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Fluorimetric Determination of Nucleic Acid Using Enhancement of Terbium-gadolinium-2-thenoyltrifluoroacetone System

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The fluorescence intensity of terbium-2-thenoyltrifluoroacetone-nucleic acid system can be enhanced by Gd^{3+}. Accordingly, a new method of determining nucleic acid was advanced. In the best conditions, the enhanced fluorescence intensity by nucleic acids was in proportion to the concentration of nucleic acids among the range of 5.0×10^{-8} g/mL to 3.0×10^{-5} g/mL for fish sperm DNA and 1.0×10^{-7} to 3.0×10^{-5} g/mL for yeast DNA. Their detection limits (S = N/3) were 4.8 and 9.0 ng/mL, respectively. The method was used for the determination of yRNA in sample and the result was quite satisfactory.

Key Words: Terbium, Gadolinium, 2-Thenoyltrifluoroacetone, Fluorescence.

INTRODUCTION

Nucleic acids are important biologic giant molecule and the carrier of genetic information. They are also the pivotal factor for species' continuation and evolution. The quantitative analysis of nucleic acids is important for the disease diagnoses and the test of therapy effects and is the common analytical item in the food examination and quality test of many biochemical medicines' separation and purification and has intrigued biochemistry and other biological subjects. But the direct determination of nucleic acids by the intrinsic fluorescence and ultraviolet absorption is severely limited by low sensitivity[1,2]. Some method based on quenching or enhancing the fluorescence of various components after the interaction with nucleic acid which named probe technique was proposed[3]. Probe technique is the important means to studying structure, function, qualitative and quantitative analyses of nucleic acids. Some probes such as organic dye[4,5], fluorescent complex[6-8] and lanthanide metal ion[9,10] have been used to improve the sensitivity and selectivity for nucleic acid determination. As rare earth ions have luminescence characteristics such as narrow spectral width, long luminescence lifetime, large stokes shift and strong binding with biological molecules, they are commonly used as fluorescence probes to study nucleic acids, which must be single-stranded[10]. However, the sensitivity is low[9,11]. Rare earth co-luminescence effect is a fluorescence enhancement effect that was first found and studied by Yang and Zhu[12]. This effect has become an important way to improve the sensitivity of rare earth ions by fluorimetry[13-15]. However, the effect is unusual to the analysis of biological material. In the present paper, a new

co-luminescence system of terbium (Tb^{3+})-gadolinium (Gd^{3+})-2-thenoyltrifluoroacetone (TTA)-nucleic acids was found and studied and results indicated that the fluorescence intensity was in proportion to the concentration of nucleic acids, the detection limits were 4.8 and 9.0 ng mL/L for fsDNA and yRNA, respectively.

EXPERIMENTAL

All reagents and solvents used in this study were analytical grade obtained from Chemical Company of China (Shanghai, China). A 10 % HMTA-HCl buffer was adjusted to 7.00 with HCl. A Gd^{3+} stock solution was prepared by dissolving Gd_2O_3 (99.99 %) in 10 mL hydrochloric acid and then diluting with water. A Tb^{3+} stock solution was prepared by dissolving Tb_4O_7 (99.99 %) in 10 mL hydrochloric acid and then diluting with water. 2-Thenoyltrifluoroacetone (TTA) stock solution was prepared by dissolving TTA with ethanol. Commercial herring sperm DNA (fsDNA, Sigma, St. Louis, MO) and yeast RNA (yRNA) stock solution were prepared by dissolving corresponding DNA with water.

Static fluorescence spectra were recorded with a LS-55 spectrofluorimeter (Perkin-Elmer, USA) in a 1 cm quartz cuvette. All pH measurements were made with a PHSJ-4A laboratory pH meter (Mettler Toledo, Shanghai, China).

General procedure: The required amount of solutions was successively added in the following order: HMTA, Tb^{3+}, Gd^{3+}, TTA and nucleic acids. The mixture was diluted with water and mixed thoroughly, then measured after 0.5 h. The excitation and emission peaks were at 372 and 545 nm, respectively. The enhanced fluorescence intensity of Tb^{3+}-Gd^{3+}-TTA-nucleic acids system was represented as $\Delta I_f = I_f - I_f^0$, where I_f and I_f^0 are the fluorescence intensity with and without nucleic acids.

