

Synthesis, structure and DNA interaction of cobalt(III) bis-complexes of 1,3-bis(2-pyridylimino)isoindoline and 1,4,7-triazacyclononane

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Abstract

The complex $[\text{CoL}_2](\text{ClO}_4) \cdot \text{MeOH}$ (**1**), where HL is the tridentate 3N ligand 1,3-bis(2-pyridylimino)isoindoline, has been isolated and its X-ray crystal structure successfully determined. It possesses a distorted octahedral structure in which both the ligands are coordinated meridionally to cobalt(III) via one deprotonated isoindoline (L^-) and two pyridine nitrogen atoms. Interestingly, the average dihedral angle between pyridine and isoindoline rings is 25.9° , indicating that the ligand is twisted upon coordination to cobalt(III). The interaction of the complex with calf-thymus DNA has been studied using various spectral methods and viscosity and electrochemical measurements. For comparison, the DNA interaction of $[\text{Co}(\text{tacn})_2]\text{Cl}_3$ (**2**), where tacn is facially coordinating 1,4,7-triazacyclononane, has been also studied. The ligand-based electronic spectral band of **1** and the $\text{N}(\sigma) \rightarrow \text{Co}(\text{III})$ charge transfer band of **2** exhibit moderate hypochromism with small or no blue shift on interaction with DNA. The intrinsic binding constants calculated reveal that the monopositive complex ion $[\text{CoL}_2]^+$ exhibits a DNA-binding affinity lower than the tripositive complex ion $[\text{Co}(\text{tacn})_2]^{3+}$. The steric clashes with DNA exterior caused by the second L^- ligand bound to cobalt(III), apart from the lower overall positive charge on the $[\text{CoL}_2]^+$ complex, dictates its DNA-binding mode to be surface binding rather than partial intercalative interaction expected of the extended aromatic chromophore of deprotonated isoindoline anion. An enhancement in relative viscosity of CT DNA on binding to **1** is consistent with its DNA surface binding. On the other hand, a slight decrease in viscosity of CT DNA was observed on binding to **2** revealing that the smaller cation leads to bending (kinking) and hence shortening of DNA chain length. The electrochemical studies indicate that the DNA-bound complexes are stabilised in the higher Co(III) rather than the lower Co(II) oxidation state, suggesting the importance of electrostatic forces of DNA interaction.

Keywords: Cobalt(III) complex; 1,3-Bis(2-pyridylimino)isoindoline; Tacn; Crystal structure; DNA binding

1. Introduction

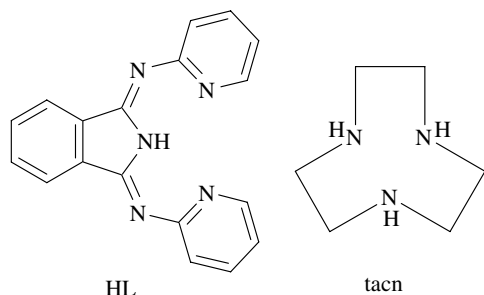
The interaction of transition metal complexes with DNA has been extensively studied in the past few years in order to develop novel non-radioactive probes of DNA structure [1,2], new therapeutic agents that cleave

DNA [3–5] and DNA-mediated electron transfer reactions [6]. These complexes offer an opportunity to explore the effects of the central metal atom, the ligands and the coordination geometries on the binding event. By varying the ligands, it is possible to modify the mode of interaction of the complex with nucleic acids and facilitate individual applications [7–9]. The application of octahedral complexes has permitted the targeting of specific DNA sites by matching the shape, symmetry and functionality of the metal complex to that of the

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DNA target. There has also been increased interest in the study of metallointercalators, which play an important role in nucleic acids chemistry for their diverse applications such as foot-printing, sequence-specific binding and reactions, new structural probes, and therapeutic agents [2,6,10–18]. 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.

