J Fluoresc (2008) 18:547–553
DOI 10.1007/s10895-007-0298-6

ORIGINAL PAPER

Synthesis, Characterization, DNA-Binding Properties of the Ln(III) Complexes with 6-Hydroxy Chromone-3-Carbaldehyde-(4′-Hydroxy) Benzoyl Hydrazone

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Received: 30 August 2007 / Accepted: 26 November 2007 / Published online: 12 December 2007

Abstract 6-Hydroxy chromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone (L) and its Ln (III) complexes, [Ln=La, Nd, Eu and Tb] have been prepared and characterized on the basis of elemental analyses, molar conductivities, mass spectra, ^{1}H NMR, thermogravimety/differential thermal analysis (TG-DTA), UV-vis spectra, fluorescence spectra and IR spectra. The formula of the complex is [Ln L·$(NO_3)_2$]·NO_3. Spectrometric titration, ethidium bromide displacement experiments and viscosity measurements indicate that Eu (III) complex bind with calf-thymus DNA, presumably via an intercalation mechanism. The intrinsic binding constant of Eu (III) with DNA was 2.48×10^5 M^{-1} through fluorescence titration data.

Keywords 6-Hydroxy chromone-3-carbaldehyde-(4-hydroxy) benzoyl hydrazone · Rare earth complexes · DNA binding

Abbreviations

CT-DNA	calf thymus DNA
L	6-hydroxy chromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone
Tris	Tris(hydroxymethyl)-aminomethane
NMR	Nuclear magnetic resonance
EB	ethidium bromide
CDC	6-hydroxy-3-carboxaldehydes chromone
UV-Vis	Ultraviolet and visble
TG-DTA	thermogravimety/differential thermal analysis

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Introduction

Binding studies of small molecules to DNA on a molecular level are very important in the development of novel chemotherapeutics and highly sensitive diagnostic agents [1–5].The interaction of transition metal complexes with DNA has attracted much attention during past decade. The metal complexes can interact non-covalently with nucleic acids by intercalation when the ligand contains planar ring systems, groove binding for large molecules, or external electrostatic binding for cations. The binding modes are dependent on the sizes and stereochemical properties of the metal complexes. Transition metal complexes have been widely exploited for serving as a probe for nucleic acid structure and showing nuclease property [6]. So the development of synthetic, sequence-selective cleavage agents and structure probes for DNA itself and for DNA-bound drugs is essential for further expected applications in molecular biology, medicine and related fields.

Fluorescent transition metals centres are particularly attractive moieties for such research for not only do they exhibit well-defined coordination geometries but they also often possess distinctive electrochemical or photophysical properties, thus enhancing the functionality of the binding agent [7]. Complexes have found a plethora of applications ranging from foot-printing agents to probes of electron transfer processes within DNA. Further, the application of these molecules necessitates isolation of structurally analogous complexes with different shapes and electronic properties, investigation of their DNA-binding properties and then the precise understanding of the structural details of their mode of interaction with the target molecule, namely, double helical DNA.

Recent study indicate that one of the successful and effective approaches in the search for new antitumor agents

from natural products is to synthesize novel compounds by simple chemical modification on the basis of natural leading compounds. Our previous work showed that the rare earth complexes of flavone benzoyl hydrazone have certain antioxidant and cytotoxic activity, and can bind to CT-DNA by intercalation [8–10]. As part of our continuing research DNA binding model of the flavone benzoyl hydrazone and its complexes, and search for new and effective antitumor agents from natural products, in this paper, we synthesized another new ligand, 6-hydroxy chromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone (Fig. 1), and its Ln (III) complexes. We described a comparative study of the interactions of Eu (III) complex with CT-DNA using UV-visible, fluorescence and viscosity measurements.