RESULTS AND DISCUSSION

Excitation and emission spectra of Tb^{3+}-TTA-yRNA, Tb^{3+}-TTA-fsDNA, Tb^{3+}-Gd^{3+}-TTA-yRNA and Tb^{3+}-Gd^{3+}-TTA-fsDNA systems were shown in Fig. 1 which indicated that with the excitation wavelength of 372 nm, the Tb^{3+}-Gd^{3+}-TTA-nucleic acids system emitted the characteristic fluorescence of Tb^{3+} with the emission peaks of 489 and 545 nm, which correspond to the transition of $^5D_4 \rightarrow {}^7F_6$ and $^5D_4 \rightarrow {}^7F_5$. The fluorescence intensity of Tb^{3+}-TTA-nucleic acids was very weak and that of Tb^{3+}-Gd^{3+}-TTA-nucleic acids was strong.

Effect of buffer solution and pH value and TTA: Effect of pH value on the fluorescence intensity was shown in Fig. 2 which indicated that the maximum ΔI_f was obtained at pH value 7.0.

The ΔIf for HMTA-HCl, NH_4Cl-NH_3, sodium barbiturate-HCl, tris-HCl, $Na_2B_4O_7$-H_2BO_3, NaH_2PO_4-Na_2HPO_4 and Na_2HPO_4-$C_4H_2O_7$ was100, 93.33, 86.67, 60.0, 5.5, 0 and 0, respectively, which showed that HMTA-HCl is the most suitable buffer solution. Further studies demonstrated the optimal concentration of HMTA was 1.0 %. Fig. 3 showed that the optimal concentration of TTA was 1.2×10^{-4} mol/L.

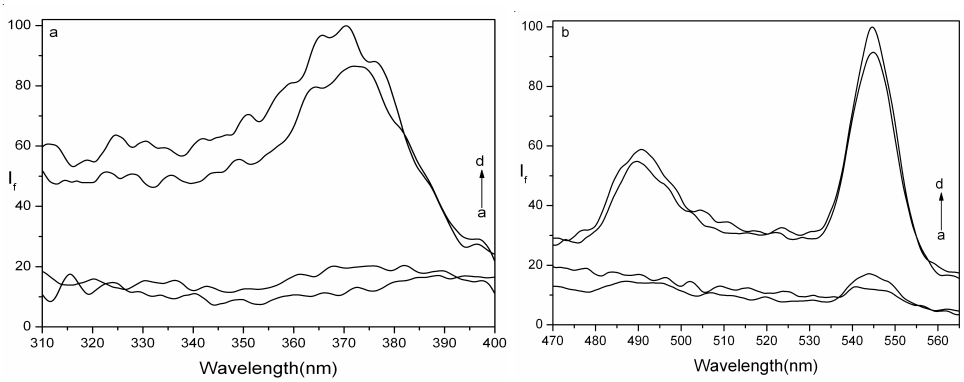

Fig. 1. Exciation (a) and emission (b) spectra; a. Tb^{3+}-TTA-yRNA b. Tb^{3+}-TTA-fsDNA; c. Tb^{3+}-Gd^{3+}-TTA-yRNA; d: Tb^{3+}-Gd^{3+}-TTA-fsDNA; Conditions: Tb^{3+}: 7.0×10^{-5} mol/L; Gd^{3+}: 1.0×10^{-6} mol/L; TTA: 1.2×10^{-4} mol/L; fsDNA: 1.0×10^{-6} g/mL; yRNA: 1.0×10^{-6} g/mL; HMTA: 10 %; pH 7.0

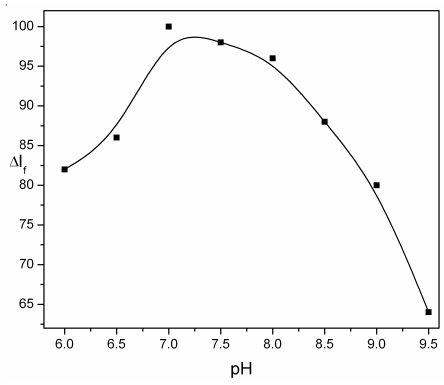

Fig. 2. Effect of pH value; Conditions: Tb^{3+}: 7.0×10^{-5} mol/L; Gd^{3+}: 1.0×10^{-6} mol/L; TTA: 1.2×10^{-4} mol/L; fsDNA: 1.0×10^{-6} g/mL; HMTA: 10 %; Effect of Gd^{3+} and Tb^{3+}

