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Synthesis, characterization and DNA interaction studies of complexes of lanthanide nitrates with tris{2-[(3,4-dihydroxybenzylidene)imino]ethyl}amine

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Abstract

A new tripodal, hydroxyl-rich ligand, tris{2-[(3,4-dihydroxybenzylidene)imino]ethyl}amine (L), and its complexes with lanthanide nitrates were synthesized. These complexes which are stable in air with the general formula of $[LnL(NO_3)_2]NO_3{\cdot}H_2O$ (Ln = La, Sm, Eu, Gd, Y) were characterized by molar conductivity, elemental analysis, IR spectra and thermal analysis. The NO_3^- groups coordinated to lanthanide monodentately, and the coordination number in these complexes may be 8. The interaction of complexes with DNA were investigated by ultraviolet and fluorescent spectra, which showed that the binding mode of complexes with DNA was intercalation, and the binding affinity with DNA were La(III) complex > Sm(III) complex > Eu(III) complex > Gd(III) complex > Y(III) complex. Based on these results, it can be shown that the La(III)complex is promising candidate for therapeutic reagents and DNA probes.

Keywords: Lanthanide; Tripodal ligand; Complexes; Synthesis; DNA interaction

1. Introduction

DNA binding studies are very important for the development of new therapeutic reagents and DNA probes [1,2]. Generally, there are three kinds of binding models for small molecules with DNA, i.e., (I) intercalation, (II) groove binding, and (III) external electrostatic binding. In these binding models, the intercalative binding is strongest, because it is a type of binding in which the intercalative molecule surface is sandwiched between the aromatic, heterocyclic base pairs of DNA [3,4].

The luminescent characteristics of lanthanide complexes and their binding affinity with DNA have led to their general application as spectroscopic probes for nucleic acids [5,6]. And compared with lanthanide metal ions, their complexes may have different binding models and stronger binding affinity. The interaction of Eu(III) and DNA have a small effect on the characteristic absorption peak of DNA in UV spectra. Hypochromicity is observed at 226 and 258 nm after the interaction of Eu-phenylalanine and DNA, and fluorescent intensity is decreased obviously. It shows that Eu-phenylalanine can strengthen the interaction with DNA [7]. However, compared with transition metal complexes, the studies of the interaction of lanthanide complexes with calf thymus DNA are few, especially when tripodal ligands are used as hosts. As our studies on complexes with tripodal ligands, a new polydentate, hydroxyl-rich tripodal ligand tris{2-[(3,4-dihydroxybenzylidene)imino]ethyl}amine (L) and its complexes with lanthanide are synthesized and characterized, and the interaction with calf thymus DNA is investigated by ultraviolet and fluorescent spectra.

2. Experimental

2.1. Reagents and apparatus

Lanthanide nitrates and tris(2-aminoethyl)amine (abbreviated as tren in the following) were prepared according to Refs. [8,9]. All other solvents and reagents used were of analytical grade. All the experiments involving interaction of the complex with DNA were performed in Tris–HCI (0.01 M, pH

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Table 1
Elemental analysis and molar conductance data

Complexes	Yield (%)	C% found (calc.)	H% found (calc.)	N% found (calc.)	Ln% found (calc.)	Λm (S cm^2 mol^{-1})
[LaL(NO$_3$)$_2$]NO$_3$·H$_2$O	73	11.25 (11.54)	37.84 (38.17)	3.91 (3.77)	16.84 (16.36)	78.5
[SmL(NO$_3$)$_2$]NO$_3$·H$_2$O	71	11.67 (11.40)	37.34 (37.67)	3.67 (3.72)	17.84 (17.44)	86.0
[EuL(NO$_3$)$_2$]NO$_3$·H$_2$O	72	11.61 (11.37)	37.62 (37.59)	3.68 (3.71)	17.23 (17.63)	79.0
[GdL(NO$_3$)$_2$]NO$_3$·H$_2$O	75	10.74 (11.30)	37.32 (37.37)	3.24 (3.69)	18.54 (18.11)	76.5
[YL(NO$_3$)$_2$]NO$_3$·H$_2$O	75	12.58 (12.27)	40.87 (40.56)	4.23 (4.00)	10.85 (11.13)	78.6

7.4). Solutions of calf thymus DNA in buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.9:1, indicating that DNA was free from protein [10]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [11]. Stock solutions were stored at 4 °C and used in no more than 4 days. Redistilled water was used to prepare buffers.

