

SYNTHESIS AND DNA INTERACTION OF A Sm(III) COMPLEX OF A SCHIFF BASE DERIVED FROM VANILLIN AND L-TRYPTOPHAN

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ABSTRACT. The interaction between the Sm(III) complex of an ionic Schiff base [HL]⁻, derived from vanillin and L-tryptophan, and herring sperm DNA at physiological pH (7.40) has been studied by UV-Vis absorption, fluorescence and viscosity methods. The binding ratios $n_{\text{Sm(III)}} : n_{\text{K[HL]}} = 1:1$ and $n_{\text{Sm(III)L}} : n_{\text{DNA}} = 5:1$ were confirmed by a mole ratio method. The calculated binding constants of [Sm(III)L]⁺ with DNA at 25 and 37 °C were $1.19 \times 10^5 \text{ M}^{-1}$ and $3.96 \times 10^4 \text{ M}^{-1}$, respectively. The thermodynamic parameters demonstrate that the interaction between [Sm(III)L]⁺ and DNA is driven mainly by enthalpy. Fluorimetric studies show that [Sm(III)L]⁺ is able to displace intercalated acridine orange. Combined with Scatchard methods, circular dichroism spectroscopy and viscosity measurements, the results indicate that the interaction between [Sm(III)L]⁺ and herring sperm DNA is mainly by intercalation with some groove binding.

KEY WORDS: Vanillin, L-Tryptophan, Sm(III) complex of Schiff base, Herring sperm DNA, Interaction

INTRODUCTION

DNA is the primary intracellular target of anticancer reagents because interaction between metal complexes and DNA can cause damage in cancer cells, blocking cell division [1, 2]. To date, much attention has been paid to binding of metal complexes containing multidentate aromatic ligands. Generally, these complexes bind to DNA by a series of non-covalent interactions, such as intercalation, electrostatic and groove binding [3, 4]. Many molecules show more than one interaction mode with DNA, depending on structural features of both the molecule and the DNA [5].

A Schiff base is obtained by the condensation between an aldehyde and an amine [6]. Complexes with transition metals are considered to be new kinds of anticancer and antiviral reagents, because of their range of biological and pharmaceutical effects, such as anticancer, antitumor and antioxidative activities as well as inhibition of lipid peroxidation [7-9].

L-Tryptophan (Trp) is an essential amino acid that is required for the biosynthesis of proteins and is important in nitrogen balance and the maintenance of muscle mass and body weight in humans [10]. The rare earth metals have wide applications in medicine. They are superior to transition metals and their complexes because they can bind to nucleic acids more efficiently [11]. Coordination with ligands reduces and can enhance physiological activity. ¹⁵³Sm is used in treatment of severe pain associated with bone cancers and Sm compounds are also used in rare earth antiphlogistic sterilization drugs.

We have studied the interaction between a tryptophan-vanillin Schiff base and herring sperm DNA, and found evidence for intercalation and groove binding [12]. In this paper, we describe the synthesis of a Sm(III) complex with the Schiff base and the determination of a series of thermodynamic parameters and binding constants for the interaction of this complex with DNA.

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EXPERIMENTAL

Apparatus

Carbon, hydrogen and nitrogen analyses were obtained by use of a Vario EL CUBE (Germany). IR spectra ($400\text{--}4000\text{ cm}^{-1}$) were recorded as KBr pellets on a Spectrum One FTIR system (USA). Absorption spectra were measured on an UV-210 spectrophotometer (Japan) and fluorescence spectra on a FL-4500 spectrofluorophotometer (Japan). Circular dichroism (CD) measurements were recorded on a Jasco-815 spectropolarimeter (Japan). The pH was recorded on a PHS-2C digital pH-meter (China) with a combined glass-calomel electrode.

