



Synthesis, Luminescence and DNA-Binding Properties of Eu(III) and Tb(III) Complexes with Amide-Based 2,3-Dihydroxynaphthalene Derivative

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The new ligand 2,2'-[2,3-naphthylenebis(oxy)]-bis(N,N-dibenzyl(acetamide)) (L) and its Eu(III) and Tb(III) picrate complexes were synthesized. The complexes were characterized by elemental analysis, IR, TG-DSC and conductivity measurements. The luminescence properties of the two complexes were investigated. The result indicates that the triplet state energy level of the ligand matches better to the resonance level of Eu(III) than Tb(III) ion. Fluorescence intensity of the Eu(III) complex was reduced with the raising coordination ability of solvent. In addition, the DNA-binding properties of the two complexes have been investigated by electronic absorption, fluorescence and cyclic voltammetry. The results suggest that both complexes can bind to DNA *via* an intercalative binding mode and their binding affinity for DNA follows the order: Eu(III) complex > Tb(III) complex.

Key Words: Amide-based 2,3-dihydroxynaphthalene, Eu(III) and Tb(III) complexes, Luminescence, DNA binding.

INTRODUCTION

In the last decades, many rare earth metal complexes have attracted considerable attention due to their long fluorescence lifetimes and strong fluorescence emissions. These complexes have been used in many areas, such as fluorescence materials, supramolecular devices, fluorescence sensors and luminescence probes¹⁻⁶. Among these studies, the luminescence properties of rare earth complexes are of special interest because they could exhibit narrow emission profiles, large Stokes shifts and high luminescent quantum efficiencies, *etc.*⁷⁻⁹. The intra-configuration $4f-4f$ transitions in rare earth ions are parity forbidden, consequently the absorption and emission spectra of the rare earth (III) ions show weak intensity. However, the population of the excited states of the rare earth (III) ions may be increased by coordination to suitable organic ligands, which act as sensitizers¹⁰.

DNA binding metal complexes are being investigated in many laboratories because they can be used as DNA structural probes, DNA dependent electron transfer probes and DNA footprinting agents, sequence-specific cleaving agents; especially they can be used as potential anticancer drug^{11,12}. Generally, metal complexes bind to DNA in a non-covalent way, such as intercalation, groove-binding and external electrostatic binding¹³. Considerable useful applications of the metal complexes require that they can bind to DNA *via* an intercalation

binding mode inducing cellular degradation^{14,15}. In this work, we introduced naphthalene groups as the basic molecular frame and obtained a new open-chain crown ether ligand 2,2'-[2,3-naphthylenebis(oxy)]bis(N,N-dibenzyl(acetamide))(L) and its europium(III) and terbium(III) picrate complexes. The fluorescence properties of the two complexes with this new ligand were studied and effects of the solvents on fluorescence properties of the Eu(III) complex were investigated. Furthermore, the interaction of the complexes with DNA was studied by absorption spectrum, fluorescence spectrum and cyclic voltammetry.

EXPERIMENTAL

Calf thymus DNA (CT-DNA) was purchased from Aladdin. All commercially available chemicals were of A.R. grade and were used without further purification. The ratio of the absorbance A_{260}/A_{280} was checked to be 1.80-1.90, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorbance at 260 nm using the molar absorption coefficient ($\epsilon_{260} = 6600 \text{ L mol}^{-1} \text{ cm}^{-1}$)¹⁶.

