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Synthesis, Characterization and Electrochemical Studies on the Interaction Mechanism between La(III) Complex and DNA

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The La(III) Schiff base complex was synthesized and characterized by elemental analysis, molar conductivity, IR and thermal decomposition. Cyclic voltammetry was used to investigate the interaction between La complex and DNA. The fundamental electrochemical characteristics of a La(III) complex have been studied and the interactivity of lanthanide complex with DNA was also studied by cyclic voltammetry and fluorescence spectrum. The results suggest that the binding ratio between the lanthanide complex and DNA was found to be 1:1 and the binding constant was 3.42×10^3 L mol^{-1}.

Key Words: Synthesis, Electrochemical studies, La(III) complex, DNA.

INTRODUCTION

The numerous biological experiments performed so far suggest that DNA is the primary intracellular target of anticancer drugs because the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death[1-3]. Of those studies, the interaction of transition metal complexes with DNA has gained much attention, owing to their possible applications as new therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation[4-10].

Over the past decades, considerable attention has been devoted to the design and synthesis luminescent lanthanide complexes due to their interesting photophysical properties, which have potential applications in sensors, liquid crystalline materials, optical fiber lasers and amplifiers, luminescent label design for specific biomolecule interactions, magnetic molecular materials and electroluminescent materials[11-15].

EXPERIMENTAL

Glycine and other reagents were of analytical reagent (AR) grade and used without further purification. Salmon sperm DNA was purchased from Shanghai Huashun Biological Engineering Company ($A_{260}/A_{280} > 1.8$). The concentration was determined by the ultra-violet absorption at 260 nm ($Z = 6600\ M^{-1}\ cm^{-1}$).

Elemental analyses were carried out with a model 2400 Perkin-Elmer analyzer. Infrared spectrum was recorded in KBr pellets using a Nicolet 170SX spectrophotometer in the 4000-400 cm^{-1} region. Molar conductivity at room temperature was measured in 10^{-3} M DMSO solution using a DDS-11A type conductivity meter at 25 ºC. The thermo gravimetric measurements were made using a Perkin-Elmer TGA7 thermo gravimeter. The heating rate was programmed to be 10 ºC/min with the protecting stream of N_2 flowing at 40 mL/min. All electrochemical measurements were carried out with Model CHI 832 Voltammetric Analyzer. A three-electrode, Ag/AgCl/KCl (salt) as reference electrode and glass carbon electrode (GCE) as working electrode.

Synthesis of the ligand: O-Vanillin was added (with stirring) to anhydrous ethanol (20 mL) to make a pellucid solution. Then, it was slowly dripped into the anhydrous ethanol solution (15 mL) containing 0.01 mol glycine (containing 0.01mol KOH) at 65 ºC (pH = 6.0-6.5), a mass of yellow grain was separated out which was collected by filtration and washed several times with anhydrous ethanol, recrystallized with methanol and then dried under vacuum for later use. The yield of the reaction was 85.2 %. Anal. calcd. (%) for C, 48.53; H, 4.08; N, 5.58;. Found: C,48.53;H, 4.15; N, 5.58. IR data (KBr, ν_{max}, cm^{-1}): 1660 (C=N); 1603, 1314 (COO^-); 1242 (Ar-O).

Synthesis of the complex: 0.5 mmol lanthanide nitrate hexahydrate of the in 15 mL of anhydrous ethanol was added dropwise into the solution of Schiff base (0.5 mmol) in 15 mL of anhydrous ethanol and was stirred at 80 ºC. The yellow solution obtained was filtered and the yellow powder was dried under vacuum. The C, H and N contents were as follows: Anal. calcd. (%) for C, 20.99; H, 2.99; N, 9.79; La, 24.28. Found: C, 20.23; H, 2.80; N, 9.67; La, 21.75. IR data (KBr, ν_{max}, cm^{-1}): 1645 (C=N); 1592, 1381 (COO^-); 1211 (Ar-O).