Materials

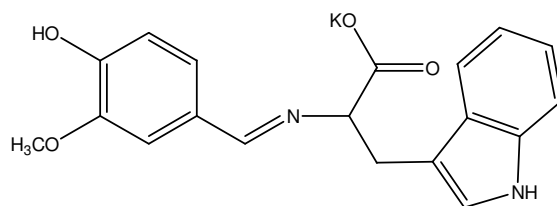
Herring Sperm DNA (hsDNA) was purchased from Sigma Biological Co. (USA) and used as received. The purity of the DNA was checked by monitoring the ratio of absorbance at 260 to that at 280 nm. The ratio A_{260}/A_{280} was 1.89, indicating that DNA was free from protein [13]. The DNA was dissolved in doubly distilled deionized water with 50 mM NaCl and dialyzed for 48 h against a buffer solution at 4 °C. The concentration of hsDNA stock solution was determined by UV absorbance at 260 nm using a molar absorption coefficient $\epsilon = 6600\text{ M}^{-1}\cdot\text{cm}^{-1}$ [13].

A tris(hydroxymethyl)aminomethane-HCl buffer (0.05 M, pH 7.40), was used to control the pH. Sm_2O_3 , obtained from Beijing Fangzheng Rare Metal Lab Company (99.99 %) (China), was dissolved in conc. HCl, then water and excess HCl were evaporated slowly to give a white powder that was added to tris-HCl buffer to prepare known concentrations of SmCl_3 . L-Tryptophan was purchased from Chengdu-China Kelong Chemical Plant (A.R.) (China). Vanillin was purchased from Xian-China Chemical Plant (A.R.) (China). Acridine orange (AO) was purchased from Shanghai-China Medicine Chemical Plant (A.R.) (China). Other reagents were at least analytical grade, and were used without further purification.

Procedures

Preparation of the potassium salt K[HL] and the Sm(III) complex SmLCl

The Schiff base (K[HL]) derived from vanillin and L-tryptophan was prepared using a method similar to that in the literature [14]. To a solution of vanillin (0.180 g, 1.2 mmol) in MeOH (10 mL), L-tryptophan (0.204 g, 1 mmol) in MeOH (15 mL) containing KOH (0.056 g, 1 mmol) was added. The solution was then magnetically stirred for 5 h at 50-60 °C on a water bath. The volume of brownish red solution was reduced *in vacuo* using rotary evaporator to about 5 mL. Then absolute ethanol was added to the solution to precipitate the solid. After several days a black product was separated, washed with Et_2O and dried *in vacuo*. From the IR spectrum and elemental analysis, the structure of K[HL] ($\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_4\text{K}\cdot 2\text{H}_2\text{O}$) is proposed in Scheme 1 [12].



Scheme 1. The proposed structure of the Schiff base K[HL] .

SmCl₃ (0.5 mmol) was dissolved in MeOH (10 mL) and the solution was added dropwise to a methanolic solution of K[HL] (15 mL, 0.5 mmol). The mixture was stirred magnetically for 2 h at 50-60 °C. The volume of the solution was reduced to 10 mL in a rotary evaporator. After several days the brown product was washed with EtOH then Et₂O and dried *in vacuo*.

Absorption spectral measurements

3.0 mL samples, in 1.0 cm quartz cells, were titrated by a stock solution of SmCl₃. Similarly 3.0 mL samples of Sm(III)L solution in 1.0 cm quartz cells were titrated with a stock solution of DNA. The titration was done manually by use of a micro-injector and incremental addition injection of 10 μL samples to minimise changes of volume. Appropriate blanks corresponding to the buffer were used as reference. The absorption spectra were measured after 5 min.

Fluorescence spectral measurements

3.0 mL samples of Sm(III)-DNA in 1.0 cm quartz cells, were titrated with a stock solution of AO and 3.0 mL samples of AO-DNA were titrated by a stock solution of Sm(III)L. Each injection was 10 μL to avoid change of volume. The widths of both the excitation slit and the emission slit were set at 5.0 nm; and the excitation wavelength was set at 411.7 nm. The fluorescence spectra were measured after 5 min.