The Eu(III) and Tb(III) ions were determined by EDTA titration using xylenol orange as an indicator. Elemental analyses for carbon, nitrogen and hydrogen were carried on a Vario EL elemental analyzer. Conductivity measurements were determined with a DDS-12A type conductivity bridge using $10^{-3} \text{ mol L}^{-1}$ solutions in DMF at 25 °C. IR spectra were recorded

on a Nicolet FIPR-8400S spectrometer in the 4000-400 cm^{-1} regions, using KBr pellets. ^1H NMR spectra were measured on a Bruker INOVA-400MHz spectrometer in $\text{DMSO-}d_6$ with TMS as internal standard. Ultraviolet visible spectra were obtained on a UV-2450 spectrophotometer. Fluorescence measurements were performed on a Hitachi RF-540 spectrophotometer. A CHI660A electrochemical analyzer in connection with a glassy carbon working electrode (GCE), a saturated calomel reference electrode (SCE) and a platinum wire counter electrode was used for the electrochemical measurements.

Synthesis of ligand and complexes: Synthesis of the ligand was prepared according to literature method¹⁷. Yield, 85 %. m.p. 132.5-132.8 $^{\circ}\text{C}$. IR (KBr, ν_{max} , cm^{-1}): 1652 (C=O), 1166 (C-O-C). ^1H NMR ($\text{DMSO-}d_6$) δ ppm: 4.483(S, 4H, -N- CH_2 -Ar), 4.584(S, 4H, -N- CH_2 -Ar), 4.991(S, 4H, O- CH_2 -C), 7.093-7.266 (m, 26H, ArH and $\text{C}_{10}\text{H}_6^-$).

For synthesis of the complexes, a solution of $\text{RE}(\text{pic})_3 \cdot 9\text{H}_2\text{O}$ (0.2 mmol) (RE = Eu or Tb) in 5 mL of ethyl acetate was added dropwise to 8 mL of chloroform of the ligand (0.2 mmol). The mixture was stirred for 5 h and yellow precipitate formed. The precipitate was collected and washed three times with ethyl acetate and chloroform, respectively. Further drying in vacuum resulted in a yellow powder.

DNA binding experiment: Absorption spectral titration experiment was performed by keeping the concentration of the complex constant while varying the concentration of DNA. Equal solution of DNA was added to the complex solution and reference solution to eliminate the absorbance of DNA itself. During the titrations, the solutions were mixed for 5 min and then the spectra were recorded.

Fluorescence quenching experiments of ethidium bromide-DNA were carried out by adding $10 \mu\text{mol L}^{-1}$ complexes solution to the samples containing $0.5 \mu\text{mol L}^{-1}$ ethidium bromide, $1.0 \mu\text{mol L}^{-1}$ DNA and *tris*-buffer (pH = 7.2). Before measurements, the system was gently shake and incubated at room temperature for 5 min. The samples were excited at 285 nm and the fluorescence spectra were obtained at an emission wavelength of 584 nm in the fluorometer.

Electrochemical experiments were performed by keeping the concentration of the complexes constant while varying the DNA concentration. Before electrochemical experiments, the GCE surface was freshly polished to a mirror prior to each experiment with $0.3 \mu\text{m}$ $\alpha\text{-Al}_2\text{O}_3$ slurry on the polishing micro-cloth and then rinsed thoroughly with distilled water to remove any remaining polishing alumina.

RESULTS AND DISCUSSION

Compositions and properties of the complexes: Analytical data for the complexes (Table-1) indicates that the complexes conform to 1:3:1 metal-to-picric-to-L stoichiometry. Both complexes are soluble in DMSO, DMF, ethanol acetonitrile, methanol and acetone, slightly soluble in ethyl acetate, THF and chloroform and insoluble in benzene and diethyl ether. The molar conductance values of the complexes in DMF (Table-1) indicate that the complexes are 2:1 ionic compound¹⁸.

IR spectra: IR spectra of the complexes are similar to each other, so it may be assumed that the complexes have the similar structures. The main infrared bands of the ligands and their complexes were presented in Table-2. The "free" ligand (L) exhibits two absorption bands at 1652 and 1126 cm^{-1} which are assigned to $\nu(\text{C}=\text{O})$ and $\nu(\text{C}-\text{O}-\text{C})$, respectively. In the complexes, the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}-\text{O}-\text{C})$ shift by 36-37 and $83\text{-}84 \text{ cm}^{-1}$, respectively, thus indicating that the C=O and ether O-atoms take part in coordination to the metal ion¹⁹.