Carbon, nitrogen and hydrogen were determined by using an Elementer Vario-EL elemental analyzer. Molar conductivity measurement was carried out with a DDSJ-308 conductometer (made in China) at 25 °C. The IR spectra was recorded on a Nicolet NEXUS 670 FT-IR spectrometer in the region of 4000–400 cm^{-1} in KBr pellets. Thermal analysis was performed on a PCT-2A thermobalance analyzer (Beijing optics apparatus factory). ^{1}H NMR spectra were recorded on a Varian VR 300 MHz spectrometer in CDCl$_3$. The FAB mass spectrum was obtained on a VG ZAB-HS mass spectrometer. UV–vis spectra were recorded on a UV 757 CRT spectrophotometer (Shanghai Precision Scientific Instrument Co., Ltd.). Fluorescence spectra were recorded on a 970 CRT spectrometer (Shanghai Precision Scientific Instrument Co., Ltd.).

2.2. Synthesis of ligand (L) Fig. 1

A solution of 1 mmol tren in 5 mL dichloromethane was added dropwise to a solution of 3 mmol 3,4-dihydroxybenzaldehyde in 15 mL of mixed solution of 2:1 (V:V) dichloromethane and methanol. The mixture was stirred at room temperature for 5 h. The precipitated solid was filtered, washed with the mixed solution of dichloromethane and methanol three times, and dried in a vacuum. Yield, 83%; FAB-MS: $m/z = 507.4$ (M + H). ^{1}H NMR (CDCl$_3$, ppm): δ 8.02(3H, d, –CH=N), 7.16–6.69(9H, m, Ar–H), 4.24(6H, s, ArOH), 3.52(6H, s, NCH$_2$–). IR(KBr pellets), ν/(cm^{-1}): 3378(ArO–H), 1648(C=N), 1280(Ar–O).

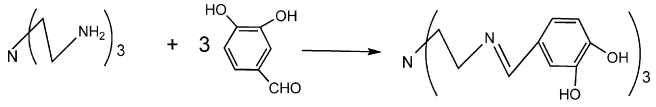

Fig. 1. The procedure of ligand (L) synthesis.

2.3. Preparation of complexes

0.5 mmol tris{2-[(3,4-dihydroxybenzylidene)imino]ethyl} amine (L) was dissolved in 10 mL methanol. To this solution was added slowly a 10 mL methanol solution of 0.5 mmol lanthanide nitrates under stirring. The solution was continually stirred for 2 h at room temperature, and then the precipitate was collected by filtration, washed with methanol for three times, and dried in a vacuum.

3. Results and discussion

3.1. Characterization of ligand (L)

The new polydentate, hydroxyl-rich ligand which derived from the 1:3 condensation of tren with 3,4-dihydroxybenzaldehyde was characterized by IR, ^{1}H NMR, and FAB-MS. The IR spectrum of the ligand shows strong band at 1650 cm^{-1}, which is attributable to stretch vibration of the ν(C=N). And the ligand exhibits band at 3378 cm^{-1} in the infrared spectrum, which is assigned to ν(ArO–H). In addition, the peak at 1280 cm^{-1} can be assigned to ν(Ar–O) of hydroxybenzene [12]. And the integral intensities of each signal in the ^{1}H NMR spectra measured in CDCl$_3$ at room temperature were found to agree with the number of different types of protons present. The signal of –CH=N proton was observed at 8.02 ppm [13], and signals appeared at 7.16–6.69, 4.24 and 3.52 ppm are assigned to protons of Ar–H, ArOH, and NCH$_2$–, respectively. According to the FAB-MS, we can know the molecule weight of this new ligand is 506.

