the following buffers were tested: trihydroxymethyl aminomethane (Tris)-HCl, NaAc-HAc, NH_4Ac-HAc, hexamethylenetetramine (HMTA)-HCl. The results indicate that 1.0 mL Tris-HCl is the most suitable buffer.

Effect of the Concentration of Y^{3+}

Effect of the Y^{3+} concentration on the fluorescence intensity is studied. The results show that the ΔF value increases with the increase of Y^{3+} concentration and reaches a maximum at 1.0×10^{-5} M. In further experiments, the Y^{3+} concentration is fixed at 1.0×10^{-5} M.

Effect of the Concentration of BPMPHD

With the change in concentration of BPMPHD, the ΔF value of this system is tested by experiment. Result showed that in the range of 3.8×10^{-5} M–5.1×10^{-5} M BPMPHD, the ΔF value of this system remains constant and reaches a maximum. The concentration of BPMPHD for further study is chosen as 4.02×10^{-5} M.

Effects of Surfactants and CTMAB Concentration

Different kinds of surfactants have different effects on ΔF value. Among the surfactants CTMAB, TX-100, SDBS, SDS, and SLS tested, their ΔF are 25.8, 1.5, −0.7, −1.5, −3.6, respectively. The concentration of all the surfactants used in this experiment was of 1.0×10^{-3} M From these results, it is concluded that CTMAB is the best surfactant.

The effect of CTMAB concentration on the ΔF is studied. Results showed that the ΔF increases with increasing CTMAB concentration, and reached a maximum in the range of 1.3×10^{-3}–2.0×10^{-3} M. When CTMAB concentration exceeds 2.0×10^{-3} M, the ΔF decreases. So we selected 1.5×10^{-3} M CTMAB for further experiments.