Circular dichroism (CD) measurements

Circular dichroism (CD) measurements were made at room temperature on a Jasco-815 spectrophotometer, with 1 cm quartz cells and recorded in the range 200-450 nm with a scan rate of 50 nm·min⁻¹.

Viscosity measurements

Viscosity experiments were made at room temperature. Various amounts of Sm(III)L was then added to a solution of DNA in the viscometer to give increasing values of $c_{\text{Sm(III)}}$ while keeping the DNA concentration constant. The flow times of the samples were repeatedly measured with an accuracy of ±0.2 s by a digital stopwatch. Each point was the average of at least three measurements. The buffer flow time (t_0) was observed, and relative viscosities for DNA in the presence and absence of Sm(III)L were calculated from the relation $\eta = (t - t_0)/t_0$, where t is the observed flow time. The data were presented as plots of $(\eta/\eta_0)^{1/3}$ versus $c_{\text{Sm(III)L}}$, where η and η_0 are the viscosity of DNA in the presence and absence of Sm(III)L, respectively.

RESULTS AND DISCUSSION

Interaction between Sm(III) and K[HL]

The UV-Vis spectra of K[HL] show three transitions: two at 213 and 280 nm; and one at 340 nm. The stoichiometry of the interaction of Sm(III) with K[HL] was determined by the mole ratio method [15] at 280 nm. The mole ratio plots of Sm(III) vs K[HL] is shown in Figure 1. The binding ratio of the complex: $n_{\text{Sm(III)}} : n_{\text{K[HL]}}$ was 1:1.

The IR spectral data of K[HL] and the complex Sm(III)L are given in Table 1. The strong band at 1627 cm⁻¹ can be assigned to the $\nu(\text{C}=\text{N})$ azomethine stretching vibration. On complexation, this band shifts to higher frequency at 1633 cm⁻¹ indicating the coordination of the azomethine nitrogen atom to the central metal ion [16]. The peaks assigned to carboxyl stretching $\nu_{\text{asym}}(\text{COO}^-)$ and $\nu_{\text{sym}}(\text{COO}^-)$ are shifted to 1601 and 1354 cm⁻¹, respectively,

indicating the linkage between the metal ion and carboxylato oxygen atom [16, 17]. The phenolic $\nu(\text{C-O})$ band was shifted to lower frequency at 1263 cm^{-1} indicating the coordination of the phenolic oxygen atom to the metal ion [16]. In the low frequency region, the band at 523 cm^{-1} is assigned to $\nu(\text{M-N})$ and the band at 466 cm^{-1} to $\nu(\text{M-O})$ [17-20]. The broad band at 3429 cm^{-1} indicates that water is present in the Schiff base, which is coordinated to the central through phenolic oxygen, imino nitrogen and carboxylato oxygen atoms. Anal. cal. for $[\text{Sm}(\text{III})\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_4(\text{H}_2\text{O})_3]\text{Cl}\cdot 2\text{H}_2\text{O}$: C, 37.27; H, 2.78; N, 4.58%. Found: C, 35.43; H, 2.72; N, 4.43%. The proposed structure of the complex $\text{Sm}(\text{III})\text{L}$ is shown in Scheme 2.