Electrochemical study on the interaction between the complex and DNA: 25 µL of 2.00×10^{-4} mol L^{-1} $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ solution was transferred into 5 mL colorimetric tubes containing 0.05 mol L^{-1} B-R buffer solution (pH 5.78) and then DNA was added. The changes on characteristics of CVs were investigated. For CV scanning, the potential scanning range was from 1.0 V to -0.2 V, the scanning rate was 0.062 V/s; the sample interval was 0.001 V and the quiet time was 2 s.

RESULTS AND DISCUSSION

The title complex $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ is yellow powder, soluble in DMSO, DMF. The molar conductivity of the complex is 83.33 Ω^{-1} cm^2 mol^{-1} in DMSO. Molar conductivity for the complex in DMSO corresponds to electrolytes[16].

The shift of ν(C=N) from 1660 cm^{-1} in the ligand to 1645 cm^{-1} in the complex, suggests that La ion is bonded with N atom in Schiff-base. The shift of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ from 1603 and 1314 cm^{-1} in the ligand to 1592 and 1381 cm^{-1} in the complex, respectively, suggests the coordination of the oxygen in the carboxylate group to the metal ion. The value of $\nu[\nu_{as}(COO^-)-\nu_s(COO^-)] = 211$ cm^{-1} indicates that the -COO^- group is coordinated to the metal ion in a mono-dentate fashion[17]. A broad absorption band at the range of 3000-3300 cm^{-1} confirms the presence of water in the complex. The appearing of Ar-O frequency (1211 cm^{-1}) is lower than 1242 cm^{-1}, which exposes that Ar-O-La in the complex. In the spectrum of the complex, five additional bands, which are not present in the spectrum of the free ligand, were observed. The bands at 1391 and 826 cm^{-1} are assigned to the metal ion in the non-coordination. The nitrate vibration of complex is seen as split bands at 1531-1539, 1225-1236 and 1026-1062 cm^{-1}. The separation of modes has been used as a criterion to distinguish between mono- and bidentate chelating nitrates. The magnitude of this separation may be indicative of a bidentate interaction of the nitrate anions with the lanthanide ions[18].

The TG and DTG curves of the complex are shown in Fig. 1, which indicate that complex decomposes in three steps. The first weight loss stage has decomposition temperature ranges of 25-115 ºC, with weight losses of 6.62 % (calcd. 6.31 %), which corresponds to the losses of two molecules of water. The second weight loss stage has decomposition temperature range of 115-280 ºC, corresponding to the fractional losses of the nitrate ion of outer of the coordination sphere with weight losses of 11.02 % (calcd. 10.87 %). The third weight loss stage showed a continuous weight loss between 280-800 ºC, which corresponds to the loss of all of the ligand and 27.6 % of the original sample remained. With its calculated weight percentage of 28.42 %, La_2O_3 is the final product.

According to the characterizations enumerated above, the possible structure of the complex is shown as Fig. 2.

Electrochemical interaction between $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ and DNA: Electrochemical study on $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ and its interaction with DNA were performed at 25 ºC. The cyclic voltammograms of $[La(GOV)(NO_3)_2]$ $(NO_3)\cdot 2H_2O$ in the absence and presence of DNA were shown in Fig. 3. The buffer used was 0.05M B-R solution (pH 5.78).