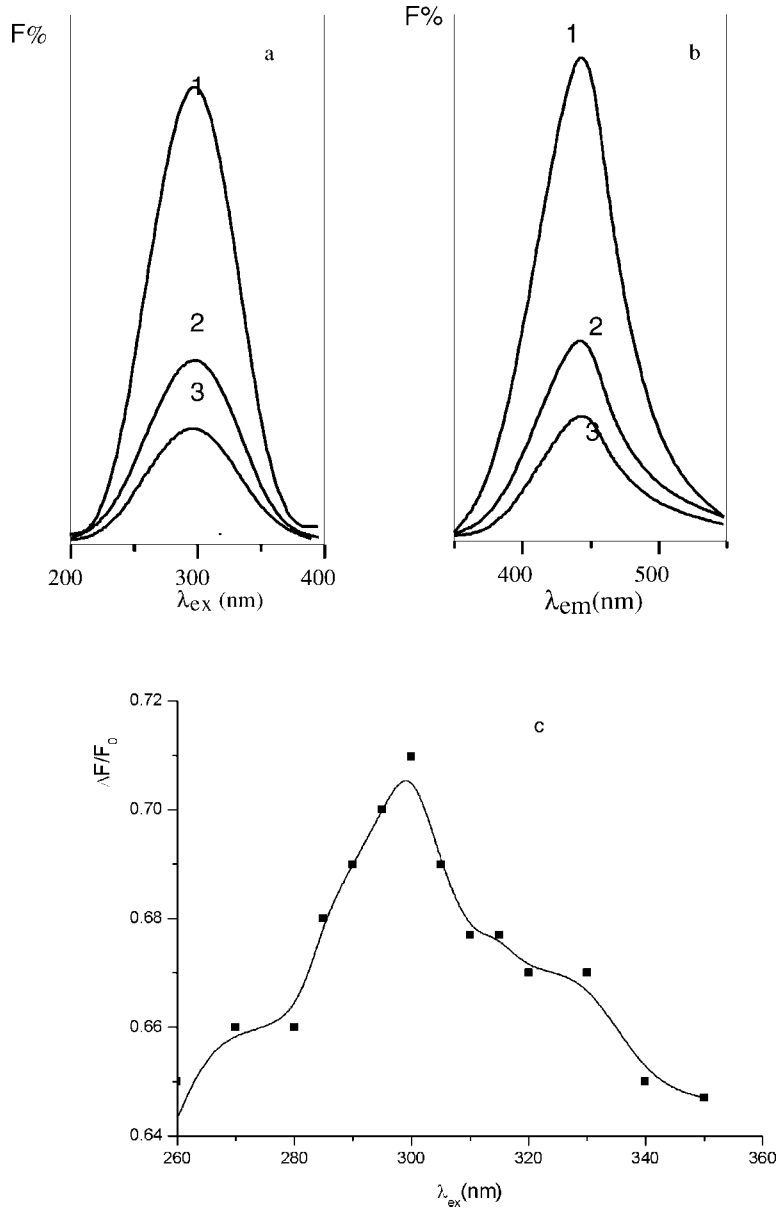

Fig. 1. Fluorescence spectra of nucleic acids on the Y-BPMPHD-CTMAB system (a) excitation spectra ($\lambda_{em} = 446$ nm); (b) emission spectra ($\lambda_{ex} = 300$ nm); (c) Effect of λ_{ex} on $\Delta F/F_0$ of the system. (1) Y^{3+}-CTMAB-BPMPHD; (2) Y^{3+}-CTMAB-BPMPHD-fsDNA; (3) Y^{3+}-CTMAB-BPMPHD-yRNA. Conditions: 1.0×10^{-5} M Y^{3+}; 4.02×10^{-5} M BPMPHD; 1.5×10^{-3} M CTMAB; 5.0×10^{-6} g/mL fsDNA; 5.0×10^{-6} g/mL yRNA; 5.0×10^{-3} M Tris pH = 5.5.

Effects of Addition Order of Reagents

The effect of addition order is tested in the experiment. Results showed that Y^{3+}-CTMAB-BPMPHD-DNA-Tris is the best addition order, which is chosen in further assay.

Stability

Tests showed that the fluorescence intensity of the system reached a maximum 20 min after adding all the reagents and remained stable for about 80 min. In this paper, 25 min was fixed.

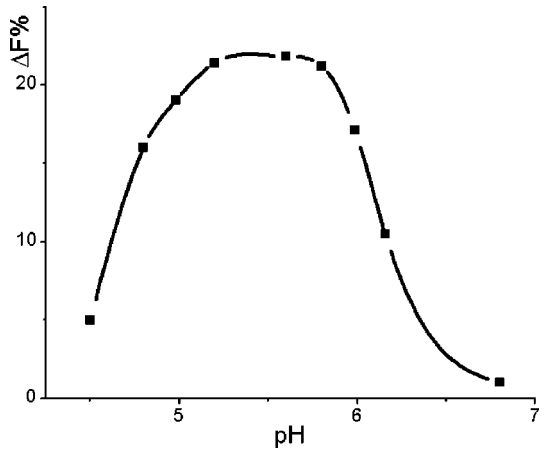

Fig. 2. Effect of pH on the fluorescence intensity. Conditions: 1.0×10^{-5} M Y^{3+}; 3.06×10^{-5} M BPMPHD; 5.0×10^{-6} g/mL fsDNA; 1.5×10^{-3} M CTMAB.

Effects of Foreign Ions

The effects of the substances including metal ions, proteins and amino acids were examined for interference. For a relative error of less than ±10%, the tolerance of some ions is listed in Table I. It shows that the metal ions commonly present in organisms such as Na^+, Ca^{2+}, K^+, and Mg^{2+} can be tolerated at high concentration levels.

ANALYTICAL APPLICATION

Calibration Curve and Determinations of Synthetic Samples

Under the optimum conditions, the calibration curve of DNA and RNA were made and shown in Table II.

Table I. Effects of Some Ions on the Fluorescence Intensity

Foreign substance	Highest permissible concentration (M)	Foreign substance	Highest permissible concentration (M)
Na^+ Cl^-	1.0×10^{-3}	Mg^{2+} SO_4^{2-}	5.0×10^{-5}
Ca^{2+} Cl^-	7.5×10^{-4}	Al^{3+} Cl^-	8.0×10^{-7}
K^+ Cl^-	1.0×10^{-4}	Pb^{2+} NO_3^-	3.2×10^{-6}
Ba^{2+} Cl^-	1.0×10^{-5}	Hg^{2+} Cl^-	2.0×10^{-7}
Zn^{2+} SO_4^{2-}	2.0×10^{-5}	Na^+ CO_3^{2-}	1.0×10^{-4}
BSA[a]	3.8[b]	L-Ala	1.0×10^{-6}
BH[c]	2.0[b]	DL-Tyr	8.5×10^{-7}

Note. Conditions: 1.0×10^{-5} M Y^{3+}; 4.02×10^{-5} M BPMPHD; 1.5×10^{-3} M CTMAB; 5.0×10^{-6} g/mL fsDNA; 5.0×10^{-3} M Tris pH = 5.5.
[a]Bovine serum albumin.
[b]$\times 10^{-6}$ g/mL.
[c]Bovine hemoglobin.