The interaction of transition metal complexes of polypyridyl ligands with DNA has been extensively studied due to their cationic character and stability in aqueous solution. Efforts have been directed towards design of complexes containing modified 2,2'-bipyridine (bipy) or 1,10-phenanthroline (phen) ligands that bind DNA primarily via base-pair intercalation [19–25]. Though Co(III) polypyridyl complexes [26–29] possess interesting metallointercalation and DNA cleavage properties in addition to binding selectivity, they have not attracted as much attention as Cu(II) [30–33] or Ru(II) complexes [34–38]. In our laboratory, we have already studied the DNA-binding properties of [Co(5,6-dmp)₃]³⁺ (5,6-dmp = 5,6-dimethyl-1,10-phenanthroline) and the mixed ligand cobalt(III) complexes containing bipy, phen, 5,6-dmp and imp (imidazo[4,5-f][1,10]-phenanthroline). We have shown that these complexes display interesting and varying DNA-binding properties and a few of them have the potential to change the conformation of CT DNA [27]. This prompted us to investigate the DNA-binding properties of the cobalt(III) bis-complex of 1,3-bis(2-pyridylimino)isoindoline (HL, Scheme 1) with extended π -aromatic ring system. This tridentate ligand is interesting since the isoindoline ring, which is almost coplanar with the two pyridine rings, is expected to insert itself partially into DNA base pairs leading to intercalative interaction. So we have isolated the complex [CoL₂](ClO₄) · MeOH (**1**), determined its X-ray crystal structure and investigated its mode and extent of DNA binding. We have also isolated



Scheme 1.

the cobalt(III) bis-complex [Co(tacn)₂]Cl₃ (**2**), where tacn (1,4,7-triazacyclononane) is a facially coordinating ligand without a planar aromatic moiety unlike the above meridionally coordinating isoindoline. It would be interesting to look at the difference in DNA-binding behaviour of the complexes caused by the difference in ligand architecture of the complexes.

So we have investigated the interaction of the complexes **1** and **2** with calf-thymus DNA (CT DNA) using a variety of spectral methods and viscometric and cyclic voltammetric measurements.

2. Experimental

2.1. Materials

Disodium salt of calf-thymus DNA (Sigma), phthalonitrile, 2-aminopyridine, cobalt(II) perchlorate hexahydrate (Aldrich) and 1,4,7-triazacyclononane (Fluka) were used as received. The complex [Co(tacn)₂]Cl₃ (**2**) was prepared according to the literature procedure [39]. Common solvents, such as ethanol, methanol and 1-butanol were used after distillation. Milli Q water was used for the preparation of the buffers. All the experiments involving CT DNA were carried out in aqueous 50 mM NaCl/5 mM Tris HCl [tris(hydroxymethyl)aminomethane hydrochloride] buffer at pH 7.1.

2.1.1. Synthesis of 1,3-bis(2-pyridylimino)isoindoline (HL)

The ligand was prepared according to the literature procedure [40]. In a round-bottom flask, phthalonitrile (1.28 g, 10 mmol), 2-aminopyridine (1.97 g, 21 mmol) and calcium chloride (0.1 g, 1 mmol) in 1-butanol (20 mL) were heated at reflux for 48 h. On cooling the solution, a crystalline solid (HL) was isolated. The crude ligand was then recrystallised from ethanol–water solution to obtain X-ray quality crystals. Yield, 0.93 g (31%). The identity of the compound was established by ¹H NMR spectrum and X-ray crystallography. The crystal structure is in good agreement with that reported recently [41].

2.1.2. Syntheses of complexes

2.1.2.1. Synthesis of [CoL₂](ClO₄) · MeOH (**1**).

The complex was prepared according to the procedure reported in the literature with slight modifications [42]. To a solution of the ligand (0.29 g, 1 mmol) in methanol (15 mL) was added dropwise a solution of cobalt(II) perchlorate hexahydrate (0.18 g, 0.5 mmol) in methanol (2 mL) with constant stirring. The orange coloured precipitate was removed by filtration and the filtrate allowed to stand. The orange coloured solution turned

deep red on standing due to aerial oxidation and deep red block-like and plate-like crystals were formed in about a week. The crystals obtained were suitable for X-ray structure analysis. The different shaped crystals were proved to be polymorphs. The block-like crystals **1** crystallizing in the monoclinic space group C_2 , while the plate-like crystals **1a** crystallised in the monoclinic space group $P2_1/a$. Yield, 0.2 g (50%). Anal. Calc. for $C_{37}H_{28}ClCoN_{10}O_5$: C, 56.46; H, 3.59; N, 17.80. Found: C, 56.40; H, 3.51; N, 17.70%.