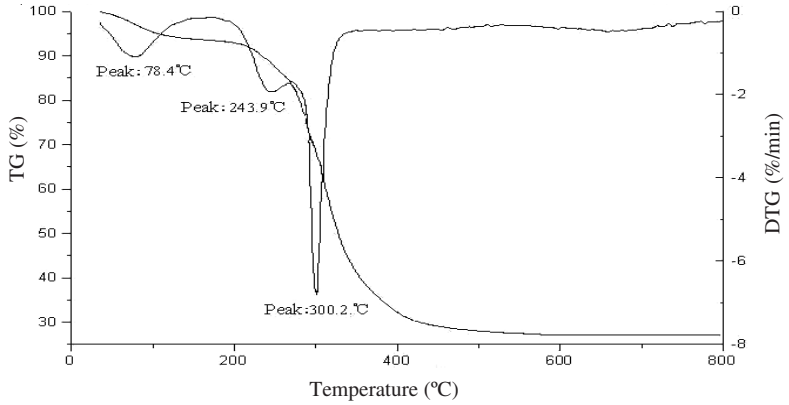

Fig. 1. Thermal analysis curves of the title compound

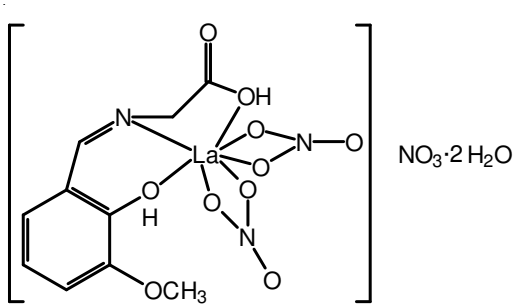

Fig. 2. Suggested structure of the complex

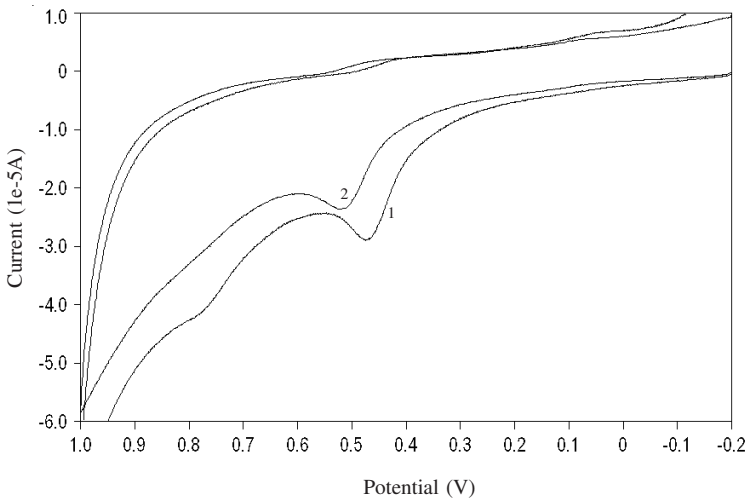

Fig. 3. Cyclic voltammetry of La(III) complex
$C_{[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O}$: 2.00×10^{-4} mol L^{-1}, C_{DNA}: (1) 0 (2) 5.20×10^{-4} mol L^{-1}

The curve 1 is a cyclic voltammetry of the title complex in the B-R buffer solution (pH = 5.78). The curve 2 is cyclic voltammetry of the mixed solution of DNA and complex in the B-R buffer solution, from which we can induce that the electric current of oxidation peak decreased and had no new oxidation peak occur, through which we can presume DNA and complex formed the new compound that wasn't electric activity compound. Due to the new compound didn't conducted, the concentration of the complex reduced so that the number of the molecule of the complex moved to the surface of electrode declined, which lead to the electric currents became weak. It is generally accepted that there are three kinds of binding modes for small molecules to DNA, which refer to intercalative binding, groove binding and electrostatic binding.

Bard[19] deemed that when the molecule inserts into the inner of DNA double helix structure, the peak currents of the oxidation of the voltammetry decreased with positive shifts of the peak potential. On the other way around, when the molecule interacted with DNA in the form of static effect, the peak currents of the voltammetry curves of the oxidation of the voltammetry decreased with negative shifts of the peak potential. In summary, we can infer that the complex interact with DNA in the form of intercalative binding.

