



## Synthesis and DNA-Binding Studies of Two Europium(III) Complexes

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Two new europium(III) complexes  $\text{EuL}_3\text{NO}_3$  and  $\text{EuL}_2\text{Cl}_2$  ( $\text{L}$  = coumarin-3-carboxylic anion), have been synthesized and characterized by IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, MS and elemental analysis. The DNA binding property of the two complexes has been investigated employing fluorescence spectroscopy. The DNA binding constants for the two complexes have been measured to be  $1.64 \times 10^4 \text{ L mol}^{-1}$  and  $1.87 \times 10^3 \text{ L mol}^{-1}$  through fluorescence quenching method, indicating that the complexes  $\text{EuL}_3\text{NO}_3$  prefer to intercalative binding to DNA. Both of the binding numbers of  $\text{EuL}_3\text{NO}_3$  and  $\text{EuL}_2\text{Cl}_2$  with DNA have been found to be 1, suggesting that each unit of complex bind with a unit of ct-DNA.

**Key Words:** Synthesis, Characterization, DNA-binding studies, Europium(III) complexes, Coumarin-3-carboxylic acid.

### INTRODUCTION

The interaction between DNA and small molecules plays an important role in life science and is associated with the transcription and replication of DNA, gene mutation and disease origins. Therefore, the study on the interaction is vital for both scientific<sup>1,2</sup> and therapeutic reasons<sup>3,4</sup>. In this respect europium(III) complexes have attracted a great deal of attention due to their strong metal to ligand charge transfer (MLCT) absorption, their unique emission characteristics<sup>5,6</sup>, the perturbation of which could be exploited to study their DNA binding properties.

In addition, the ligands can also create some interesting differences in the space configuration and the electron-density distribution of metal complexes<sup>7</sup>, which will result in differences in spectral properties and the DNA-binding behaviours of these complexes<sup>6,8</sup>. So, well designed organic ligands enable a fine tuning of special properties of the metal ions. The chemistry of coumarin-3-carboxylic acid and its derivatives has attracted special interest due to their photophysical properties<sup>9,10</sup> and wide bioactivities<sup>11-13</sup>. They can also act as a tetradentate ligand binding to metal ion<sup>14</sup>, while such structures may have strong affinities of binding to DNA through intercalations<sup>6</sup>.

Herein, based on the above conception, we described the synthesis and DNA binding property of two new europium(III) mixed ligand complexes, using coumarin-3-carboxylic acid as the main ligand.

### EXPERIMENTAL

All reagents for synthesis were commercially available and employed as received or purified by standard methods prior to use. Infrared spectra were recorded with a Perkin Elmer spectrophotometer on KBr disks. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR studies were carried out by the BRUKER AVANCE 500 instrument and the mass spectral studies were done using BRUKER ESQUIRE HCT instrument. Elemental analyses were determined in the Carlo Erba model 1106 instrument. Melting points were recorded using an electrothermal-WRS-1A melting point apparatus and were uncorrected. The fluorescence spectra were recorded in the RF-5301 fluorospectrophotometer and the fluorescence life were recorded in the FLS-920 luminoscope instrument.

#### General procedure

**Synthesis of coumarin-3-carboxylic acid:** Under the room temperature, the mixture of salicylaldehyde (100 mmol), ethanol (50 mL), diethyl malonate (140 mmol) and hexahydropyridine (0.8 mL) was blended together in a round flask and reacted under ultrasonic irradiation (Power 250 w, frequency 40 kHz) for 15 min. This reaction system was then filtered to give a white-solid product. Then the white-solid product was treated with the mixture of ethanol (30 mL) and aqueous solution of sodium hydroxide (50 mL, at the concentration of 4 mol/L) and refluxed for 1 h. The reaction mixture was then poured into 200 mL ice-water and the pH was adjusted

to 4-5 by periodically adding the aqueous solution of HCl (4.0 mol/L). The mixture was filtered to give white crystals of coumarin-3-carboxylic acid.

**Synthesis of complex  $\text{EuL}_3\text{NO}_3$  and  $\text{EuL}_2\text{Cl}_2$ :** To the ethanol solution (30 mL) of coumarin-3-carboxylic acid (1 mmol), 5 mL aqueous solution of europium(III) nitrate (0.95 mmol) was added. The mixture was refluxed for 0.5 h and filtered to give white powder of  $\text{EuL}_3\text{NO}_3$ . The synthetic procedure of  $\text{EuL}_2\text{Cl}_2$  was consistent with that of  $\text{EuL}_3\text{NO}_3$ , while the europium(III) nitrate was replaced by europium(III) chloride.