Table 2
Major infrared spectral data for the [LnL(NO$_3$)$_2$]NO$_3$·H$_2$O (cm^{-1})

Compounds	ν(OH)	ν(C=N)	ν(Ar–O)	ν_1(NO$_3^-$)	ν_2(NO$_3^-$)	ν_3(NO$_3^-$)	ν_4(NO$_3^-$)	ν_5(NO$_3^-$)	ν_5-ν_1
Ligand	3378	1649	1280						
[LaL(NO$_3$)$_2$]NO$_3$·H$_2$O	3421	1651	1229	1312	1033	1383	759	1422	110
[SmL(NO$_3$)$_2$]NO$_3$·H$_2$O	3423	1650	1231	1311	1034	1383	764	1421	110
[EuL(NO$_3$)$_2$]NO$_3$·H$_2$O	3424	1651	1231	312	1033	1383	763	1422	110
[GdL(NO$_3$)$_2$]NO$_3$·H$_2$O	3426	1650	1232	1314	1035	1383	755	1422	108
[YL(NO$_3$)$_2$]NO$_3$·H$_2$O	3419	1650	1232	1314	1033	1383	768	1422	108

Table 3
Data of TG–DTA spectra of some complexes

Complexes	T_{endo} (°C)	T_{1exdo} (°C)	T_{2exdo} (°C)	Residue (calc.)%
$[LaL(NO_3)_2]NO_3{\cdot}H_2O$	97	260	481	20.24 (19.20)
$[GdL(NO_3)_2]NO_3{\cdot}H_2O$	79	270	374	21.75 (20.96)

3.2. Composition of complexes

The metal ion was determined by EDTA titration using xylenol orange as indicator. The results of elemental analyses (Table 1) indicate that the composition of the complexes is $Ln(NO_3)_3{\cdot}L{\cdot}H_2O$ (Ln = La, Sm, Eu, Gd, Y) and the ratio of Ln/L in all complexes are 1:1.

3.3. Solubility and molar conductance

These complexes are soluble in DMSO, DMF, and poorly soluble in methanol, ethanol, acetone or chloroform. The molar conductance values of the complexes measured in DMF (1×10^{-3} mol L^{-3}) at 25 °C are in the range of 76–86 S cm^2 mol^{-1} (Table 1), indicating 1:1 electrolytes [14].

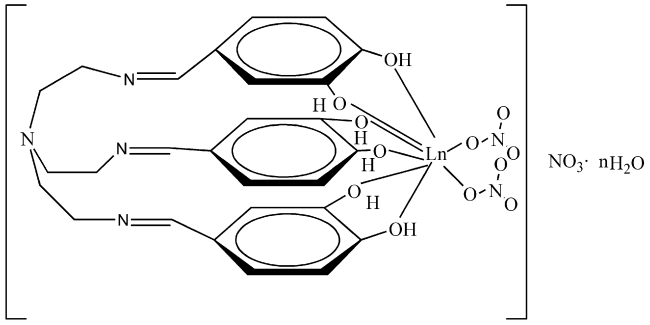

Fig. 2. The possible structure of the complex Ln^{3+}-L.

3.4. Infrared spectra

The infrared spectral data of ligand L and its complexes are listed in Table 2. IR spectra of complexes are similar to