Binding ratio and binding constant of DNA-[La(GOV)$(NO_3)_2$] $(NO_3)\cdot 2H_2O$: To study the binding ratio and binding constant between [La(GOV)$(NO_3)_2$]$(NO_3)\cdot 2H_2O$ and DNA, it was assumed the interaction of DNA and [La(GOV)$(NO_3)_2$]$(NO_3)\cdot 2H_2O$ only produced one single complex: DNA-nML, as shown in the following equation[20]:

$$DNA + nML \rightleftharpoons DNA - nML \quad (n = 1, 2, 3, \ldots \text{ or } 1, 1/2, 1/3, \ldots)$$

The equilibrium constant β could be expressed as eqns. 1-7

$$\beta = \frac{[DNA - nML]}{[DNA][ML]^n} \tag{1}$$

$$\Delta I_{p,max} = KC_{DNA} \tag{2}$$

$$\Delta I_p = K[DNA - nML] \tag{3}$$

$$[DNA] + [DNA - nML] = C_{DNA} \tag{4}$$

$$\Delta I_{p,max} - \Delta I_p = K(C_{DNA} - [DNA - nML]) \tag{5}$$

$$\Delta I_{p,max} - \Delta I_p = K[DNA] \tag{6}$$

$$\frac{1}{\Delta I_p} = \frac{1}{\Delta I_{p,max}} + \frac{1}{\beta \Delta I_{p,max}[ML]^n} \tag{7}$$

According to the eqn. 7, different n might result in different curves of ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{n-}$. With the suitable n, the curve of ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{n-}$ should be a straight line if there was only one complex formed when $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{n-}$ bound to DNA. From the slope and intercept of the straight line, the binding constant β could be calculated and the n could be regarded as the binding ratio.

The dependence of the oxidation peak current (I_{pa}) on the analytical concentration of $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ in the absence (curve 1) and presence (curve 2) of DNA was shown in Fig. 4. The relationship between ΔI_{pa} (the difference of I_{pa1}, I_{pa2}, $I_{pa} = I_{pa1}-I_{pa2}$) and the analytical concentration of $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ was also displayed (curve 3).

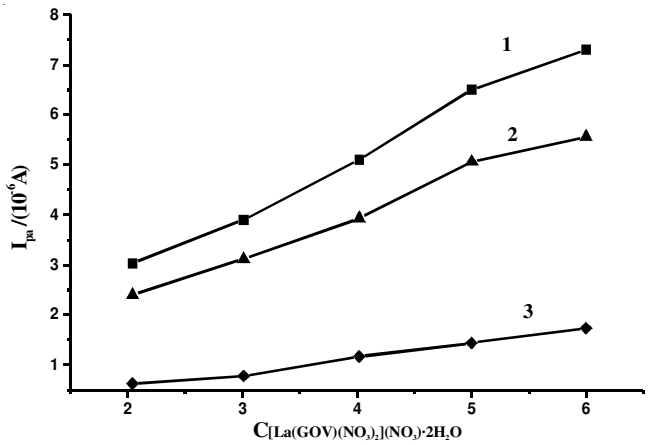

Fig. 4. Relationship curve of I_{pa1}, I_{pa2} and I_{pa} *vs.* $C[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$
1. C_{DNA}: 0, 2. C_{DNA}: 1.4×10^{-4} mol L^{-1}, 3. $\Delta I_{pa} = I_{pa1} - I_{pa2}$

The curves of ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{-0.5}$, ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{-}$, ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{2-}$, were displayed in Fig. 5, where $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ represented the equilibrium concentration of $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ and calculated from data in Fig. 4, For n = 0.5 and 2, the curves bent down and up, respectively. While for n = 1, the curve was a straight line (λ = 0.999), indicating the forming of a 1:1 association between $[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O$ and DNA. From the slope and intercept of the straight line, the binding constant β was calculated to be 3.42×10^3 L mol^{-1}.