The OH out-of-plane bending vibration of the free Hpic at 1265 cm^{-1} disappears in the spectra of the complexes indicating that the H atom of the OH group is replaced by rare earth (III) ions. The vibration $\nu(\text{C}-\text{O})$ of at 1265 cm^{-1} is shifted towards higher frequency by 10 cm^{-1} in the complex. This is due to the following two effects. Firstly, after forming the RE-O bond, the π -bond character in the C-O bond increases. Secondly, coordination of the oxygen atom of L to RE(III) causes the π -character to be weakened. The free Hpic has $\nu_{\text{as}}(\text{NO}_2)$ and $\nu_{\text{s}}(\text{NO}_2)$ at 1555 and 1342 cm^{-1} , respectively, which splits into two bands in the complexes. This indicates some of the O-atoms in the nitro group of Pic^- take part in coordination²⁰.

Thermal analysis: The complexes are stable in air. DSC and TG curves obtained for the complexes don't exhibit endothermic peak before $300 \text{ }^{\circ}\text{C}$, which indicates that there are not lattice water and coordination water in the two complexes. On the basis of above evidence and analysis, the constitutions for the Eu(III) complex and Tb(III) complex are $\text{Eu}(\text{pic})_3\text{L}$ and $\text{Tb}(\text{pic})_3\text{L}$, respectively.

Luminescence properties of the complexes: Under identical experimental conditions, the emission spectra of the Eu(III) and Tb(III) complexes (concentration: $0.1 \mu\text{mol L}^{-1}$) in acetonitrile solution are shown in Fig. 1. The maximum of excitation was observed at a wavelength of 378 nm for both complexes. As shown in Fig. 1, the emission spectra of the Eu(III) complex show characteristic emission bands of Eu(III)

TABLE-1
ELEMENTAL ANALYTICAL DATA AND MOLAR CONDUCTANCE VALUES FOR THE Eu(III) AND Tb(III) COMPLEXES

Complexes	Analysis (%) Found (calcd.)				Λ_{M} ($\text{S cm}^2 \text{ mol}^{-1}$)
	N	C	H	Metal	
Eu(III) complex	10.83 (10.48)	48.38 (48.98)	3.32 (3.20)	10.17 (10.34)	158.51
Tb(III) complex	10.69 (10.43)	48.68 (48.75)	3.29 (3.18)	10.51 (10.76)	160.27

TABLE-2
KEY IR BANDS (cm^{-1}) OF Eu(III) AND Tb(III) COMPLEXES

Compound	$\nu(\text{C}=\text{O})$	$\nu(\text{C}-\text{O}-\text{C})$	$\nu(\text{C}-\text{O})$	$\nu_{\text{as}}(-\text{NO}_2)$	$\nu_{\text{s}}(-\text{NO}_2)$		
Picric acid	–	–	1265	1555	1342	–	–
L	1652	1166	–	–	–	–	–
Eu(III) complex	1616	1082	1275	1575	1539	1362	1326
Tb(III) complex	1615	1083	1276	1575	1540	1363	1328

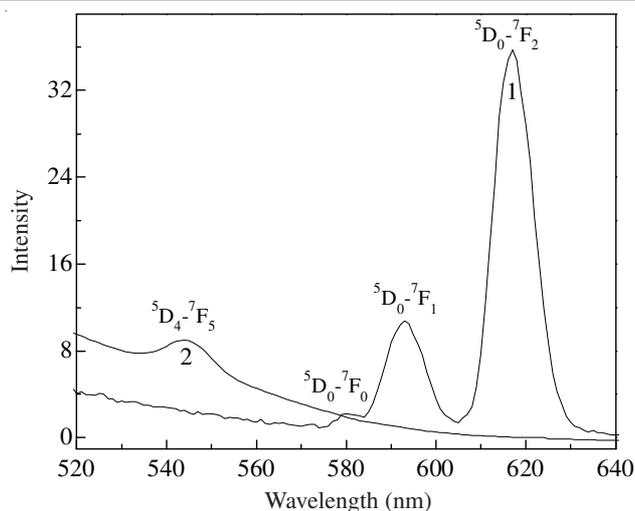


Fig. 1. Emission spectra of the Eu(III) complex (1) and Tb(III) complex (2) in acetonitrile solution