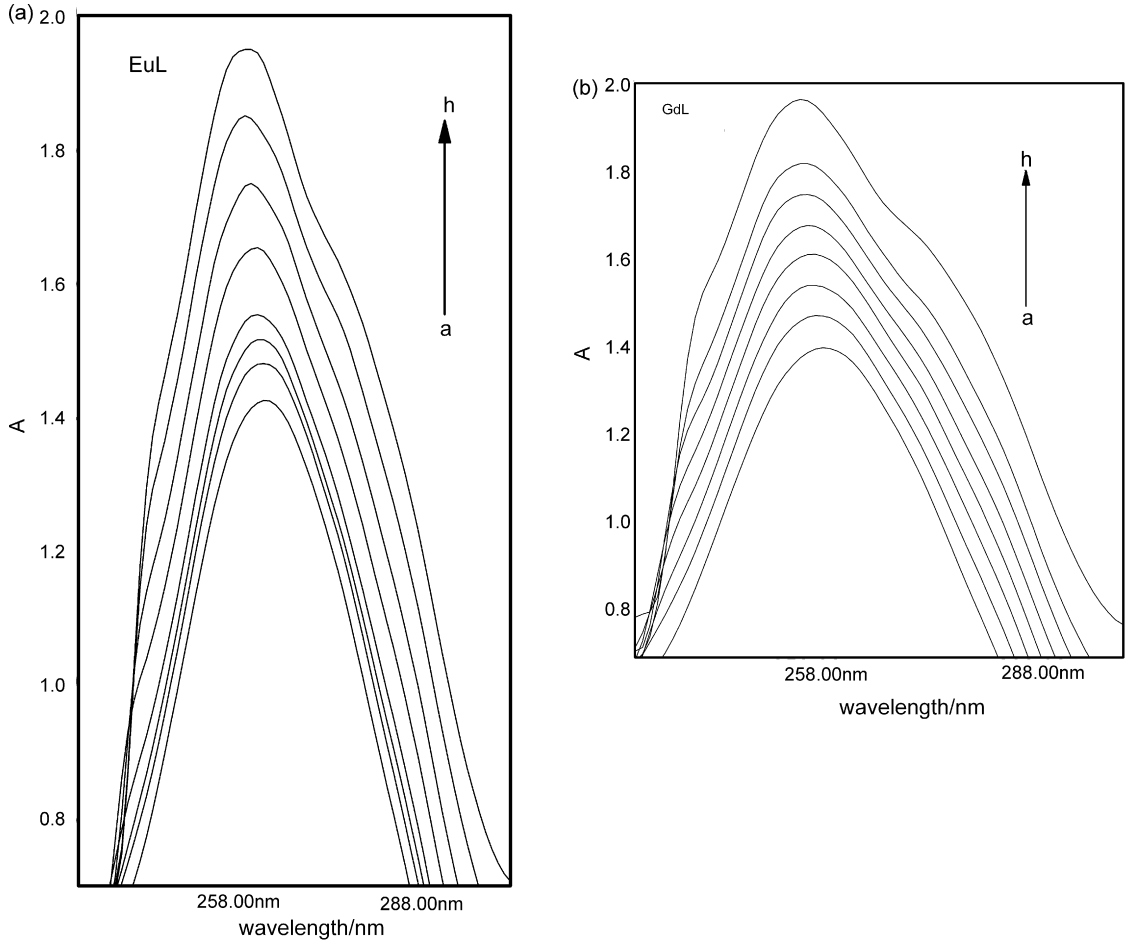

Fig. 3. (a) Absorption spectra of the DNA solution in the absence and presence of Eu(III) complex. C_{DNA}: 2.1×10^{-4} mol/L. C_{EuL}: a, 0; b, 1.47; c, 4.40; d, 7.33; e, 10.3; f, 14.7; g, 22.0; h, 29.4×10^{-5} mol/L. (b) Absorption spectra of the DNA solution in the absence and presence of Gd(III) complex. C_{DNA}: 2.1×10^{-4} mol/L. C_{GdL}: a, 0; b, 1.47; c, 4.40; d, 7.33; e, 10.3; f, 14.7; g, 22.0; h, 29.4×10^{-5} mol/L. (c) Absorption spectra of the DNA solution in the absence and presence of La(III) complex. C_{DNA}: 2.1×10^{-4} mol/L. C_{LaL}: a, 0; b, 1.47; c, 4.40; d, 7.33; e, 10.3; f, 14.7; g, 22.0; h, 29.4×10^{-5} mol/L. (d) Absorption spectra of the DNA solution in the absence and presence of Sm(III) complex. C_{DNA}: 2.1×10^{-4} mol/L. C_{SmL}: a, 0; b, 1.47; c, 4.40; d, 7.33; e, 10.3; f, 14.7; g, 22.0; h, 29.4×10^{-5} mol/L. (e) Absorption spectra of the DNA solution in the absence and presence of Y(III) complex. C_{DNA}: 2.1×10^{-4} mol/L. C_{YL}: a, 0; b, 1.47; c, 4.40; d, 7.33; e, 10.3; f, 14.7; g, 22.0; h, 29.4×10^{-5} mol/L.