Experimental

Materials

Acetic anhydride, hydroquinone, safranin and EDTA, were produced in China. NBT, MET, $VitB_2$, CT-DNA and EB were purchased from Sigma Chemical Co.. All chemicals used were of analytical grade. The rare earth (III) nitrates were derived from their oxide (99.9%) acquired from Nong Hua (P.R.C.).

Instrumentation

Carbon, hydrogen, and nitrogen were analyzed on an Elemental Vario EL analyzer. The metal contents of the complex were determined by titration with EDTA. Infrared spectra (4,000–400 cm^{-1}) were determined with KBr disks on a Therrno Mattson FTIR spectrometer. The UV-visible spectra were recorded on a Varian Cary 100 Conc spectrophotometer. ^1H NMR spectra were measured on a Varian VR 300-MHz spectrometer, using TMS as a reference in DMSO-d_6. Mass spectra were performed on a VG ZAB-HS (FAB) instrument. The fluorescence spectra were recorded on a Hitachi RF-4500 spectrofluorophotometer.

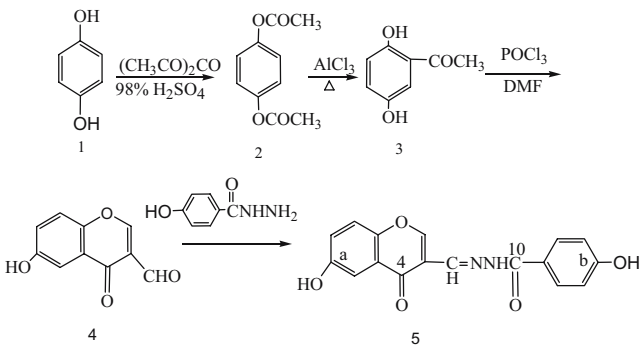

Fig. 1 Scheme of the synthesis of the ligand

The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6,600 M cm) at 260 nm. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [11, 12]. Absorption titration experiments were performed by maintaining the metal complex concentration constant (20 mM) and varying the nucleic acid concentration.

To compare quantitatively the affinity of the compound bound to DNA, the intrinsic binding constants K_b of the two compounds to DNA were obtained by the luminescence titration method. Fixed amounts of compound were titrated with increasing amounts of DNA, over a range of DNA concentrations from 2.5 to 20.0 μM. The concentration of the bound compound was calculated using Eq. 1 [13]:

$$c_b = c_t\left[\left(F - F^0\right)\left(F^{\max} - F^0\right)\right] \quad (1)$$

Where C_t is the total compound concentration, F is the observed fluorescence emission intensity at given DNA concentration, F^0 is the intensity in the absence of DNA, and F^{max} is the fluorescence of the totally bound compound. Binding data were cast into the form of a Scathchard plot [14] of r/C_f versus r, where r is the binding ratio $C_b/[DNA]_t$ and C_f is the free ligand concentration.. All experiments were conducted at 20 °C in a buffer containing 5 mM Tris–HCl (pH 7.1) and 50 mM NaCl concentrations.

Further support for complex binding to DNA by intercalation mode is given through the emission quenching experiment. EB is a common fluorescent probe for DNA structure and has been employed in examinations of the mode and process of metal complex binding to DNA [15]. A 2-ml solution of 10 μM DNA and 0.33 μM EB (at saturating binding levels [16]) was titrated by 5–30 μM complex (λ_{ex}=500 nm, λ_{em}=520.0–650.0 nm).

According to the classical Stern–Volmer equation [17]:

$$F_0/F = K_q[Q] + 1$$

Where F_0 is the emission intensity in the absence of quencher, F is the emission intensity in the presence of quencher, K_q is the quenching constant, and $[Q]$ is the quencher concentration. The shape of Stern–Volmer plots can be used to characterize the quenching as being predominantly dynamic or static. Plots of F_0/F versus $[Q]$ appear to be linear and K_q depends on temperature.