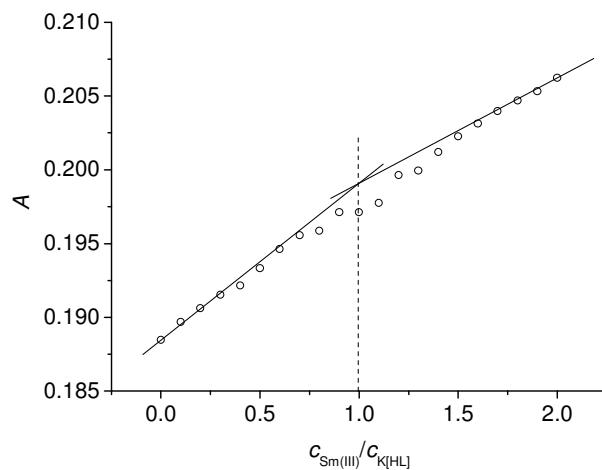
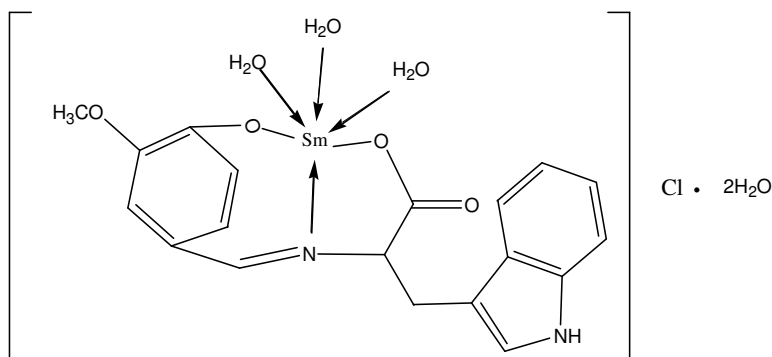


Figure 1. Mole ratio plots of $\text{Sm}(\text{III})\text{-K}[\text{HL}]$ in a tris-HCl buffer (pH 7.40); $c_{\text{K}[\text{HL}]} = 2.00 \times 10^{-5}\text{ M}$, $\lambda = 280\text{ nm}$.

Table 1. Infrared spectral data of Schiff base ligand and its $\text{Sm}(\text{III})$ complex (cm^{-1}).

Compound	$\nu(\text{O-H})$	$\nu(\text{C=N})$	$\nu_{\text{asym}}(\text{COO}^-)$	$\nu_{\text{sym}}(\text{COO}^-)$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
$\text{K}[\text{HL}] \cdot 2\text{H}_2\text{O}$	3398	1627	1593	1390	-	-
$\text{Sm}(\text{III})\text{L}(\text{H}_2\text{O})_3\text{Cl} \cdot 2\text{H}_2\text{O}$	3429	1633	1601	1354	523	466



Scheme 2. The proposed structure of the complex $\text{Sm}(\text{III})\text{L}$.

Binding of Sm(III)L complex to DNA

The binding of small molecules to DNA has been characterized classically through absorption titrations [21]. Both red (hypochromic) and blue (hyperchromic) shifts are observed.

UV-Vis absorption spectra were obtained by titration of a solution of Sm(III)L with DNA (Figure 2). In the absence of DNA, the spectrum of Sm(III)L shows three peaks: at 213, 279 and 340 nm. With the addition of DNA, the intensities of the peaks at 213 nm and 279 nm absorbance increased, but that at 340 nm decreased. An isochromatic point was observed at 333 nm. These results indicate the presence of intercalation between Sm(III)L and DNA.

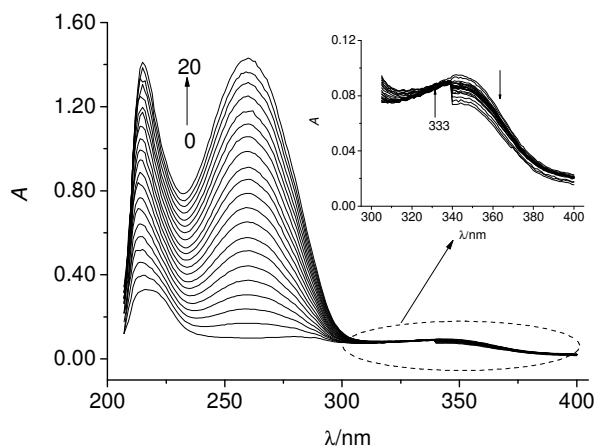


Figure 2. Absorption spectra of Sm(III)L with various molar amounts of DNA (pH 7.40) at 25 °C; from curve 0-20, $c_{\text{Sm(III)L}} = 1.00 \times 10^{-5}$ M, $c_{\text{DNA}} = 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28, 0.30, 0.32, 0.34, 0.36, 0.38$ and 0.40×10^{-5} M.