**Coumarin-3-carboxylic acid:** Yield 97 %; m.p. 189-191 °C; anal. calcd. (%) for  $\text{C}_{10}\text{H}_6\text{O}_4$ : C, 63.16; H, 3.18. Found (%): C, 63.33; H, 3.41. MS (ESI)  $m/z$ : 191  $[\text{M} + 1]^+$ ; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3462 (COOH), 3056 (Ar, C=CH), 1743, 1683 (C=O), 1613, 1569 (Ar, C=CH);  $^1\text{H}$  NMR (500 MHz, DMSO,  $\delta$ , ppm): 13.25 (s, 1H, COOH), 8.70 (s, 1H, C=CH), 7.87 (d,  $J = 7.7$  Hz, 1H, Ar-H), 7.69 (t,  $J = 7.7$  and 7.9 Hz, 1H, Ar-H), 7.35-7.39 (dd,  $J = 8.3$  and 7.5 Hz, 2H, Ar-H);  $^{13}\text{C}$  NMR (125 MHz, DMSO,  $\delta$ , ppm): 116.5, 118.4, 118.7, 125.2, 130.6, 134.7, 148.8, 154.9, 157.2, 164.4.

**Complex  $\text{EuL}_3\text{NO}_3$ :** Yield 90.2 %; m.p. >300 °C; anal. calcd. (%) for  $\text{C}_{30}\text{H}_{15}\text{NO}_{15}\text{Eu}$ : C, 46.11; H, 1.93; N, 1.79. Found (%): C, 45.98; H, 1.75; N, 2.01. MS (ESI)  $m/z$ : 782  $[\text{M} + 1]^+$ ; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3432 (COOH), 3066 (Ar, C=CH), 1671, 1657 (C=O), 1613, 1599, 1576 (Ar, C=CH), 1562, 1452 ( $\text{NO}_3^-$ );  $^1\text{H}$  NMR (500 MHz, DMSO,  $\delta$ , ppm): 7.90 (d,  $J = 8.3$  Hz, 1H, Ar-H), 7.71 (t,  $J = 7.5$  and 7.7 Hz, 1H, Ar-H), 7.23-7.26 (dd,  $J = 7.4$  and 4.5 Hz, 2H, Ar-H), 5.84 (s, 1H, C=CH);  $^{13}\text{C}$  NMR (125 MHz, DMSO,  $\delta$ , ppm): 116.3, 118.3, 118.7, 125.1, 129.8, 133.9, 146.2, 149.0, 152.5, 155.1.

**Complex  $\text{EuL}_2\text{Cl}_2$ :** Yield 86.7 %; m.p. > 300 °C; anal. calcd. (%) for  $\text{C}_{20}\text{H}_{10}\text{O}_8\text{Cl}_2\text{Eu}$ : C, 39.96; H, 1.68. Found (%): C, 39.77; H, 1.83. MS (ESI)  $m/z$ : 602  $[\text{M} + 1]^+$ ; IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3429 (COOH), 3068 (Ar, C=CH), 1670, 1656 (C=O), 1614, 1599, 1562 (Ar, C=CH);  $^1\text{H}$  NMR (500 MHz, DMSO,  $\delta$ , ppm): 7.86 (d,  $J = 7.9$  Hz, 1H, Ar-H), 7.70 (t,  $J = 7.1$  and 8.4 Hz, 1H, Ar-H), 7.25-7.29 (dd,  $J = 7.4$  and 7.3 Hz, 2H, Ar-H), 6.08 (s, 1H, C=CH);  $^{13}\text{C}$  NMR (125 MHz, DMSO,  $\delta$ , ppm): 116.3, 118.4, 118.7, 124.9, 129.7, 133.5, 145.5, 152.8, 155.3, 157.7.

**Detection method:** The DNA-binding property the two complexes were investigated by fluorescence quenching spectroscopy. At room temperature, the two complexes and ct-DNA were dissolved in DMF-water (7:3, v:v) to the proper concentration. After addition of ct-DNA (0.1 mL) solution to complex solution (3.9 mL), respectively, the mixed was allowed to stand for 0.5 h (until the stable) with intermittent shaking and the fluorescence spectroscopy was scanned in the RF-5301 fluorospectrophotometer.

## RESULTS AND DISCUSSION

The structures of compounds synthesized were identified by FT-IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, MS and micro analysis. The characterized data of the synthesized compounds were given in experimental section.