ion at 580, 593 and 617 nm, assigned to $^5D_0-^7F_0$, $^5D_0-^7F_1$ and $^5D_0-^7F_2$ transitions, respectively. Among these bands, the emission at 617 nm from $^5D_0-^7F_2$, electronic dipole transition is the strongest, which suggests low symmetry around the Eu(III) ion²¹. Therefore, the peak height at 617 nm is used to measure the fluorescence intensity of the Eu(III) complex. For the Tb(III) complex, the emission spectra show only characteristic emission band of Tb(III) ion at 544 nm, assigned to $^5D_4-^7F_5$ transition. Comparing the spectra of Eu(III) and Tb(III) complex, the luminescence intensity of europium complex at 617 nm is greatly stronger than that of terbium complex at 544 nm and the luminescence intensity of the Eu(III) complex is about 4 times that of the Tb(III) complex. Based on antenna effect²², the intensity of the luminescence of rare earth (III) complexes is related to the efficiency of the intramolecular energy transfer between the triplet energy level of the ligand and the emitting level of the central ion, which depends on the gap between the two levels. So we consider that the triplet-state level of this ligand matches better with the lowest excited state level of Eu(III) than with that of Tb(III).

The fluorescence characteristics of the europium complex in acetonitrile, acetone, ethanol, methanol and DMF solutions (concentration: $0.1 \mu\text{mol L}^{-1}$) were listed in Fig. 2. It can be seen that in acetonitrile solution the complex has the strongest luminescence and then in acetone, ethanol, methanol and DMF solutions. This is due to the coordinating effects of solvents, namely solvate effect²³. The amide-based portion of the ligands forms a covered conformation suitable for the uptake of the europium ion, but this ajar cavity could not prevent absolutely the solvent molecules from entering. Along with the raising coordination abilities of solutions for the europium ion, the oscillatory motions of the entering molecules consume more energy which the ligands triplet level transfers to the emitting level of the europium ion. Hence, the energy transfer could not be carried out perfectly.

DNA binding studies

Electronic absorption titration: To examine the binding mode of the complexes with DNA, the absorption spectra of the complexes in the absence and presence of DNA were recorded.

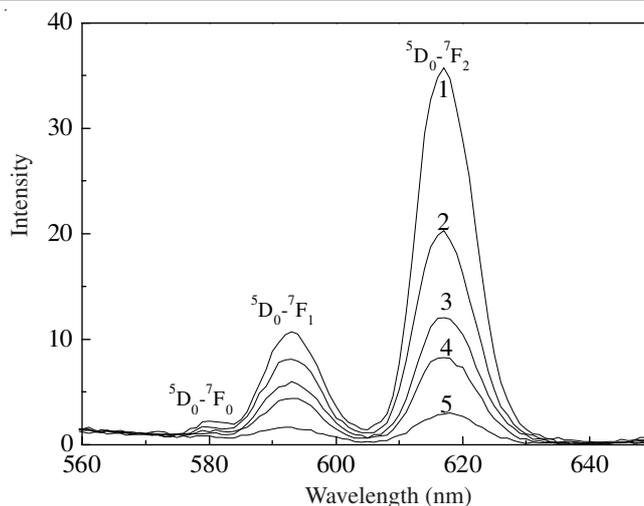


Fig. 2. Emission spectrum of the Eu(III) complex in different solutions at room temperature. (1) acetonitrile, (2) acetone, (3) ethanol, (4) methanol and (5) DMF