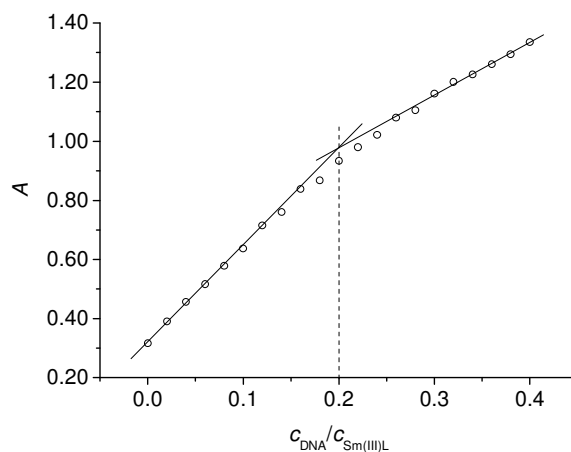


Figure 3. Mole ratio plots of Sm(III)L-DNA in a tris-HCl buffer (pH 7.40) at 25 °C; $c_{\text{Sm(III)L}} = 1.00 \times 10^{-5}$ M, $\lambda = 213$ nm.

In order to determine the stoichiometry of the formation of the Sm(III)L-DNA complex, the mole ratio experiment [15] was applied to the peak 213 nm (Figure 3). The binding ratio of the complex $n_{\text{Sm(III)L}} : n_{\text{DNA}} = 5:1$. The apparent molar absorption coefficient of Sm(III)L-DNA ϵ was $4.81 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

Thermodynamic parameters

The binding constant of Sm(III)L with DNA was determined using the following relationship [22-23]:

$$1/(A_0 - A) = 1/A_0 + 1/(K \times A_0 \times c_{\text{DNA}}) \quad (1)$$

where A_0 and A are the absorbance of Sm(III)L in the absence and in the presence of DNA, respectively. K is the binding constant between Sm(III)L and DNA and c_{DNA} refers to the concentration of DNA. The plots of $1/(A_0 - A)$ versus $1/c_{\text{DNA}}$ were linear at 25 and 37 °C and the binding constants derived from the slope were calculated (Figure 4): $K_{\text{B}}^{\ominus}{}_{25} = 1.19 \times 10^5 \text{ M}^{-1}$, $K_{\text{B}}^{\ominus}{}_{37} = 3.96 \times 10^4 \text{ M}^{-1}$.

The thermodynamic parameters show the binding mode [24]. If the binding enthalpy change (ΔH) is invariant over a small temperature range [25] the standard molar binding enthalpy (ΔH_m^{\ominus}), is estimated from the relationship:

$$\ln K_2^{\ominus}/K_1^{\ominus} = -\Delta_r H_m^{\ominus} (1/T_2 - 1/T_1)/R \quad (2)$$

where K_1^{\ominus} and K_2^{\ominus} are the standard binding constants of Sm(III)L and DNA at 25 and 37 °C, respectively. Then $\Delta_r H_m^{\ominus}$ is $-6.93 \times 10^5 \text{ J} \cdot \text{mol}^{-1}$: the binding of Sm(III)L to DNA is exothermic. From this and the value of the equilibrium constant: ΔG_m^{\ominus} is calculated as $-2.90 \times 10^4 \text{ J} \cdot \text{mol}^{-1}$ and ΔS_m^{\ominus} is $-2.23 \times 10^3 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. The negative value of ΔG_m^{\ominus} is consistent with a spontaneous process, and the negative values of ΔH_m^{\ominus} and ΔS_m^{\ominus} indicate that the binding is mainly enthalpy driven.