Viscosity experiments were conducted on an Ubbdlodhe viscometer, immersed in a thermostated water-bath maintained to 25.0 °C. Titrations were performed for the Eu (III) complex (1–5 μM), and compound was introduced into DNA solution (5 μM) present in the viscometer. Data were

Table 1 Fluorescence data of the Eu (III) complex at room temperature

	State	Slit (nm)	λ_{ex} (nm)	λ_{em} (nm)	aRFI	Assignment
Eu(III) complex	Solid	1	344	571	11.6	$^5D_0 \rightarrow {}^7F_0$
				582	37.6	$^5D_0 \rightarrow {}^7F_1$
				616	81.9	$^5D_0 \rightarrow {}^7F_2$
	acetone	5	379	592	261.4	$^5D_0 \rightarrow {}^7F_1$
				615	706	$^5D_0 \rightarrow {}^7F_2$
	acetonitrilet			592	125	$^5D_0 \rightarrow {}^7F_1$
				615	254	$^5D_0 \rightarrow {}^7F_2$
	$CHCl_3$			592	198	$^5D_0 \rightarrow {}^7F_1$
				616	484	$^5D_0 \rightarrow {}^7F_2$
	DMF			614	82	$D_0 \rightarrow {}^7F_2$
	DMSO			614	72	$D_0 \rightarrow {}^7F_2$

[a] RFI is relative fluorescence intensity

presented as $\left(\eta'/\eta_0'\right)^{1/3}$ versus the ratio of the concentration of the compound and DNA, where η' is the viscosity of DNA in the presence of compound and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution corrected from the flow time of buffer alone (t_0), $\eta' = t - t_0$ [18, 19].

Preparation of ligand (L)

CDC was prepared according to the literature methods [20]. An ethanol solution containing 4-hydroxy benzoyl hydrazine (1.52 g, 10 mmol) was added dropwise to another ethanol solution containing CDC (1.90 g, 10 mmol). The mixture was stirred for 2 h at room temperature and a white precipitate formed. The precipitate was collected by filtration and washed with ethanol. Recrystallistation from 1:1 (*V*/*V*) DMF/H_2O gave the ligand (L), which was dried in a vacuum. Yield: 90%. Mp 231–232 °C. FAB-MS: m/z= 325$[M+H]^+$. Anal. Calcd for $C_{17}H_{12}N_2O_5$: C, 62.96; H, 3.7; N, 21.47 Found: C, 62.12; H, 3.78; N, 21.02. ^{1}H NMR (DMSO-d_6 300 MHz): δ 11.92 (1H, br, NH), 11.85 (1H, s, a-OH), 10.14 (1H, s, b-OH), 8.77 (1H, s, 2-H), 8.60 (1H, s, CH=N), 7.93–6.91 (7H, m, PhH, 5, 7, 8-H). IR ν_{max} (cm^{-1}): $\nu_{(carbonyl)\ C=O}$: 1624, $\nu_{(hydrazonic)\ C=O}$: 1,646, $\nu_{C=N}$: 1,596 cm^{-1}.

Preparation of complexes

The ligand (1.0 mmol, 0.324 g) and the Eu (III) nitrate (0.5 mmol, 0.217 g) were added together in ethanol (10 ml). The mixtures were stirred at 60 °C. After 5 min, the mixtures solution was filtrated to move residue and continued stirring for 24 h at room temperature. A white precipitated, the Eu (III) complex, was separated from the solution by suction filtration, purified by washing several times with ethanol, and dried for 24 h in a vacuum. The La (III), Nd(III) and Tb(III) was prepared by the same method. Anal. Calcd for Eu (III) complex $C_{34}H_{24}N_7O_{19}Eu$ (%): C, 41.67 (41.38); H, 2.35 (2.43); N, 9.79 (9.94); Eu, 16.14 (15.41). Λ_m ($s.cm^2.mol^{-1}$): 104.8. IR ν_{max} (cm^{-1}): $\nu_{(carbonyl)\ C=O}$: 1,609, $\nu_{(hydrazonic)\ C=O}$: 1,635, $\nu_{C=N}$: 1,572, ν_{NO3}: 1,479, 1,383, 1,324, 1,186, 838. Thermal analyses: $T_{Decomp.}$ (°C): 237, 316, 390; Residue Calcd(%): 17.15