In the IR spectra of the complexes, noticeable changes were observed compare with the ligand coumarin-3-carboxylic acid, especially in the frequencies of the  $\nu(\text{C}=\text{O})$  and

$\nu(\text{COOH})$  vibrations. Upon complexation,  $\nu(\text{C}=\text{O})$  frequencies shifted to the right as 27-73  $\text{cm}^{-1}$  and the peaks weakened relatively, while  $\nu(\text{COOH})$  frequencies shifted to the left as 30-34  $\text{cm}^{-1}$ . In  $^1\text{H}$  NMR spectra of the complexes, the peaks of COOH group disappear and the peaks of C=CH group shifted to high field. In  $^{13}\text{C}$  NMR spectra of the complexes  $\text{EuL}_3\text{NO}_3$  and  $\text{EuL}_2\text{Cl}_2$ , carbon peaks besides the oxygen atom changed obviously. The results indicated that the ligand coumarin-3-carboxylic acid was bonded with europium(III) through the carboxylic acid and carbonyl groups. Besides, the MS and micro analysis data were consistent with their structure.

The fluorescence-quenching spectroscopy was employed to study DNA-binding property of two europium(III) complexes. Upon addition of ct-DNA into the DMSO- $\text{H}_2\text{O}$  (v:v = 7:3) solution of the two complexes, the fluorescence intensity decreased gradually (Fig. 1).

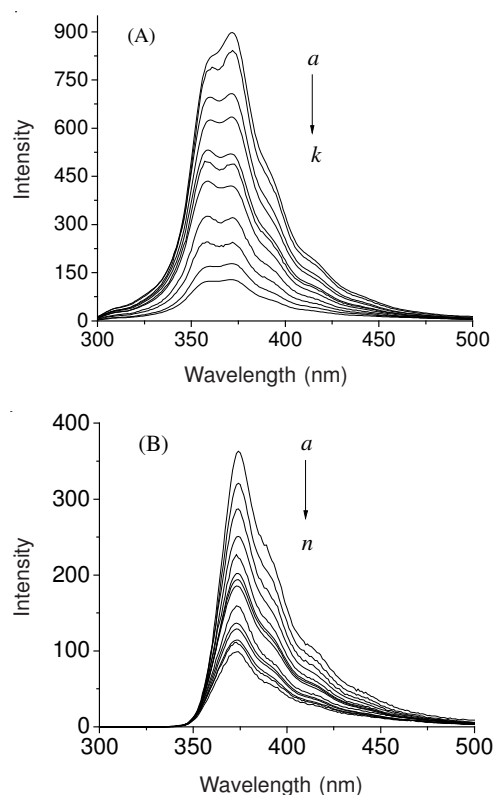


Fig. 1. Fluorescence spectra of complexes  $\text{EuL}_3\text{NO}_3$  (A) and  $\text{EuL}_2\text{Cl}_2$  (B) quenched by DNA (The concentration of europium(III) complexes  $\text{EuL}_3\text{NO}_3$  and  $\text{EuL}_2\text{Cl}_2$  were both  $1.0 \times 10^{-4} \text{ mol L}^{-1}$ ; the concentrations of DNA a  $\rightarrow$  k (a  $\rightarrow$  n) were: 0, 0.68, 0.96, 1.37, 1.77, 2.05, 2.73, 3.41, 4.08, 5.18, 6.98  $\times 10^{-4}$  (0, 0.68, 1.37, 2.05, 2.75, 3.41, 4.10, 4.78, 5.46, 6.14, 6.83, 7.51, 8.19, 8.87  $\times 10^{-4}$ )  $\text{mol L}^{-1}$ , respectively.)

Fluorescence quenching can be static, deriving from the formation of a ground state complex between the fluorophore and quencher or dynamic, resulting from collisional encounters between the fluorophore and quencher<sup>15</sup>. Supposing it as dynamic quenching, Stern-Volmer eqn. 1<sup>15</sup> was employed to analyse the fluorescence quenching mechanism (Fig. 2(A)).

$$\frac{F_0}{F} = 1 + K_q \tau_0 c(Q) = 1 + K_{sv} c(Q) \quad (1)$$

where  $F_0$  and  $F$  were the steady-state fluorescence intensities in the absence and presence of quencher (ct-DNA), respectively,

TABLE-1  
DATA OF FLUORESCENCE SPECTRUM, THE BINDING CONSTANTS  $K_A$  AND BINDING NUMBERS OF THE COMPLEXES

Compound	$\lambda_{ex}$ (nm)	$\lambda_{em}$ (nm)	$c(\text{DNA})_{\max}$ (mol L <sup>-1</sup> )	$K_{sv}$ (L mol <sup>-1</sup> )	$K_a$ (L mol <sup>-1</sup> s <sup>-1</sup> )	$K_a$ (L mol <sup>-1</sup> )	n
EuL <sub>3</sub> NO <sub>3</sub>	260	372	$6.98 \times 10^{-4}$	$9.40 \times 10^4$	$2.23 \times 10^{11}$	$1.64 \times 10^4$	0.95
EuL <sub>2</sub> Cl <sub>2</sub>	258	347	$8.87 \times 10^{-4}$	$3.08 \times 10^4$	$9.72 \times 10^{10}$	$1.87 \times 10^3$	1.09