Warning: Perchlorate salts are dangerous and should be handled with care and in only small quantities.

2.2. Physical methods

Disodium salt of calf-thymus DNA (Sigma) was stored at 4 °C and was used as received. Solutions of DNA in 50 mM NaCl/5 mM Tris–HCl (pH 7.1) gave the ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.9, indicating that the DNA was sufficiently free of protein [43]. Concentrated stock solutions of DNA were prepared in 50 mM NaCl–5 mM Tris HCl (pH 7.1) and the concentration of DNA in nucleotide phosphate was determined by UV absorbance at 260 nm after 1:100 dilutions. The extinction coefficient ϵ_{260} was taken as $6600 \text{ M}^{-1} \text{ cm}^{-1}$ [44]. Stock solutions were stored at 4 °C and used after no more than 4 days.

Absorption spectra were recorded on a Varian Cary 300 UV–Vis spectrophotometer using cuvettes of 1 cm path length. Absorption spectral titrations were performed using DNA stock solutions pretreated with the cobalt complexes to take care of dilution effects. Emission intensity measurements were carried out using JASCO FP 6500 spectrofluorimeter. DNA (20 μM) was pretreated with ethidium bromide (EthBr) in the mixing ratio of $[\text{DNA}]/[\text{EthBr}] = 1$ for 30 min at 27 °C. The metal complexes (20 μM) were then added to this mixture and their effect on the emission intensity measured. For viscosity measurements, the viscometer was thermostated at 25 °C in a constant temperature bath. The concentration of DNA was 500 μM and the flow times were determined with a Schott Gerate AVS 310 automated viscometer.

All voltammetric experiments were performed in a single compartment cell with a three electrode configuration on a EG&G PAR 273 potentiostat–galvanostat equipped with an PIV computer. The working electrode was a glassy carbon disk (0.384 cm^2) and the reference electrode a saturated calomel electrode. A platinum plate was used as the counter electrode. The supporting electrolyte was 50 mM NaCl/5 mM Tris–HCl buffer at pH 7.1. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements; during measurements a stream of N_2 gas was passed over the solution. All experiments were carried out at

25.0 ± 0.2 °C maintained by a Haake D8-G circulating bath.

2.3. X-ray crystallography

2.3.1. Data collection and refinement

Suitable single crystals of the cobalt(III) complex $[\text{CoL}_2](\text{ClO}_4) \cdot \text{MeOH}$ (**1**), were obtained as dark red blocks. The intensity data were collected at 153 K on a Stoe Mark II-Image Plate Diffraction System [45] equipped with a two-circle goniometer and using Mo $K\alpha$ graphite-monochromated radiation. Image plate distance 100 mm, ω rotation scans 0–180° at ϕ 0°, and 0–85° at ϕ 90°, step $\Delta\omega = 1.2^\circ$, 2θ range 2.29–59.53°, $d_{\min} - d_{\max} = 17.779 - 0.716 \text{ \AA}$. The structure was solved by Direct methods using the programme SHELXS-97 [46]. The refinement and all further calculations were carried out using SHELXL-97 [47]. The H-atoms were included in calculated positions and treated as riding atoms using SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2 . An empirical absorption correction was applied using the DELREFABS routine in PLATON [48]; transmission factors: $T_{\min}/T_{\max} = 0.220/0.685$. It was found that the plate-like crystals were a polymorph, **1a**, of complex **1**. The data for **1a** were collected as described above and the structure solved and refined in a similar manner. The crystal data and refinement details for both the polymorphs are collected in Table 1.