Table II. Analytical Parameters of This Method

Nucleic acids	Linear range (g/mL)	r^a	LOD[b] (g/mL)	RSD[c] (%) ($n = 11$)
fsDNA	4.5×10^{-8}–5.0×10^{-7}	0.9988	14×10^{-9}	1.8
	5.0×10^{-7}–1.2×10^{-5}	0.9944		
yRNA	3.2×10^{-8}–3.0×10^{-5}	0.9848	21×10^{-9}	1.6

[a]r = correlation coefficient.
[b]LOD = limit of detection.
[c]RSD = relative standard deviation.

Table II shows that there are good linear relationships between ΔF and the concentration of nucleic acid over a wide range. Their detection limits reach ng/mL levels. The standard addition method was used for determination of synthetic samples. From Table III, it can be seen that the results obtained are satisfactory. This method can be applied for the determination of actual sample (yRNA), which is made from yeast solution by dilute alkaline method [16]. In comparison with the results of ultraviolet spectrophotometry, the results of the method are satisfactory.

A comparison between this probe and other common probes for nucleic acids in sensitivity and selectivity is summed in Table IV. It can be seen that this method is better than the most of well-known staining methods in the sensitivity. In addition, this probe has no carcinogenicity compared to using ethdium bromide (EB) as the probe. EB is widely used as a general purpose-staining agent for DNA, but is a carcinogen. Therefore this method would become a valuable tool for studying the biological properties of nucleic acids.

MECHANISM FOR THE FLUORESCENCE QUENCHING

Generally, organic dyes interact with nucleic acids according to the following model: intercalative binding, groove binding, electrostatic binding and long range assembly on the molecular surface of nucleic acids that does

Table III. Recoveries of Nucleic Acids in Synthetic Samples

Nucleic acid	Foreign co-existing substance	Sample added (μg/mL)	Found (μg/mL)	RSD (%)	Recovery (%)
fsDNA	BSA, Na^+, K^+	0.5	0.492	4.9	98.4
yRNA	BSA, Na^+, K^+	4.1	4.09	1.1	99.8

Note. Conditions: BSA, 0.6 μg/mL, Na^+, 1.0×10^{-6} M, K^+, 1.0×10^{-6} M.

Table IV. Comparison with Common Fluorescence Probes for Nucleic Acids

Fluorescence probe	Nucleic acids	LOD (ng/mL)	Reference
Ethdium Bromide	DNA	10	17
Safranine T(ST)	FsDNA	14	18
Eu^{3+}-tetracycline	FsDNA	10	6
Tb^{3+}-phenanthroline	fsDNA/yDNA	200/200	12
Al^{3+}-8-hydroxyquinoline	fsDNA/yRNA	13/130	19
Vitamin K_3	ctDNA/yRNA	10/26	20
This probe	fsDNA/yRNA	14/21	

not involve intercalative or groove binding. RNA does not have a groove and helix structure, but both DNA and RNA can quench the fluorescence intensity of the Y-BPMPHD-CTMAB system. In addition, after adding nucleic acid, the absorbance of the Y-BPMPHD-CTMAB complex at 268 nm increases. Hence we can conclude that the groove binding and intercalative binding are not the reason for the fluorescence quenching. The fluorescence quenching data were plotted shown in Fig. 3 according to the Stern-Volmer equation [21]:

$$F_0/F = 1 + K[Q] \quad (1)$$

Where F_0 and F are the fluorescence intensities of the systems in the absence and presence of DNA, respectively; K is the association equilibrium constant; and $[Q]$ is the concentration of the quencher (fsDNA). According to equation (1), the K of this system at 16°C and 40°C is calculated to be 0.215 and 0.078 mL/μg, respectively. On the basis of the above result, it is considered that the quenching is a static process. This indicates that the Y-