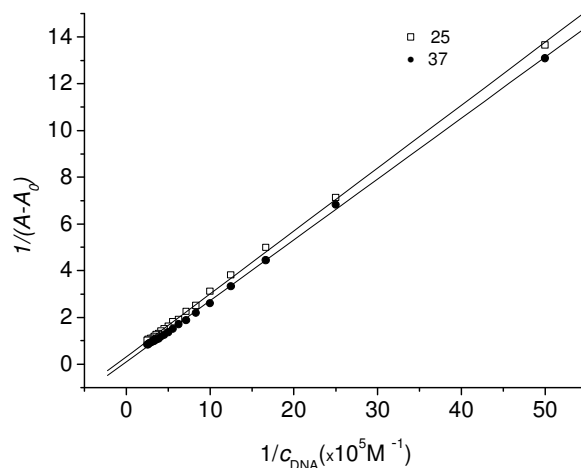


Figure 4. Double reciprocal plots of Sm(III)L-DNA in a tris-HCl buffer at 25 and 37 °C, respectively (pH 7.40); $c_{\text{Sm(III)L}} = 1.00 \times 10^{-5} \text{ M}$.

Fluorescence measurements with acridine orange (AO) as probe

Since the fluorescence of the Sm(III)L complex is weak both in the presence and absence of DNA, the above results were supplemented by competitive AO binding studies. Because of its planar aromatic chromophore, AO can insert between two adjacent base pairs in a DNA helix.

The emission spectra of Sm(III)L bound to DNA in the absence and the presence of AO are given in Figure 5. There is a significant increase in the fluorescence intensity and a red shift $\Delta\lambda$ of 4 nm as the AO inserts between the base pairs.

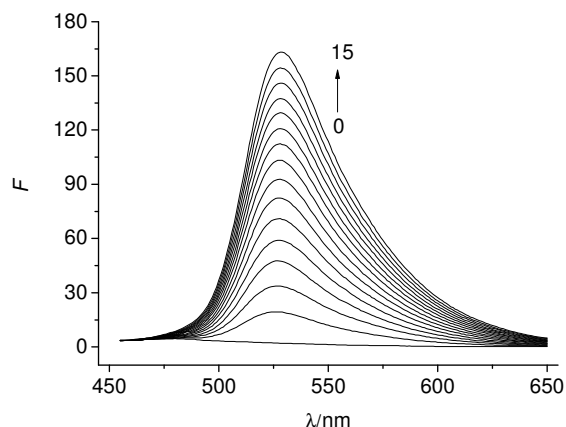


Figure 5. Emission spectra of Sm(III)L-DNA with various concentrations of AO (pH 7.40; $\lambda_{\text{ex}} = 411.7$ nm); from curve 0-15, $c_{\text{DNA}} = 2.00 \times 10^{-6}$ M, $c_{\text{AO}} = 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28,$ and 0.30×10^{-5} M.

The emission spectra of AO bound to DNA in the absence and the presence of Sm(III)L are given in Figure 6. If Sm(III)L has the same binding mode with DNA as AO, competition between AO and complex would be shown by a change in the fluorescence spectrum [26]. Figure 6, shows that the fluorescence of DNA-AO is efficiently quenched, suggesting that Sm(III)L substitutes for AO i.e. that Sm(III)L intercalates into DNA, consistent with the results above [27].

Scatchard method

Data for binding studies were processed by the Scatchard procedure [28]. The binding of AO to DNA is expressed using the following equation:

$$r_{\text{AO}}/c_{\text{AO}} = K(n - r_{\text{AO}}) \quad (3)$$

where r_{AO} is the number of moles of AO bound per mole of DNA, c_{AO} is the molar concentration of free AO, n is the number of binding sites per DNA and K is the intrinsic association constant. If Sm(III)L interacts with DNA by intercalation, the intercept on the abscissa (n) should remain constant as the slope (K) changes in the Scatchard plots. If Sm(III)L interacts with DNA by groove or electrostatic binding, K should remain constant as n changes. If the binding mode is mixed, both n and K should change in the Scatchard plots [29].

Various Sm(III)L/DNA mole ratios $c_{\text{Sm(III)L}}/c_{\text{DNA}} = 0.00, 0.30, 0.60, 0.90$ were used. Further, experiments without or with addition of NaCl were compared. NaCl is not an anionic quencher

of DNA [30], so its influence on the Sm(III)L-AO-DNA system comes only from ionic strength. Results are shown in Table 2.