In general, a complex binding to DNA through intercalation usually shows hypochromism and red shift, due to the stacking interaction between the aromatic chromophore and DNA base pairs²⁴. The extent of the spectral change is related to the strength of binding²⁵. The electronic absorption spectra of the two complexes in the absence and presence of DNA are given in Fig. 3. As shown, in the presence of DNA, the absorption bands of the Eu(III) and Tb(III) complexes at 378 nm exhibited hypochromism of 20.23 and 15.45 %, respectively. The hypochromism observed for the complexes are all accompanied by a red shift of 3 nm. The binding of complexes to DNA led to decrease in the absorption intensities with a small amount of red shifts in the UV-visible absorption spectra. After intercalating the base pairs of DNA, the π^* orbital of the intercalated ligand can couple with the π orbital of the DNA base pairs, thus, decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in the hypochromism. All these results suggest that the complexes can interact with DNA quite probably by intercalating the compounds into DNA base pairs. Thus, it is concluded that both complexes can interact with DNA *via* a same mode (intercalation) and the Eu(III) complex bind to DNA more strongly than the Tb(III) complex.

Ethidium bromide (EB) fluorescence displacement experiments: To further investigate the interaction of the complexes with DNA, a competitive binding experiment using ethidium bromide as a probe was carried out. The ethidium bromide fluorescence displacement experiment has been widely used to investigate the interaction of metal complexes with DNA. As one of the most sensitive probes, ethidium bromide binds to DNA by the intercalation mode²⁶. The intrinsic fluorescence intensity of DNA is very low and that of ethidium bromide in *tris*-HCl buffer is also not high because of solvent quenching, but the fluorescence intensity of ethidium bromide tends to increase when intercalatively bound to DNA due to its burial in the hydrophobic region of DNA. If the complex intercalates into DNA, it will lead to a decrease in

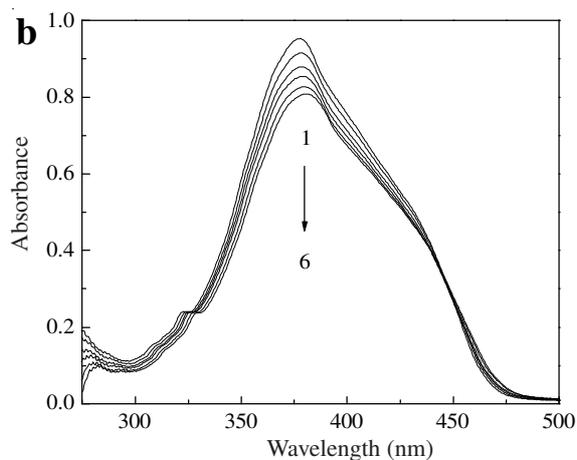
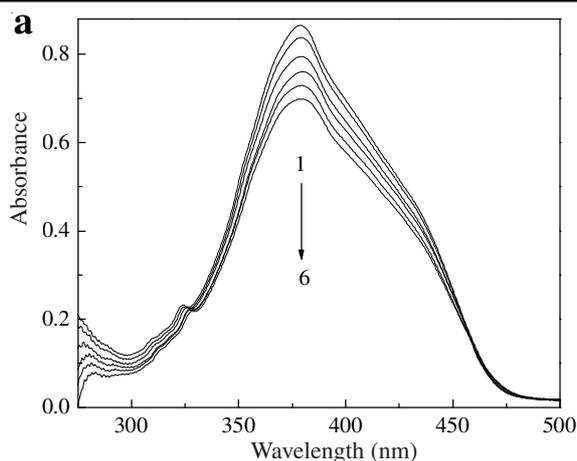