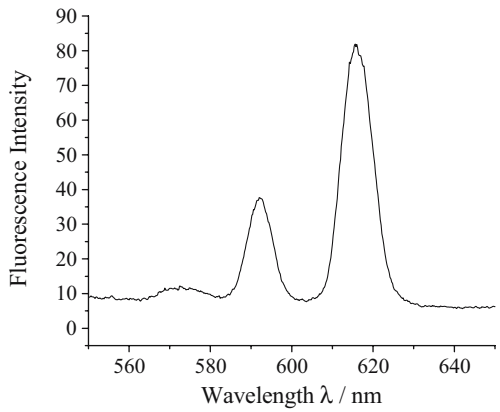

Fig. 2 Emission spectrum of the Eu (III) complex in solid state at room temperature

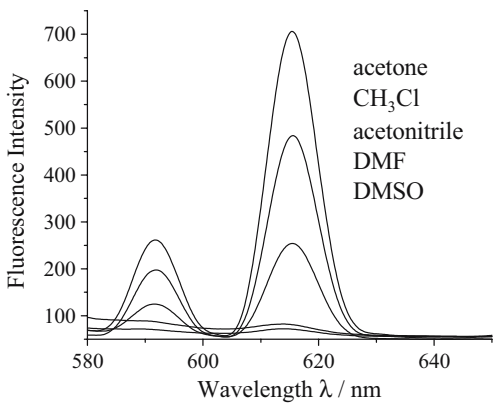

Fig. 3 Emission spectra of the Eu (III) complex in different solutions at room temperature

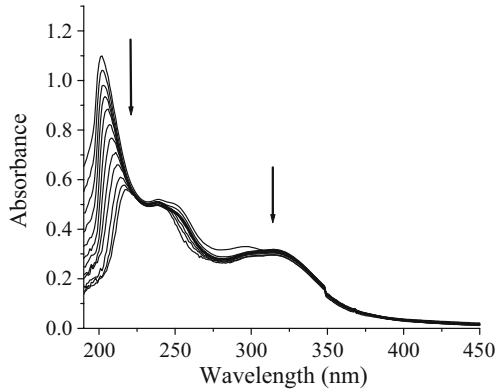

Fig. 4 Electronic spectra of Eu (III) complex (10 μM) in the presence of increasing amounts of CT-DNA. [DNA]=0–55 μM. Arrow shows the absorbance changes upon increasing DNA concentration