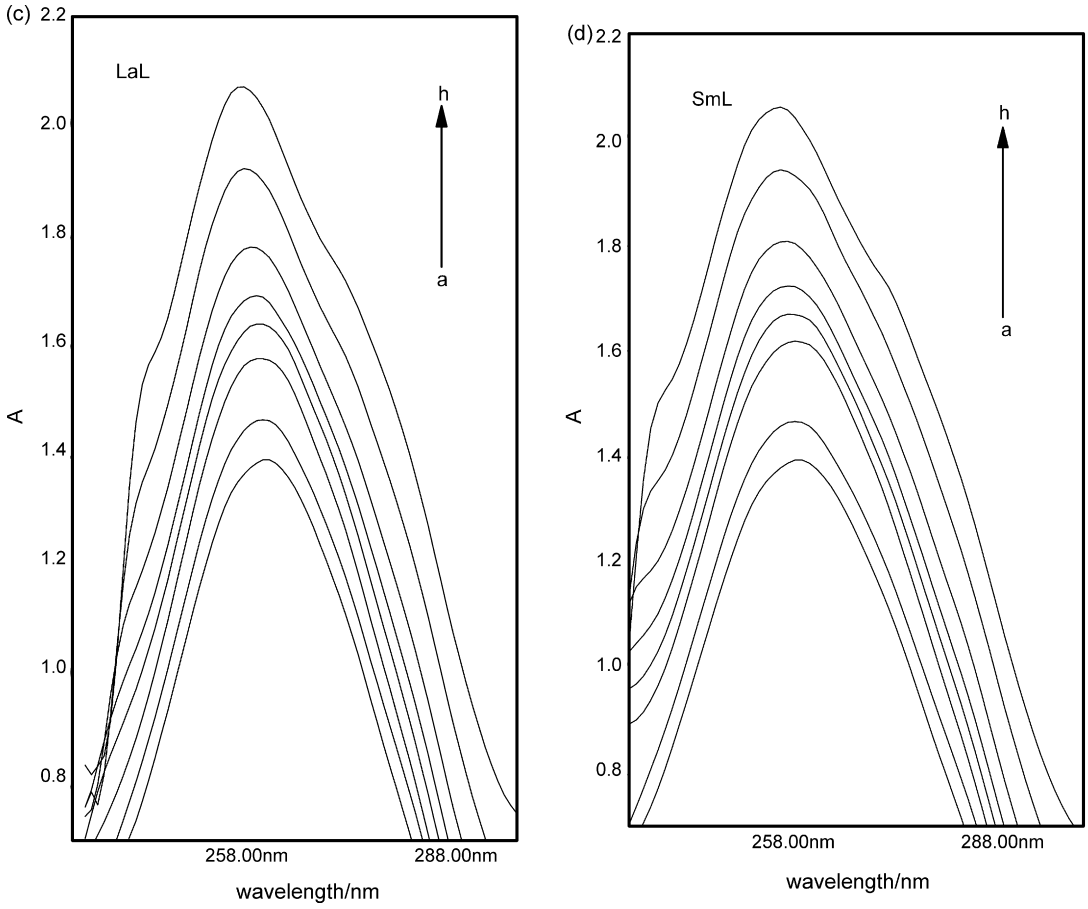

Fig. 3. (*Continued*)

each other, indicating that the complexes have similar structures. In the complexes, the bands due to ν(OH) are observed at 3419–3425 cm^{-1}, so it is apparent that ν(ArO–H) bands in these complexes are shifted to lower frequencies about 41–48 cm^{-1}, indicating that the oxygen atoms in hydroxybenzene take part in coordination to the lanthanide ions. In comparison with the free ligand, the ν(C=N) bands in the complexes shift only 1 or 2 cm^{-1} to lower frequencies, indicating that the nitrogen atoms do not coordinate to the metal ions.