Fig. 3. Absorption spectra of the Eu(III) complex (a) and Tb(III) complex (b) in *tris*-HCl buffer (pH = 7.2) upon addition of DNA. [Complex] = 10 mmol L⁻¹, [DNA] = 10 mmol L⁻¹. The arrow shows the absorbance changes upon increasing DNA concentration

the binding of ethidium bromide to DNA, hence quenching the fluorescence of the ethidium bromide-DNA mixture²⁷. As shown in Fig. 4, the fluorescence intensity at 584 nm for the DNA-ethidium bromide system decreased with increasing complexes concentration. These results indicate that the complexes could partially displace ethidium bromide from the DNA-ethidium bromide system, as often observed in intercalative complex-DNA modes⁸.

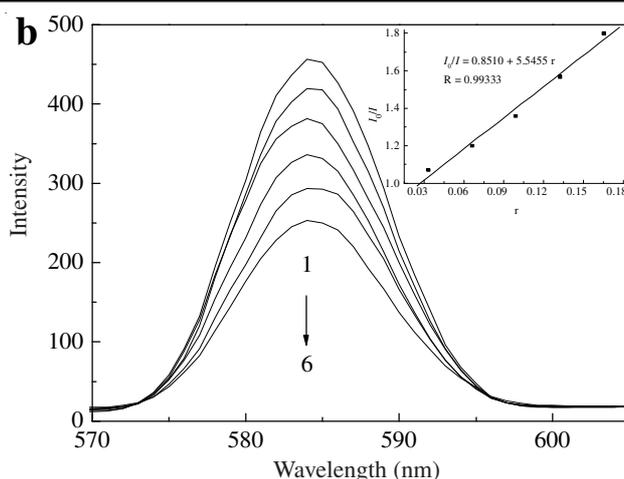
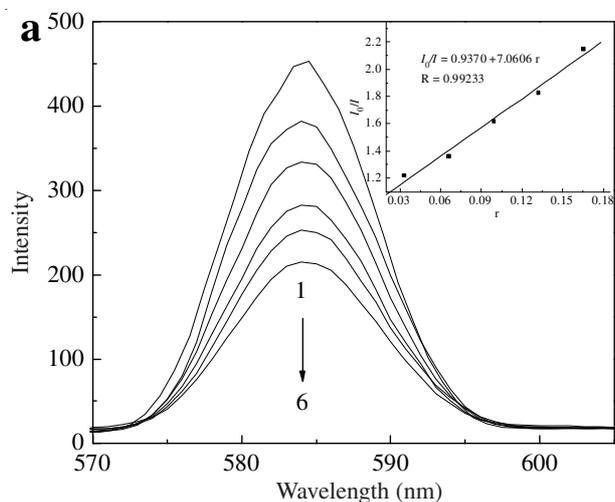


Fig. 4. Fluorescence spectra of ethidium bromide-DNA system with the Eu(III) complex (a) and Tb(III) complex (b). [Complex]/[DNA] (1-6) = 0, 0.033, 0.066, 0.099, 0.132, 0.165. The arrows show the intensity changes upon increasing concentrations of the complexes. Inset: plot of I_0/I versus r . r = [Complex]/[DNA]

In order to measure the binding strength of the complexes with CT-DNA, the Stern-Volmer constant K_{sq} was obtained from the linear Stern-Volmer equation²⁹:

$$\frac{I_0}{I} = 1 + K_{sq} r$$

where I_0 and I are the fluorescence intensities in the absence and presence of the complexes, respectively and r is the concentration ratio of the complex to DNA. The K_{sq} is the linear Stern-Volmer constant and the K_{sq} values can be obtained by the slope. For the Eu(III) complex and Tb(III) complex, the K_{sq} values are 7.0606 and 5.5455, respectively, in agreement with the order derived from the electronic absorption spectra.

Cyclic voltammograms: Electrochemical measure, as a trenchancy method in studying electron transition, can effectively complement the spectral experimental results. Consequently, the interaction mechanism of a complex with DNA can be profoundly comprehended³⁰. The cyclic voltammogram is the most commonly used method.