(17.84). ES-MS [CH3OH, m/z]: 923.9$\{EuL\cdot(NO_3)_2\}\cdot NO_3-NO_3^-\}^+$, 860.9$\{EuL\cdot(NO_3)_2\}\cdot NO_3-2NO_3^--H\}^+$, 797.7 $EuL\cdot(NO_3)_2\}\cdot NO_3-3NO_3^--2H\}^+$ and 399.6$\{EuL\cdot(NO_3)_2\}\cdot NO_3-3NO_3^--H\}^{2+}$. Anal. Calcd for La (III) complex $C_{34}H_{24}N_7O_{19}$ La (%): C, 41.45 (41.94); H, 2.23 (2.46); N, 10.02 (10.07); La, 14.35 (14.27). Λ_m (s.cm^2.mol^{-1}): 100. IR ν_{max} (cm^{-1}): $\nu_{(carbonyl)\ C=O}$: 1,606, $\nu_{(hydrazonic)\ C=O}$: 1,624, $\nu_{C=N}$: 1,572, ν_{NO_3}: 1,478, 1,383, 1,324, 1,174, 841. Thermal analyses: $T_{Decomp.}$ (°C): 247, 379, 444; Residue Calcd(%): 15.89 (16.74). Anal. Calcd for Nd (III) complex $C_{34}H_{24}N_7O_{19}Nd$ (%): C, 41.25 (41.70); H, 2.30 (2.45); N, 9.59 (10.01); Nd, 15.14 (14.74). Λ_m (s.cm^2.mol^{-1}): 105. IR ν_{max} (cm^{-1}): $\nu_{(carbonyl)\ C=O}$: 1,609, $\nu_{(hydrazonic)\ C=O}$: 1,625, $\nu_{C=N}$: 1,572, ν_{NO3}: 1,479, 1,383, 1,324, 1,186, 838. Thermal analyses: $T_{Decomp.}$ (°C): 239, 306, 423; Residue Calcd(%): 16.90 (17.19). Anal. Calcd for Tb (III) complex $C_{34}H_{24}N_7O_{19}Tb$ (%): C, 41.35 (41.09); H, 2.39 (2.41); N, 9.90 (9.86); Eu, 15.84 (16.00). Λ_m (s.cm^2.mol^{-1}): 106. IR ν_{max} (cm^{-1}): $\nu_{(carbonyl)\ C=O}$: 1,609, $\nu_{(hydrazonic)\ C=O}$: 1,623, $\nu_{C=N}$: 1,572, ν_{NO_3}: 1,479, 1,383, 1,324, 1,186, 838. Thermal analyses: $T_{Decomp.}$ (°C): 235, 336, 420; Residue Calcd(%): 17.95 (18.42).

Results and discussion

The complexes were prepared by direct reaction of ligand with the appropriate mole ratios of Ln (III) nitrate in ethanol. The yields were good to moderate. The desired Ln (III) complexes were separated from the solution by suction filtration, purified by washing several times with ethanol. The complexes are air stable for extended periods and soluble in methanol, DMSO, and DMF; slightly soluble in ethanol; insoluble in benzene, water and diethyl ether. The Eu (III) complex has been unambiguously characterized through mass spectral analysis. The mass spectrum of Eu (III) complex shows peaks at m/z of 923.9, 860.9, 797.9 and 399.6 which can be assigned to the ion pair $\{EuL\cdot(NO_3)_2\}\cdot NO_3-NO_3^-\}^+$, $\{EuL\cdot(NO_3)_2\}\cdot NO_3-2NO_3^--H\}^+$, $\{EuL\cdot(NO_3)_2\}\cdot NO_3-3NO_3^--2H\}^+$ and $\{EuL\cdot(NO_3)_2\}\cdot NO_3-3NO_3^--H\}^{2+}$, respectively. The molar conductivities of the complexes are around 100–107 S cm^2 mol^{-1} in DMF, showing that complexes are 1:1 electrolytes [21]. The elemental analyses and molar conductivities show that formula of the complexes conform to $[Ln\ L_2\cdot(NO_3)_2]\cdot NO_3$.

IR spectra

The $\nu_{(hydrazonic)}$ (C=O) and $\nu_{(carbonyl)}$ (C=O) vibrations of the free ligand are at 1,649 and 1,632 cm^{-1}, respectively; for the complexes these peaks shift to 1,624 and 1,606 cm^{-1}, $\nu_{(ligand\ -\ complex)}$ is equal to 23–27 and 21–25 cm^{-1}. The band at 590 cm^{-1} is assigned to ν (M–O). These shifts and the new band demonstrate that the oxygen of carbonyl has formed a coordinative bond with the rare earth ions. The band at 1,598 cm^{-1} for the free ligand is assigned to the ν (C=N) stretch, which shifts to 1,574 cm^{-1} for its complexes. Weak bands at 437 cm^{-1} are assigned to ν (M–N). These shifts and the new band further confirm that the nitrogen of the imino-group bonds to the rare earth ions. The absorption bands of the coordinated nitrates were observed at about 1,480 (ν_{as}) and 839 (ν_s) cm^{-1}. The ν_3 (E′)