Table 1
Crystal data and structure refinement details for $[\text{CoL}_2]\text{ClO}_4 \cdot \text{MeOH}$ (**1**) and the polymorph **1a**

	1	1a
Empirical formula	$C_{37}H_{28}ClCoN_{10}O_5$	$C_{37}H_{28}ClCoN_{10}O_5$
Formula weight	787.07	787.07
Crystal system	Monoclinic	Monoclinic
Space group	$C2$	$P2_1/c$
a (Å)	8.5954(5)	8.7959(11)
b (Å)	17.8663(14)	11.8166(11)
c (Å)	21.6317(13)	33.107(5)
α (°)	90	90
β (°)	99.172(5)	90.801(11)
γ (°)	90	90
V (Å ³)	3279.5(4)	3440.7(7)
Z	4	4
Mo $K\alpha$, λ (Å)	0.71073	0.71073
D_{calc} (mg m^{-3})	1.594	1.519
Goodness of fit on F^2	1.046	0.805
$F(000)$	1616	1616
Residuals [$I > \sigma_2(I)$]		
R^a	0.0445	0.0510
wR_2^b	0.1040	0.0924

$$^a R = \sum(|F_o| - |F_c|) / \sum|F_o|$$

$$^b R = [\sum(w(F_o^2 - F_c^2)^2) / \sum(wF_o^4)]^{1/2}$$

3. Results and discussion

3.1. Description of the structure of $[\text{CoL}_2](\text{ClO}_4) \cdot \text{MeOH}$ (**1**)

The molecular structure of complex **1** is depicted in Fig. 1, along with atom numbering scheme. Selected bond lengths and bond angles are given in Table 2. The compound crystallised in the non-centrosymmetric space group C_2 as a racemic or inversion twin. The asymmetric unit consists of two halves of two independent and discrete $[\text{CoL}_2]^+$ cations (A and B, both possessing C_2 symmetry), an uncoordinated perchlorate anion and one disordered molecule of methanol. The complex cations consist of two tridentate anionic ligands (L^-), each coordinated meridionally to cobalt(III) through the isoindoline and two pyridine nitrogen atoms. The coordination geometry of the two cations can be described as distorted octahedral with all the six nitrogen atoms of both the ligands occupying the apices. In cation A, the Co–N3 bond distance (1.889 Å) is significantly shorter than the Cu–N_{py} bond distances (1.983 and 1.986 Å) indicating that the deprotonated N3 atom of isoindoline is strongly coordinated to cobalt(III). The average Co–N_{py} bond length of 1.984 Å is in the range found for the related $[\text{CoL}(\text{OBz})(\text{OO}-t\text{-Bu})]$ complexes [49,50]. The N5–Co–N1 (177.2°) and N3–Co–N3 (176.3°) bond angles are very close to the ideal angle of 180° indicating that the planar ligand avoids severe distortions around Co(III). The average dihedral angle between pyridine and indoline rings is 25.9° indicating that the ligand is twisted upon coordination to cobalt(III).

The $[\text{CoL}_2](\text{ClO}_4) \cdot \text{MeOH}$ crystal (**1**) used for X-ray diffraction was selected randomly amongst the block-like crystals. On examining a plate-like crystal, it was found that the complex had crystallised in another space

Table 2
Selected bond lengths (Å) and bond angles (°) for $[\text{CoL}_2]\text{ClO}_4$ (**1**) and the polymorph **1a**

1 (cation A)		1a	
Co(1)–N(3)	1.889(3)	Co(1)–N(8)	1.885(4)
		Co(1)–N(3)	1.887(3)
Co(1)–N(5)	1.983(3)	Co(1)–N(5)	1.971(3)
		Co(1)–N(6)	1.972(4)
Co(1)–N(1)	1.986(3)	Co(1)–N(1)	1.982(3)
		Co(1)–N(10)	1.986(4)
N(3)–Co(1)–N(3)#1	176.3(2)	N(8)–Co(1)–N(3)	176.28(16)
N(3)–Co(1)–N(5)	88.26(12)	N(8)–Co(1)–N(5)	88.86(15)
N(3)#1–Co(1)–N(5)	89.19(12)	N(3)–Co(1)–N(5)	88.45(15)
		N(8)–Co(1)–N(6)	88.31(16)
		N(3)–Co(1)–N(6)	89.15(15)
N(5)–Co(1)–N(5)#1	91.58(17)	N(5)–Co(1)–N(6)	90.45(14)
N(3)–Co(1)–N(1)#1	92.59(13)	N(8)–Co(1)–N(1)	93.25(15)
N(3)–Co(1)–N(1)	90.06(12)	N(3)–Co(1)–N(1)	89.53(15)
N(5)–Co(1)–N(1)#1	177.20(13)	N(5)–Co(1)–N(1)	177.03(14)
N(5)–Co(1)–N(1)#1	90.64(11)	N(6)–Co(1)–N(1)	91.70(15)
		N(8)–Co(1)–N(10)	90.08(16)
		N(3)–Co(1)–N(10)	92.54(15)
		N(5)–Co(1)–N(10)	91.15(14)
		N(6)–Co(1)–N(10)	177.70(15)
N(1)#1–Co(1)–N(1)	87.19(18)	N(1)–Co(1)–N(10)	86.75(14)