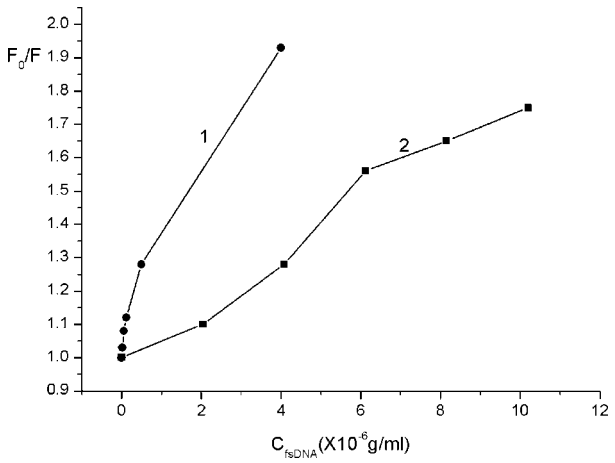

Fig. 3. Fluorescence quenching of the system by DNA. (1) 16°C (2) 40°C. Conditions: 1.0×10^{-5} M Y^{3+}; 4.02×10^{-5} M BPMPHD; 1.5×10^{-3} M CTMAB; 5.0×10^{-3} M Tris pH = 5.5.

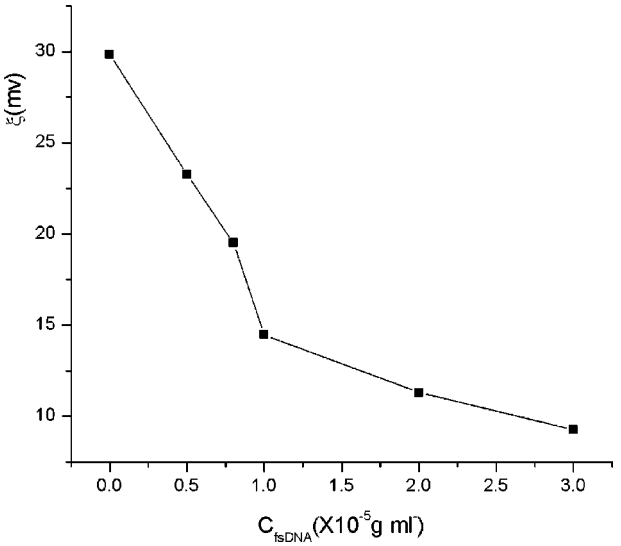

Fig. 4. Effect of concentration of DNA on ξ(mv) of Y-BPMPHD-CTMAB-DNA system. Conditions: 5.0×10^{-6} M Y^{3+}; 2.01×10^{-5} M BPMPHD; 7.5×10^{-4} M CTMAB; 2.5×10^{-3} M Tris pH = 5.5.

BPMPHD-CTMAB can form a large complex with nucleic acid through the electrostatic attraction. Figure 4 is the ξ of Y-CTMAB-BPMPHD system at different concentration of fsDNA. From Fig. 4 we can see that the ξ of Y-CTMAB-BPMPHD system is about 29.83 mv. With the addition of DNA which is electro-negative to the system, the ξ decreased gradually and tend to zero, which proved the electrastatic interaction between Y-BPMPHD-CTMAB and DNA.

We considered that Y^{3+} in Y-BPMPHD-CTMAB could react with phosphate group of nucleic acid by electrostatic binding, which not only weakened the binding between Y^{3+} and BPMPHD, but also may destroy the structure of Y-BPMPHD complex [15]. Therefore, the fluorescence intensity of this system decreases.

CONCLUSION

It is found that nucleic acids can quench the fluorescence of Y-BPMPHD-CTMAB system. Basis on the fluorescence quenching, a new method for determination nucleic acids using metal complex of Y-BPMPHD-CTMAB as fluorescence probe is proposed. The detection limits are 14.0 ng/mL for fsDNA and 21.0 ng/mL for yRNA. This method is used for the determination of yRNA in actual sample, and the result obtained is satisfactory. This method is sensitive comparison with other common probes. The mechanism is also studied. It is considered that the Y-BPMPHD-CTMAB can form a large complex with nucleic acid through electrostatic attraction.

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