The absorption bands assigned to the coordinated nitrate groups ($C_{2\nu}$) were observed at about 1310 cm^{-1} (ν_1), 1031 cm^{-1} (ν_2), 1420 cm^{-1} (ν_5) in these complexes, respectively. In addition, the separation of two strongest frequency bands $|\nu_5–\nu_1|$ is approximately 110 cm^{-1}, clearly establishing that the coordinated NO^{3-} groups in the solid complexes coordinate to the lanthanide ions mono-dentately [15]. However, in the majority of the structurally characterized lanthanide nitrate complexes, the nitrato ligands are bidentate [16]. And the noteworthy band at 1380 cm^{-1} in the spectra of complexes indicates that free nitrate group (D_{3h}) is present [17], in agreement with the results of the molar conductivity experiments. Furthermore, there are no bands that show H_2O coordinate to metal ions in the complexes, which is in accordance with the results of TG–DTA.

3.5. Thermal analyses

The TG–DTA curves of all the complexes are similar. The thermal analysis data of some complexes are listed in Table 3. The combined TG and DTA studies were performed on the La(III) complex. The TG curve shows weight loss in the temperature of 97 °C, attributed to elimination of the water molecules which is accompanied by an endothermic peak at 97 °C in the DTA thermogram. The complex has no melting point. The complex decomposes since there are two exothermic peaks at 260 and 481 °C, losing weight about 79.76% (Ca. 80.80%) in the TG curve. Beyond the temperature of 750 °C, the curves of TG and DTA have no change, possibly due to the complex decomposed to Ln_2O_3.

According to the data of the elemental analyses, molar conductivity, ^{1}H NMR spectra, IR spectra and TG–DTA, the possible molecular structures of the complexes are shown in Fig. 2. The coordination number for L complexes may be 8.

4. Interaction of complexes with DNA

4.1. UV–vis spectra

The calf thymus DNA absorbs at 260–280 nm and all the complexes have no absorption at 240–300 nm. So addition com-

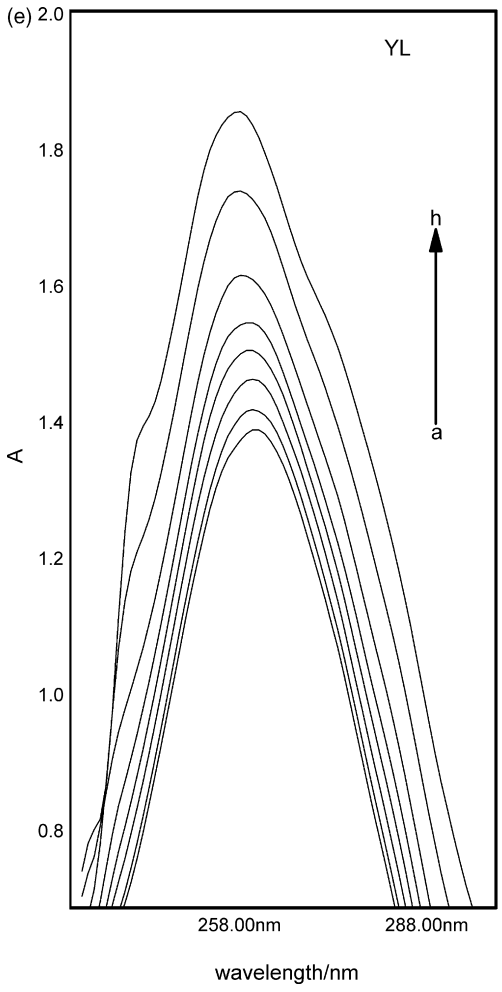

Fig. 3. (*Continued*).

plexes to the DNA solution directly can be used to examine the reaction of DNA with the complexes by the change of absorption of DNA. When these complexes in different concentration are added into DNA solution, respectively, the absorptivity of the DNA solution increases considerably. Furthermore, the emission peak of DNA shift to lower wavelength step by step in the course of adding complexes into DNA. The results can be seen in Fig. 3(a)–(e).

The shift of the emission peak and the enhancement of its intensity may be largely due to the fact that the complexes intercalated into the base group pairs of DNA and destroyed the H-binding of purine bases and pyrimidine bases, which may cause the slight change of the conformation of DNA [18]. Based on the above investigation, it can be found that all the complexes can bind to DNA, and have strong binding affinity with DNA.