Symmetry operation: (#1) $-x, y, -z + 1$.

group, that is, a polymorph of complex **1** was obtained. The molecular structure of this polymorph **1a**, also a methanol solvate, is depicted in Fig. 2 and the bond lengths and bond angles were found to be very similar to those for **1** (Table 2).

3.2. Interaction of cobalt(III) complexes with CT DNA

3.2.1. Electronic absorption spectral studies

The electronic absorption spectrum of complex **1** in 5 mM Tris–HCl/50 mM NaCl buffer exhibits bands around 446, 366 and 253 nm (Fig. 3). Since the bands

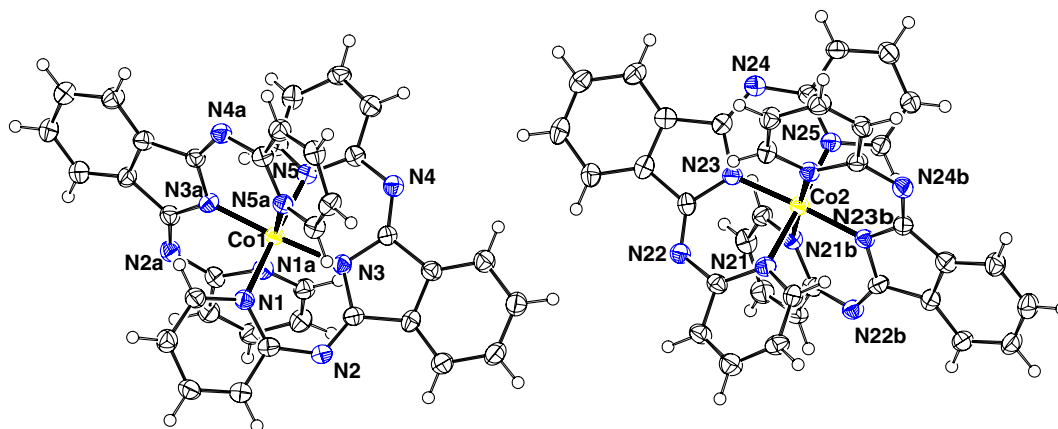


Fig. 1. ORTEP drawing of the two independent $[\text{CoL}_2]^+$ cations of **1**, both possessing C_2 symmetry, showing the atom numbering scheme and thermal ellipsoids (50% probability level). The hydrogen atoms have been omitted for clarity. [Symmetry operations: (a) $-x, y, -z + 1$; (b) $-x + 1, y, -z$.]

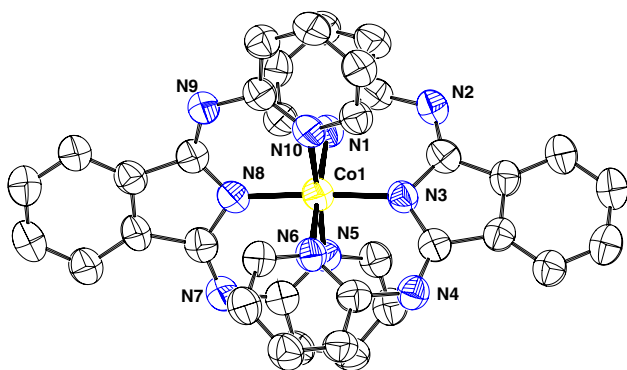


Fig. 2. ORTEP drawing of the $[\text{CoL}_2]^+$ cation of **1a**, showing the atom numbering scheme and thermal ellipsoids (50% probability level). The hydrogen atoms have been omitted for clarity.