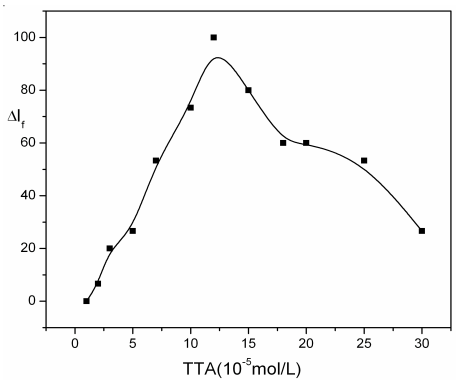

Fig.3. Effect of TTA; Conditions: Tb^{3+}: 7.0×10^{-5} mol/L; Gd^{3+}: 1.0×10^{-6} mol/L; fsDNA: 1.0×10^{-6} g/mL; HMTA: 10 %; pH 7.0

Effect of Gd^{3+} and Tb^{3+}: It was found that Gd^{3+}, La^{3+}, Eu^{3+} and Y^{3+} can enhance the fluorescence intensity of Tb^{3+}-TTA-fsDNA system to a certain degree. The ΔI_f for Gd^{3+}, La^{3+}, Eu^{3+} and Y^{3+} was100, 94.5, 96.6 and 90.7, respectively. Fig. 4 showed that the optimal concentration of Gd^{3+} was 1.0×10^{-6} mol/L. Fig. 5 showed that the optimal concentration of Tb^{3+} was 7.0×10^{-5} mol/L.

Addition order and time evolution effect: Effect of addition order was tested and results indicated the optimum addition order was TTA,Tb^{3+}, Gd^{3+}, nucleic acids and HMTA.

Effect of time evolution on the fluorescence intensity was studied and the results showed that ΔI_f reached a maximum after 20 min and remained stable for over 10 h. Therefore, this system exhibited good stability.

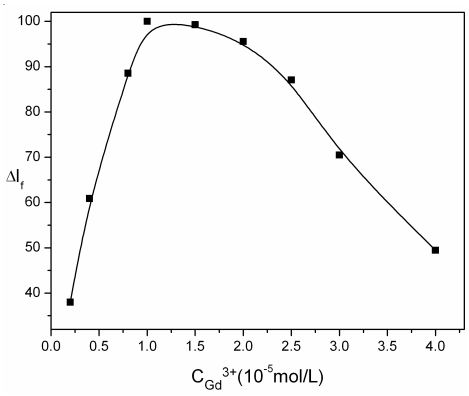

Fig. 4. Effect of Gd^{3+}; Conditions: Tb^{3+}: 7.0 × 10^{-5} mol/L; TTA: 1.2 × 10^{-4} mol/L; fsDNA: 1.0 × 10^{-6} g/mL; HMTA: 10 %; pH 7.0

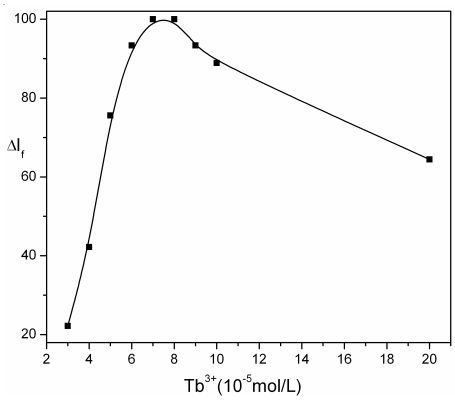

Fig. 5. Effect of Tb^{3+}; Conditions: Gd^{3+}: 1.0 × 10^{-6} mol/L; TTA: 1.2 × 10^{-4} mol/L; fsDNA: 1.0 × 10^{-6} g/mL; HMTA: 10 %; pH 7.0

Effect of foreign substances: Interference of foreign substances was tested and shown in Table-1. It was found that most of ions had little effect on the determination of 1.0 × 10^{-6} g/mL fsDNA within the permissible ± 5 % error.