The cyclic voltammogram of the two complexes without and with DNA were studied at the scan rate of 50 mV s⁻¹ and the results are shown in Fig. 5. It can be seen from the Fig. 5, in the absence of DNA (curve 1), the complexes have been found to show a quasi-reversible redox process corresponding to Eu(III)/Eu(II) and Tb(III)/Tb(IV). In the presence of DNA (curve 2), the oxidation-reduction currents of them decline, which indicates that the complexes interact with DNA. The phenomena that the oxidation-reduction currents of the complexes decline in the presence of DNA suggests there is binding action between the complexes molecules and DNA molecules, which makes the concentration of free complex molecules in solution decreased. Consequently the complex molecules that transfer to the electrode surface in unit time decline and ultimately the peak currents decline³¹. In addition, the peak currents of the Eu(III) complex and Tb(III) complex decrease by 34.21 and 7.24 %, respectively, so we can infer the Eu(III) complex binds to DNA more strongly than the Tb(III) complex. This result is in agreement with the above spectral results.

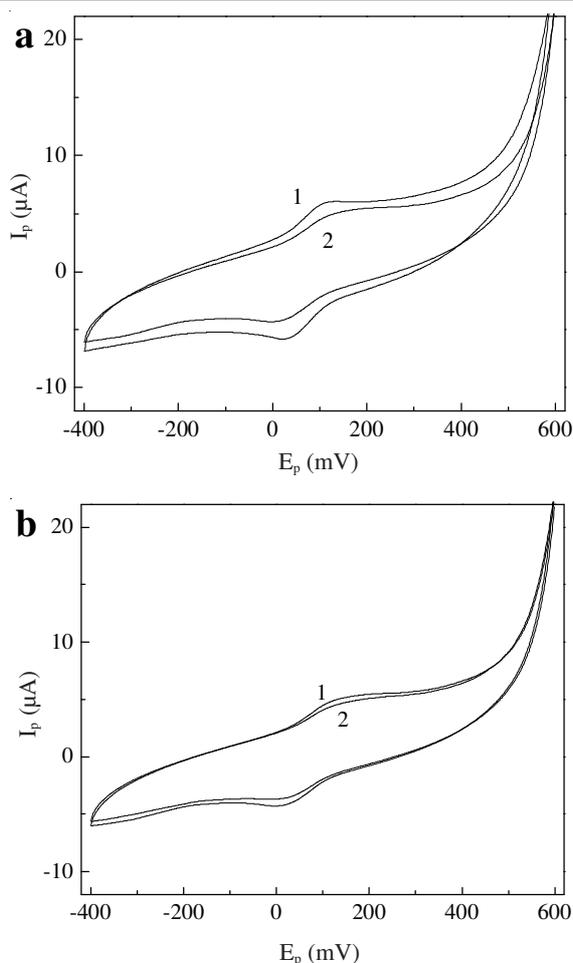


Fig. 5. Cyclic voltammograms of the Eu(III) complex (a) and Tb(III) complex (b) in the absence (1) and presence (2) of DNA [Complex] = 60 mmol L⁻¹, [DNA] = 100 mmol L⁻¹

Conclusion

In this paper, we report the preparation, fluorescence properties and DNA-binding of the Eu(III) and Tb(III) complexes with a new amide-based ligand, 2,2'-[2,3-naphthylenebis-(oxy)]bis(N,N-dibenzyl). Under the excitation, the Eu(III) complex exhibited characteristic fluorescence and the different solvents may affect the fluorescence of europium ions. The lowest triplet state energy level of the ligand matches better to the resonance level of Eu(III) than Tb(III) ion. The interaction of the complexes with DNA was examined by absorption, emission and cyclic voltammetry. The result obtained suggests an intercalative mode of DNA binding for the two complexes and the Eu(III) complex binds to DNA more strongly than the Tb(III) complex.

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