$k_q$  the quenching rate constant of the biological molecule,  $c(Q)$  the concentration of quencher (DNA),  $\tau_0$  the average lifetime of the molecule without any quencher and the fluorescence lifetime of the complexes EuL<sub>3</sub>NO<sub>3</sub> and EuL<sub>2</sub>Cl<sub>2</sub> were measured to be 0.422 and 0.317 ms by the FLS-920 instrument, respectively.  $K_{SV}$  was the Stern-Volmer quenching constant. Accordingly, eqn. 1 was applied to determine  $K_{SV}$  by linear regression of a plot of  $F_0/F$  against  $c(Q)$  and the Stern-Volmer plots were shown in Fig. 2(A).

In Table-1,  $k_q$  was much greater than the value of the maximum scatter collision quenching constant  $2.0 \times 10^{10}$  L mol<sup>-1</sup> s<sup>-1</sup><sup>16</sup>, which indicated that the fluorescence quenching was caused by a specific interaction. Therefore, the mechanism of fluorescence quenching was a static quenching procedure and the quenching data must be analyzed according to the modified Stern-Volmer eqn. 2<sup>15,17</sup>.

$$\frac{F_0}{F_0 - F} = \frac{F_0}{\Delta F} = \frac{1}{f_a k_a c(Q)} + \frac{1}{f_a} \quad (2)$$

In this case,  $\Delta F$  was the difference of fluorescence intensity in absence and presence of the quencher at the concentration  $c(Q)$ ,  $f_a$  the fraction of accessible fluorescence and  $K_a$  was the binding constants for the quencher-acceptor system.

The modified Stern-Volmer plots were shown in Fig. 2(B) and the corresponding quenching constants  $K_a$  of the complexes EuL<sub>3</sub>NO<sub>3</sub> and EuL<sub>2</sub>Cl<sub>2</sub> were found to be  $1.64 \times 10^4$  L mol<sup>-1</sup> and  $1.87 \times 10^3$  L mol<sup>-1</sup> (Table-1), indicating that complex EuL<sub>3</sub>NO<sub>3</sub> prefer to intercalative binding to DNA. It suggested that the ligands and complexation patterns play an important role in the binding to DNA. The binding numbers of EuL<sub>3</sub>NO<sub>3</sub> and EuL<sub>2</sub>Cl<sub>2</sub> with DNA was found to be 0.95 and 1.05 (approximately equal to 1, Table-1), respectively, according to the eqn. 3<sup>15,18</sup>. It suggests that each unit complex bind with a unit of ct-DNA.

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_A + n \log c(Q) \quad (3)$$

Since the interaction between DNA and small molecules plays an important role in life science, the studies above are expected to be much useful in the design of a new kinds of europium(III) complexes analogs with novel biomedical functions and DNA-binding properties.

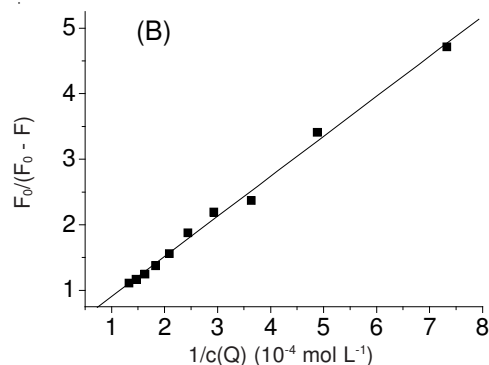
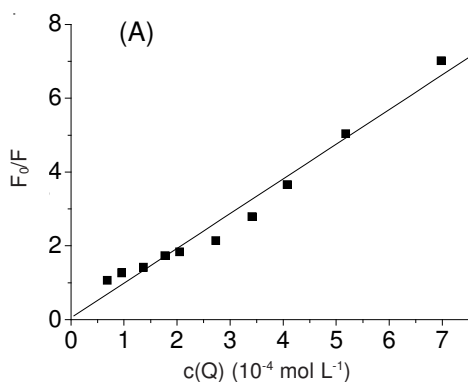


Fig. 2. Stern-Volmer (A) and Lineweaver-Burk (B) plots of the fluorescence quenching of complex EuL<sub>3</sub>NO<sub>3</sub>

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