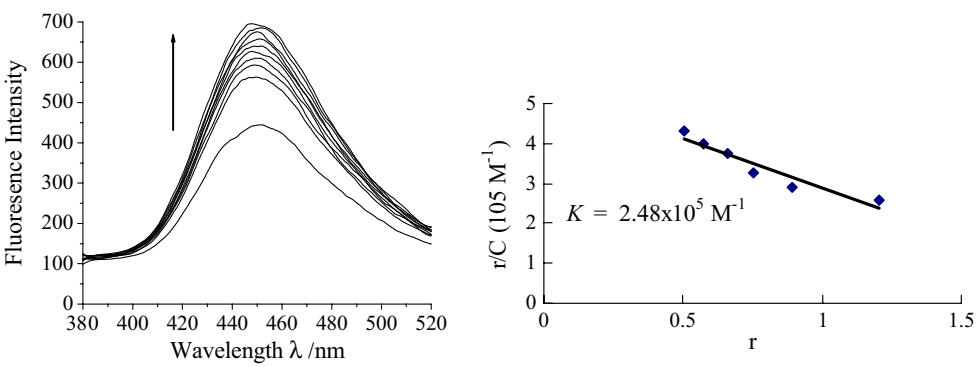

Fig. 5 The emission enhancement spectra of Eu (III) complex (10 μM) in the presence of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 μM CT-DNA. *Arrow* shows the emission intensities upon increasing DNA concentration. *Inset* Scatchard plot of the fluorescence titration data of Eu (III) complex, K=2.48×10^5 M^{-1}

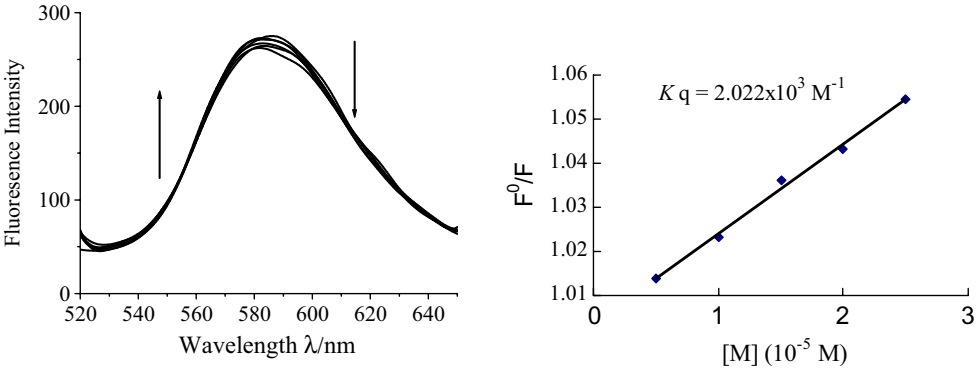

Fig. 6 The emission spectra of DNA-EB system (10 μM and 0.32 μM EB), λ_{ex}=500 nm, λ_{em}=520.0–650.0 nm, in the presence of 0, 5, 10, 15, 20, 25 μM Eu (III) complex. Arrow shows the emission intensities changes upon increasing Eu (III) complex concentration. Inset: Stern–Volmer plot of the fluorescence titration data of Eu (III) complex, K_q=2.02×10^3 M^{-1}

free nitrates appear at 1,384 cm^{-1} in the spectra of the complex. In addition, the separation of the two highest frequency bands $|\nu_4 - \nu_1|$ is approximately 156 cm^{-1}, and accordingly the coordinated NO_3^- ion in the complexes are a bidentate ligand [19].

Thermal analyses

The complexes begin to decompose at 237 °C or so and there are three exothermic peaks appear around 237–390 °C. The corresponding TG curves show a series of weight loss. Under 200 °C, there are no endothermic peak and no weight loss on corresponding TG curves. It indicates that there are no crystal or coordinate solvent molecules. While being hated to 800 °C, the complexes become their corresponding oxides. The residues are in accordance with calculation.