TABLE-1
EFFECT OF FOREIGN SUBSTANCES

Substances	Concentration coexisting (10^{-5} mol/L)	Change of ΔI_f (%)	Substances	Concentration coexisting (10^{-5} mol/L)	Change of ΔI_f (%)
Zn^{2+}, Cl^-	5.2	4.4	L-His	3.1	5.0
Al^{3+}, Cl^-	8.5	-4.9	DL-Glu	2.2	5.0
Fe^{3+}, Cl^-	1.3	4.9	L-Asn	2.5	4.9
K^+, Cl^-	90	4.8	DL-lys	3.8	4.8
Ca^{2+}, Cl^-	7.9	5.0	GMP	1.5	3.7
Mg^{2+}, SO_4^{2-}	3.2	4.7	L-Phe	1.8	4.2
Mn^{2+}, SO_4^{2-}	4.8	4.1	Pro	3.5	4.7
NH_4^+, Cl^-	150	4.6	DL-Thr	8.4	4.7
Na^+, Cl^-	200	4.0			

Conditions: Tb^{3+}: 7.0 × 10^{-5} mol/L; Gd^{3+}: 1 × 10^{-6} mol/L; TTA: 1.2 × 10^{-4} mol/L; fsDNA: 1.0 × 10^{-6} g/mL; HMTA: 10 %; pH 7.0

Analytical applications: Under the optimum condition defined, the calibration graphs for nucleic acids were shown in Table-2 which indicated that there was a linear relationship between ΔI_f and the concentration of nucleic acids in the range of 5.0 × 10^{-8}-3 × 10^{-5} g/mL for fsDNA, 1.0 × 10^{-7}-3 × 10^{-5} g/mL for yRNA. Their detection limits (S/N = 3) are 4.8 and 9.0 ng/mL, respectively.

A comparison between this probe and other common probes for nucleic acids in sensitivity was summed up in Table-3. It can be seen that the sensitivity of this method is higher than most of the well known fluorimetric methods for the determination

TABLE-2
ANALYTICAL PARAMETERS

DNA	Linear range (g/mL)	Linear regression equation (g/mL)	Correlation coefficient	Detection limit (ng/mL)
fsDNA	5.0×10^{-8}-3×10^{-5}	$\Delta I_f = 5.48\times10^6C+7.46$	0.993	4.8
yRNA	1.0×10^{-7}-3×10^{-5}	$\Delta I_f = 2.62\times10^6C+6.95$	0.996	9.0

Conditions: Tb^{3+}: 7.0×10^{-5}m ol/L; Gd^{3+}: 1×10^{-6} mol/L; TTA: 1.2×10^{-4} mol/L; HMTA: 10%; pH 7.0

TABLE-3
COMPARISON OF THIS METHOD WITH REPORTED METHOD

Fluorescent probe	Nucleic acids	Determination limit (ng/mL)	References
Pico Green	dsDNA	0.025	16
Magdala red	ctDNA/smDNA/yRNA	6.0/7.0/15.0	17
Heptamethylene cyanine	DNA	6.8	18
Brilliant cresyl blue	smDNA/yRNA	7.0-25.0	19
Berberine	ctDNA/yRNA	50/80	20
Eu^{3+}-Oxytetracycline	fsDNA/ctDNA	11.2/15.1	21
AlS_4Pc^2	ctDNA/smDNA/yRNA	17/24/98	22
Al(III) –SAP	fsDNA/ctDNA/yRNA	52/49/62	23
Tb^{3+}-Gd^{3+}-TTA-NA	fsDNA/yRNA	4.8/9.0	This work

Tb^{3+}-Gd^{3+}-TTA-NA=Terbium-gadolinium-2-thenoyltrifluoro-acetone (TTA)-nucleic acids (NA)

of nucleic acids. In addition, the reagents used in this method are easily available in the market. The cost are low in comparison to more common methods with high sensitivity such as PicoGreen method.

The standard addition method was used for the determination of yRNA. The yRNA actual sample was made from yeast solution using an alkaline method[24] and analyzed using this method and compared with UV spectrophotometric method. The results (Table-4) showed that the accuracy and precision of the method were quite satisfactory.