It is seen that both n and K change as the concentration of Sm(III)L is increased suggesting that Sm(III)L interacts with DNA by a mixed binding mode. Moreover n is increased by addition of NaCl. Generally, n is reduced in the presence of NaCl, by electrostatic interaction [31], so the interaction is by groove binding rather than electrostatic.

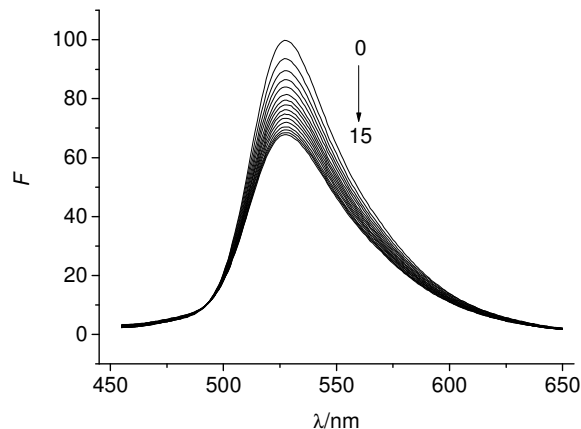


Figure 6. Emission spectra of DNA-AO with various concentrations of Sm(III)L (pH 7.40; $\lambda_{\text{ex}} = 411.7$ nm); from curve 0-15, $c_{\text{DNA-AO}} = 1.34 \times 10^{-6}$ M, $c_{\text{Sm(III)L}} = 0.00, 0.67, 1.33, 2.00, 2.67, 3.33, 4.00, 4.67, 5.33, 6.00, 6.67, 7.33, 8.00, 8.67, 9.33,$ and 10.00×10^{-6} M.

Table 2. Data of Scatchard equation of the interaction between Sm(III)L and DNA.

Curve	Rt	NaCl %	Scatchard	$K/(\text{M}^{-1})$	n
a	0.00	5.00	$2.61 \times 10^5 - 1.56 \times 10^7 r_{\text{AO}}$	1.56×10^7	0.0168
		0	$3.21 \times 10^5 - 2.63 \times 10^7 r_{\text{AO}}$	2.63×10^7	0.0122
b	0.30	5.00	$2.42 \times 10^5 - 1.07 \times 10^7 r_{\text{AO}}$	1.07×10^7	0.0226
		0	$1.43 \times 10^5 - 8.08 \times 10^6 r_{\text{AO}}$	8.08×10^6	0.0178
c	0.60	5.00	$2.79 \times 10^5 - 1.01 \times 10^7 r_{\text{AO}}$	1.01×10^7	0.0276
		0	$2.14 \times 10^5 - 9.60 \times 10^6 r_{\text{AO}}$	9.60×10^6	0.0224
d	0.90	5.00	$3.97 \times 10^5 - 1.17 \times 10^7 r_{\text{AO}}$	1.17×10^7	0.0339
		0	$1.95 \times 10^5 - 5.97 \times 10^6 r_{\text{AO}}$	5.97×10^6	0.0327

Rt = $c_{\text{Sm(III)L}}/c_{\text{DNA}}$; $c_{\text{DNA}} = 1.00 \times 10^{-6}$ M; $c_{\text{AO}} = 3.00 \times 10^{-5}$ M.

Circular dichroism spectroscopy

Circular dichroism (CD) spectroscopy is a useful tool to diagnose changes in DNA morphology during drug-DNA intercalations, since the positive band due to base stacking and the negative one due to right-handed helicity are quite sensitive to the interaction mode [32, 33]. The CD spectroscopy of DNA in the presence of the Sm(III)L is shown in Figure 7. The positive band at 276 nm and negative one at 249 nm indicate that the DNA remains right-handed. The intensity of both bands is increased relative to that of free DNA. Simple groove binding and electrostatic interaction show little or no perturbation on the base-stacking and helicity bands, while intercalation enhances the intensities of both bands arising from the right-handed B conformation of CT-DNA (CT-DNA is referred to calf thymus DNA.) as observed for the classical intercalator methylene blue [34]. Thus, the CD spectroscopy of the Sm(III)L-DNA

system shows an intercalation involving π - π^* stacking and stabilization of the right-handed B-form of DNA [32, 34].