Fluorescence studies

The fluorescence characteristics of the europium complex in solid state and in $CHCl_3$, acetone, acetonitrile, DMF and DMSO solutions (concentration: 1.0×10^{-4} mol l^{-1}) are listed in Table 1. It can be seen that the Eu (III) complex shows strong emission when excited with 425 nm radiation in the solid state (Fig. 2). Fluorescence arises from ligand-to-cation energy transfer, mainly from the lowest resonance level. The most intensity ratio value η ($^5D_0 \rightarrow {}^7F_2/{}^5D_0 \rightarrow {}^7F_1$) is 2.17, indicating a low symmetry for the electrostatic field surrounding Eu (III). It could be seen from Fig. 3 that in acetone solution the Eu (III) complex has the strongest luminescence, and then in $CHCl_3$, acetonitrilet, DMF and DMSO. This is due to the coordinating effects of solvents, namely solvate effect. Together with the raising coordination abilities of acetone, acetonitrile, DMF and DMSO for the lanthanide ions, the oscillatory motions of the entering molecules consume more energy which the ligand triplet level transfer to the emitting level of the lanthanide ion. Thus, the energy transfer could not be carried out perfectly.

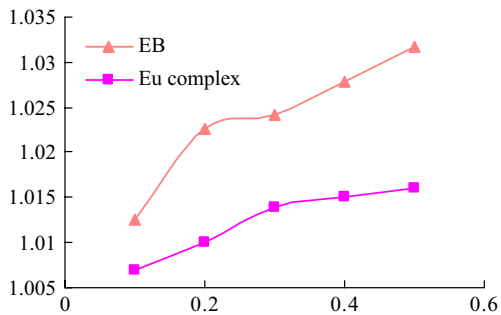

Fig. 7 Effect of increasing amounts of Eu (III) complex and EB on the relative viscosity of calf thymus DNA at 25.0 °C

Electronic absorption titration

The electronic absorption spectra of complex in the presence of increasing amounts of DNA in 5 mM Tris, 50 mM NaCl, pH 7.2 buffer are shown in Fig. 4. In the UV region, the intense absorption bands with maxima of 202 and 296 nm were attributed to intraligand π–π* transition of the coordinated groups in comparison with the spectrum of Eu (III) complex. In the presence of CT-DNA, the absorption bands of Eu (III) complex at 202 and 296 nm exhibited hypochromism of about 50.32 and 10.60% and bathochromism of about 16 and 7 nm, respectively. The spectroscopic variations are strongly indicative of the intercalation of the Eu (III) complex with DNA, involving a strong π-stacking interaction between the complex and the DNA base pairs. It can both insert into DNA base pairs.

Fluorescence spectra studies

In Tris buffer, Eu (III) complex has fluorescence around 450 nm when excited at 328 nm. If DNA solution was added to the Eu (III) complex solution, enhanced fluorescence is observed when excited under the given conditions. Figure 5 shows the emission spectra of Eu (III) complex in the presence and absence of DNA. The stronger enhancement for Eu (III) complex may be largely due to the increase of the molecular's planarity of the complex and the decrease of the collisional frequency solvent molecules

with the complexes usually lead to emission enhancement. According to the Scathchard equation, a plot of r/C_f versus r gave the binding constants 2.48×10^5 M^{-1} from the fluorescence data for Eu (III) complex. This value is lower than the one reported for Eu (III) complex (K_b=3.55×10^6 M^{-1}) [10]. These different values indicate that the position of substituting group can affect the binding affinity that complex binds to DNA.