TABLE-4
ANALYSIS OF yRNA SAMPLES

Sample	Methods	Concentration (mg/mL)	Average (mg/mL)	RSD (%)
yRNA	Proposed method	19.5, 19.8, 20.1, 20.3, 19.0	19.24	0.51
	UV method	20.3, 21.5, 20.7, 20.9, 21.0	20.88	0.44

Conditions: Tb^{3+}: 7.0×10^{-5} mol/L; Gd^{3+}: 1×10^{-6} mol/L; TTA: 1.2×10^{-4} mol/L; HMTA: 10%; pH 7.0.

Conclusion

In this paper, a new fluorimetric method for determination of nucleic acids was reported. Under optimum conditions, the enhanced intensity of fluorescence was in proportional to the concentration of nucleic acids among wide range.

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REFERENCES

1. W.E. Schy and M.J. Pleva, *Anal. Biochem.*, **180**, 314 (1989).
2. Z.X. Guo and H.X. Shen, *Analyst*, **124**, 1093 (1999).
3. Y. Liu, C.Q. Ma, K.A. Li and S.Y. Tong, *Anal. Chim. Acta*, **379**, 39 (1999).
4. J. Kapuscinski and B. Skoczylas, *Anal. Biochem.*, **83**, 282 (1997).
5. W.Y. Li, J.G. Xu and X.Q. Guo, *Anal. Lett.*, **30**, 245 (1997).
6. C.G. Lin, J.H. Yang, G.L. Zhang and X. Wu, *Anal. Chim. Acta*, **392**, 291 (1999).
7. J.H. Yang, C.G. Lin and G.L. Zhang, *Spectrochim. Acta A*, **54**, 2019 (1998).
8. X.J. Liu, Y.Z. Li and Y.X. Ci, *Anal. Chim. Acta*, **345**, 213 (1997).
9. J.H. Yang, C.L. Tong and N.Q. Jie, *Chin. Biochem. J.*, **12**, 143 (1996).
10. C.G. Lin, J.H. Yang, X. Wu, G.L. Zhang and R.T. Liu, *Anal. Chim. Acta*, **403**, 209 (2000).
11. K.L. Patty and T. Claudia, *J. Am. Chem. Soc.*, **121**, 1 (1999).
12. J.H. Yang and G.Y. Zhu, *J. Shandong Univ.*, **21**, 133 (1986).
13. Y.Y. Xu and I. Hemmila, *Anal. Chim. Acta*, **256**, 9 (1992).
14. J.H. Yang, H.M. Ge, N.Q. Jie and X.Z. Ren, *Spectrochim. Acta A*, **51**, 185 (1995).
15. J.H. Yang, H.M. Ge, N.Q. Jie and X.Z. Ren, *Analyst*, **120**, 1705 (1995).
16. L.S. Victoria, J.J. Laurie, T.Y. Stephen and H.P. Richard, *Anal. Biochem.*, **249**, 228 (1997).
17. H.H. Yang, Q.Z. Zhu, Q.Y. Chen, D.H. Li and J.G. Xu, *Fresenius' J. Anal. Chem.*, **366**, 303 (2000).
18. H. Zheng, X.L. Chen, M.H. Hu, D.H. Li and J.G. Xu, *Anal. Chim. Acta*, **461**, 235 (2002).
19. H. Zheng, X.L. Chen, C.Q. Zhu and D.H. Li, *Microchem. J.*, **64**, 263 (2002).
20. G.Q. Gong, Z.X. Zong and Y.M. Song, *Spectrochim. Acta A*, **55**, 1903 (1999).
21. R.T. Liu, J.H. Yang and X. Wu, *J. Luminescence*, **96**, 201 (2002).
22. D.H. Li, X.L. Chen, Y. Fang and J.G. Xu, *Analyst*, **126**, 518 (2001).
23. Y.M. Hao and H.X. Shen, *Spectrochim. Acta*, **56A**, 1013 (2000).
24. Y.X. Peng, W.H. Zhu and J.H. Chen, Experimental Biochemistry, The People's Education Press, Beijing, China, p. 79 (7989).

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