4.2. Fluorescence spectra

Further exploration of the binding of complexes with DNA, fluorescence spectra studies were carried out on DNA pretreated with EB by varying the concentration of the added complexes. EB is employed as a probe in examination of the reaction, and it bind to DNA by intercalation [19]. In contrast to DNA, the addition of complexes to the solution of EB has no effect on the emission intensity and emission peak of EB.

Fig. 4 shows the emission spectra of the EB–DNA system in the absence and presence of La(III) complexes. The emission intensity of EB–DNA system decreases when the concentration of La(III) complex increases. This phenomenon indicates that there is a strong binding and a large affinity in the complex with the calf thymus DNA.

The addition of complexes caused the quenching fluorescence of the EB–DNA system. This case can be considered as the complexes directly reacted with the DNA of DNA–EB system, which leads to the EB molecules left the EB–DNA system, and the emission intensity of EB–DNA system decreased [20]. So the binding constant K of complexes to DNA can be determined from the following equation: $F_0/F = 1 + K[\mathrm{M}]$. Where, [M], F_0 and F correspond to the concentration of quenching agent, the emission intensity of the EB–DNA system before and after the addition of complexes, respectively. K is the apparent binding constant, which is given by the ratio of the slop to intercept [21]. According to the equation, in the plots of F_0/F versus [M], $K = 3.55 \times 10^3 [\mathrm{M}^{-1}]$ for La(III) complex is the highest, and the lowest binding constant K is 2.10×10^3

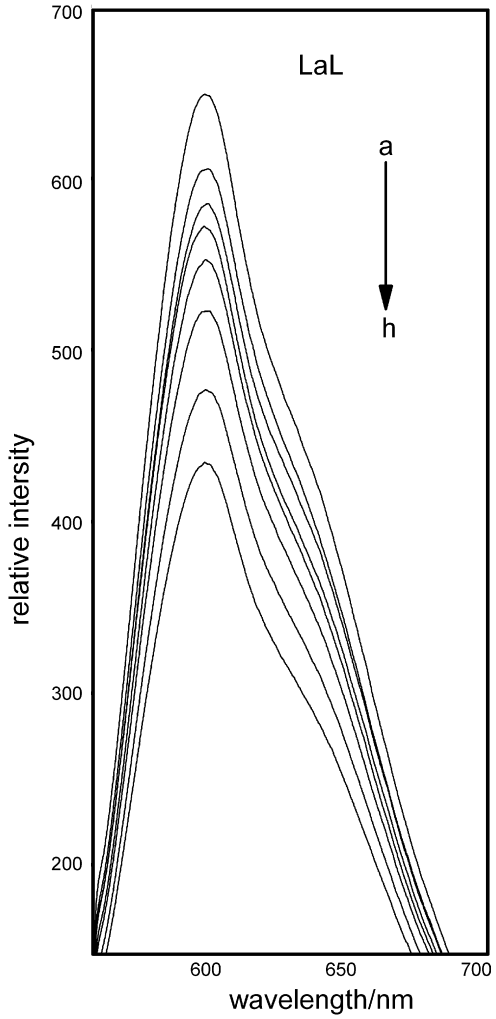

Fig. 4. Emission spectra of the EB–DNA system in the absence and presence of La(III) complex, C_{EB}:6.34×10^{-6} mol/L.C_{DNA}:2.25×10^{-4} mol/L. Excited at 540 nm, C_{LaL}: a, 0; b, 1.47; c, 2.94; d, 4.41; e, 5.88; f, 7.35; g, 11.0; h, 14.7×10^{-5} mol/L.