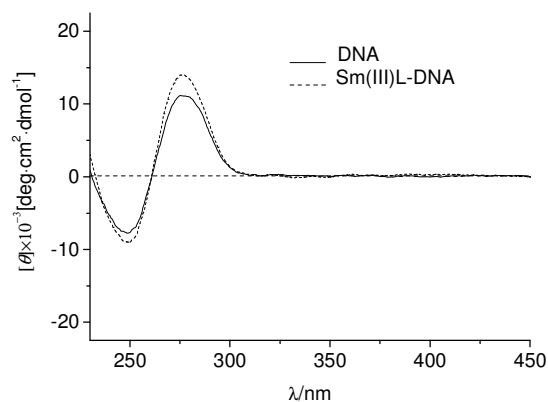


Figure 7. Circular dichroism spectra of DNA in the presence of Sm(III)L; $c_{\text{DNA}} = 1.00 \times 10^{-5}$ M, $c_{\text{Sm(III)L}} = 5.00 \times 10^{-5}$ M.

Viscosity measurements

In the absence of crystallographic structural data, hydrodynamic measurements, being sensitive to length change, are regarded as the most critical tests for a binding model in solution [35].

Classical intercalation is known to cause a significant increase in the viscosity of a DNA solution, as the base-pairs are forced away from each other, the double helix unwinds and the DNA lengthens. In contrast, a partial intercalation mode could bend the DNA helix and reduce its effective length and, concomitantly, the viscosity. Non-intercalation binding causes no obvious increase of DNA viscosity [35, 36].

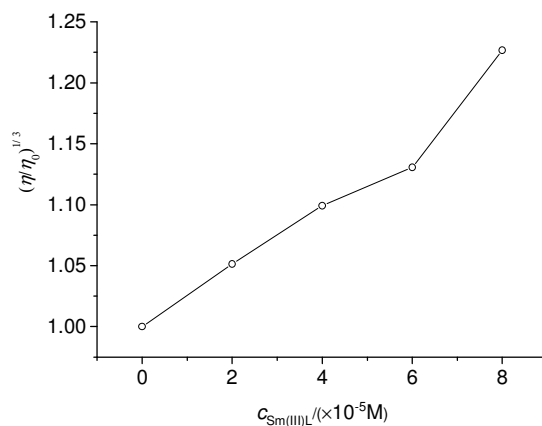


Figure 8. The effect of increasing amounts of Sm(III)L on the relative viscosities of DNA at 25 °C; $c_{\text{DNA}} = 1.00 \times 10^{-4}$ M.

The values of $(\eta/\eta_0)^{1/3}$ (where η_0 and η are the specific viscosity contributions of DNA in the absence and the presence of the Sm(III)L, respectively) are plotted against $c_{\text{Sm(III)L}}$ in Figure 8. Addition of Sm(III)L increases the relative specific viscosity of DNA, consistent with lengthening of the DNA double helix by intercalation.

CONCLUSIONS

The Sm(III) complex of a Schiff base derived from vanillin and L-tryptophan has been synthesized. Its interaction with hsDNA has been studied by UV-Vis absorption, fluorescence, and CD spectroscopy and viscosity methods. The binding constants of Sm(III)L with DNA were measured at 25 and 37 °C, and the thermodynamic parameters were calculated. From a variety of experiments, it is suggested that Sm(III)L can bind to DNA mainly through intercalation but with some groove binding. The binding studied here will provide information helpful to the understanding of the mechanism of interaction of small molecules with nucleic acids and should be useful in the development of new drugs and DNA probes.

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