Fluorescence spectrum of the interactivity of the complex and DNA: The 1-(*o*-cyanostyryl)-4-(*p*-cyanostyryl)benzene (EB) is one of fluorescent reagent. When it inserts into the inner of DNA double helix structure, the system of EB-DNA can give off the fluorescence and hindrance the copy of DNA. If the molecule can insert DNA, the molecules compete with EB

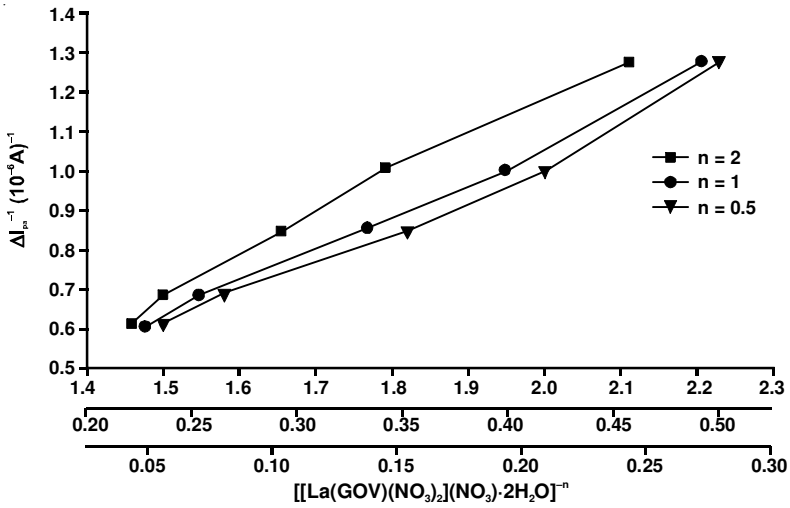

Fig. 5. Relationship curve of ΔI_{pa}^{-} *vs.* $[[La(GOV)(NO_3)_2](NO_3)\cdot 2H_2O]^{-n}$

at the bonding point of DNA, the EB releases. As the EB is free, the fluorescence intensity of the system become weak, through which we can determine if the molecule inserts into the inner of DNA double helix structure. Fig. 6 shows different concentration of complex interacted with EB-DNA (excitation wavelength: 540 nm pH = 5.78).

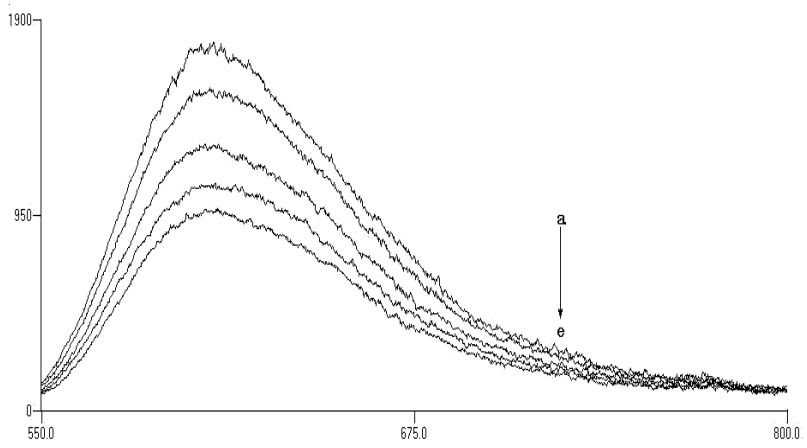

Fig. 6. Fluorescence spectra of different concentration complex interaction with EB-DNA system
a-C_{Comp} = 0 mol/L; b-C_{Comp} =5.00 × 10^{-5} mol/L; c-C_{Comp} = 1.00 × 10^{-4} mol/L; d-C_{Comp} = 1.50 × 10^{-4} mol/L; e-C_{Comp} = 2.00 × 10^{-4} mol/L

From Fig. 6, we concluded that when added the complex into the system of EB-DNA, the fluorescence of the system of EB-DNA obviously trailed off, that is these indicated complex combined with DNA, competing with EB. When added the complex in the system of EB-DNA, the EB replace by the complex. According, the complex and EB may be combine with DNA at the same spot and proved the complex inserted into the inner of DNA double helix structure[21].

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