The DNA-binding modes of compound were further monitored by a fluorescent EB displacement assay. It is well known that EB can intercalate nonspecifically into DNA which causes it to fluoresce strongly. Competitive binding of other drugs to DNA and EB will result in displacement of bound EB and a decrease in the fluorescence intensity. This fluorescence-based competition technique can provide indirect evidence for the DNA-binding mode. Figure 6 shows the emission spectra of DNA-EB system with increasing amounts of the Eu (III) complex. The emission intensity of the DNA-EB system (λ_{em}= 582 nm) decreased apparently as the concentration of the Eu (III) complex increased, and an isobathic point appeared at 538 nm. The quenching plots illustrate that the quenching of EB bound to DNA by the complexes is in good agreement with the linear Stern–Volmer equation. The emission band at 584 nm of the DNA-EB system decreased in intensity with an increase in the concentration of the compound, which indicated that the compound could displace EB from the DNA-EB system. The resulting decrease in fluorescence was caused by EB changing from a hydrophobic environment to an aqueous environment. Such a characteristic change is often observed in intercalative DNA interactions [13]. The quenching plots illustrate that the quenching of EB bound to DNA by the complexes is in good agreement with the linear Stern–Volmer equation. In the plots of F_0/F versus $[Q]$, K_q is given by the ratio of the slope to the intercept. The K_q value for the Eu (III) complex is 2.02×10^3 M^{-1}.The binding of synthesized Eu (III) complex with DNA-EB results in displacement of EB from DNA double helix to the solution which is accompanied by a quenching of EB fluorescence. Based on this phenomenon we can determine the binding constants of the complexes with DNA (Fluorescent intercalator displacement assay). The binding constants were calculated using following Eq. 2 [21]:

$$\begin{aligned} C_{\mathrm{Eth}} &= 2(F^0 - F)\cdot Et/F^0 \\ r_{\mathrm{Eth}} &= (Et - C_{\mathrm{Eth}})/C_{DNA} \\ r_{\mathrm{M}} &= n - r_{\mathrm{Eth}} - r_{\mathrm{Eth}}/K_{\mathrm{Eth}}\cdot C_{\mathrm{Eth}} \\ C_M &= Mt - r_{\mathrm{M}}\cdot C_{\mathrm{DNA}} \\ K_M &= [(n - r_{\mathrm{Eth}})\cdot K_{\mathrm{Eth}}\cdot C_{\mathrm{Eth}}/r_{\mathrm{Eth}} - 1]/C_{\mathrm{M}} \end{aligned} \quad (2)$$

Where F^0 is observed fluorescence emission intensity of DNA-EB system; F is the intensity in the absence of compound; E_t is the total EB concentration; C_{Eth} is the free EB concentration; C_M is the bound compound concentration, and Mt is the total compound concentration. From above equation, the binding constants of complex is 5.20×10^5 M^{-1}, respectively. The value is almost equal to the binding constants determined by the fluorescence titration experiments, so it may be concluded that the complex binds DNA viaintercalation mode.

Viscosity measurements

To further confirm the interaction mode of the Eu (III) complex with DNA, a comparative viscosity study between the EB and the complex were carried out (Fig. 7). The effects of the compounds together with EB on the viscosity of DNA are shown in Fig. 7. It is found that the viscosity of DNA increases steadily with the increase of the concentration of the compounds, which is similar to that of a classical intercalator EB. This result demonstrates that the complex and EB bind to DNA through the same way, i.e., the classical intercalation mode, which also parallels the pronounced hypochromism and spectral red shift of the complex in the absorption spectrum experiment.

Conclusion

A new ligand (CDC), 6-hydroxy chromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone, and its Ln (III) complexes have been prepared and characterized. The DNA-binding properties of the Eu (III) complex was investigated by absorption, fluorescence and viscosity measurements. The results support the fact that the compound bind to CT-DNA via intercalation. These findings clearly indicate that lanthanide-based complexes have many potential practical applications, like the development of nucleic acid molecular probes. This work has also unequivocally demonstrated that that the position of substituting group can affect the binding affinity that complex binds to DNA.

Acknowledgements This work is supported by the National Natural Science Foundation of China (20475023).

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