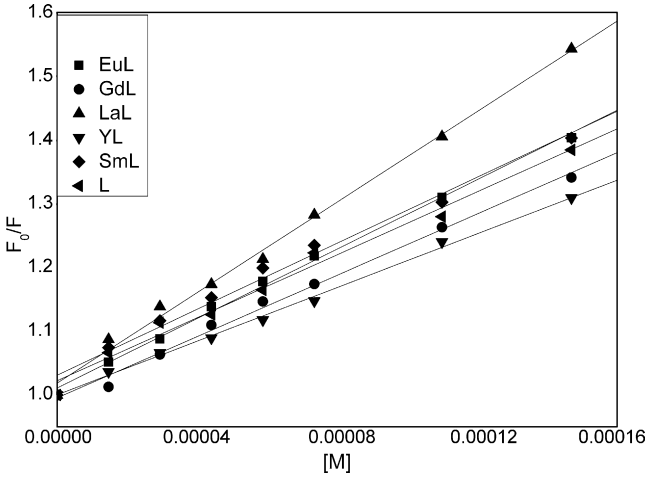

Fig. 5. The fluorescence quenching curve of the complexes to DNA–EB.

$[M^{-1}]$ for the Y(III)complex. The binding constant K of complexes to DNA are shown in Fig. 5. By above investigation, it can be shown that the binding affinity with DNA is La(III) complex > Sm(III) complex > Eu(III) complex > Gd(III) complex > Y(III) complex.

5. Conclusion

The present studies show that the novel tripodal ligand and its complexes have been synthesized and characterized. The results of these characterization methods show that these complexes are 1:1 (Ln/L), and their coordination number is 8.

The interaction studies of complexes with calf-thymus DNA suggest that the complexes intercalated into the base group pairs of DNA, have strong binding affinity with DNA. And according to fluorescence data, the binding constant for the reaction at 298 K are obtained, which show that the binding affinity with DNA is La(III) complex > Eu(III) complex > Sm(III) complex > Gd(III) complex > Y(III) complex. Based on those results, it can be shown that the La(III)complex is promising candidate for therapeutic reagents and DNA probes.

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References

[1] P.J. Dandlier, R.E. Holmlin, J.K. Barton, Science 274 (1997) 1465.
[2] D.S. Sigman, A. Mazumder, D.M. Perrin, Chem. Rev. A 93 (1993) 2295.
[3] C.V. Kumar, H.E. Asuncion, J. Am. Chem. Soc. 115 (1993) 8547.
[4] X.Q. He, Q.Y. Lin, R.D. Hu, X.H. Lu, Spectrochim. Acta A 68 (2007) 184–190.
[5] P. Yang, R. Ren, B.Sh. Yang, Chem. Res. Appl. 6 (1994) 22.
[6] Y.L. Zhao, F.Y. Zhao, Chin. J. Rare Earths 21 (2000) 5.
[7] H.B. Shen, L.H. Kuai, L.H. Ni, et al., J. Rare Earths 15 (1997) 300.
[8] Q.D. Tan, W.G.M.G. He, et al., Chem. J. Chin. Univ. 7 (1986) 1067.
[9] S. Kimura, S. Young, J.P. Collman, Inorg. Chem. 9 (1970) 1183.
[10] J. Marmur, J. Mol. Biol. 3 (1961) 208.
[11] M.F. Reichmann, S.A. Rice, C.A.J. Am. Chem. Soc. 76 (1954) 3047.
[12] W.B. Yuan, L. Yan, R.D. Yang, Chin. J. Appl. Chem. 21 (2004) 829.
[13] H. Yang, W.H. Sun, Z. Li, L. Wang, Synth. Comm. 32 (2002) 2395.
[14] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
[15] E. Katsoulakoua, V. Bekiarib, C.P. Raptopoulouc, Spectrochim. Acta A 61 (2005) 1627–1638.
[16] X.L. Tang, W. Dou, W.Sh. Liu, Spectrochim. Acta A 68 (2007) 349–353.
[17] K. Nakamoto, Infrared and Raman Spectroscopy of Inorganic and Coordination Compounds, John Wiley & Sons, New York, 1986.
[18] Ch.Q. Jiang, J.X. He, J.Sh. Wang, Spectrosc. Spect. Anal. 22 (2002) 103.
[19] J.K. Veal, R.L. Rill, J. Biochem. 80 (1991) 1132.
[20] Y.Zh. Kang, H.B. Shen, Y.Q. Luo, et al., J. Chin. Rare Earths 22 (2002) 23.
[21] Y.M. Song, X.L. Lu, M.L. Yang, et al., J. Transit. Met. Chem. 30 (2005) 4.