at 446 and 366 nm are not very intense, the intense band around 253 nm arising from ligand-based transition was used for the absorption spectral titration with CT DNA. On the incremental addition of DNA, the band shows hypochromism ($\Delta\epsilon$, 15% at $R = [\text{DNA}]/[\text{Co}] = 15$) accompanied by a small blue shift of 3 nm (Table 3). On the other hand, the cobalt(III) complexes $[\text{Co}(\text{phen})_2(\text{imp})]^{3+}$ ($\Delta\epsilon$, 27% at $R = [\text{DNA}]/[\text{Co}] = 15$) [22],

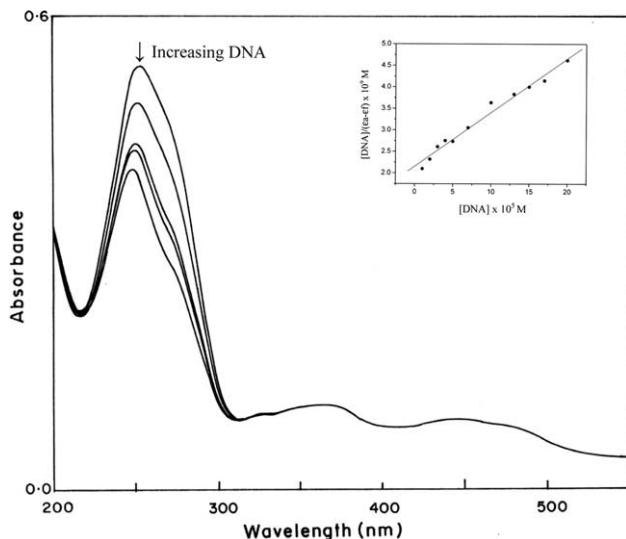


Fig. 3. Absorption spectra of $[\text{CoL}_2]^+$ **1** in 5 mM Tris-HCl/50 mM NaCl (pH 7.1) in the presence of increasing amounts of CT DNA ($R = 0-25$). Inset: Plot of $[\text{DNA}]/(\epsilon_A - \epsilon_F) \times 10^9 \text{ M}$ vs. $[\text{DNA}] \times 10^5 \text{ M}$.

$[\text{Co}(\text{phen})_2(\text{PIP})]^{3+}$ (PIP = 2-phenylimidazo[4,5-f][1,10]-phenanthroline, $\Delta\epsilon$, 28% at $R = 15$) [22] and $[\text{Co}(\text{phen})_2(\text{HPIP})]^{3+}$ (HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-f][1,10]-phenanthroline, $\Delta\epsilon$, 47% at $R = 15$) [29] exhibit higher hypochromism with a red shift. They have been suggested to bind to DNA strongly through intercalation of the extended heterocyclic aromatic chromophores of the ligands in between the DNA base pairs. So it is obvious that complex **1**, which exhibits hypochromism lower than these metallointercalators, is involved in DNA surface binding rather than intercalative interaction. The electronic absorption spectrum of complex **2** exhibits an intense charge transfer band at 225 nm, which is assignable to the $\text{N}(\sigma) \rightarrow \text{Co}(\text{III})$ charge transfer transition. The band exhibits a lower hypochromism ($\Delta\epsilon$, 10% at $R = [\text{DNA}]/[\text{Co}] = 40$) without any red shift on the incremental addition of CT DNA (Fig. 4) indicating that complex **2** also binds on the DNA surface.

In order to quantitatively compare the affinities of the complexes to bind to CT DNA, the intrinsic binding constant K_b was determined by using the equation [51],

$$[\text{DNA}]/(\epsilon_A - \epsilon_F) = [\text{DNA}]/(\epsilon_0 - \epsilon_F) + 1/K_b(\epsilon_0 - \epsilon_F),$$

where $[\text{DNA}]$ is the concentration of DNA in base pairs, ϵ_A corresponds to the extinction coefficient observed ($A_{\text{obsd}}/[\text{Co}]$) for the MLCT band, ϵ_F corresponds to the extinction coefficient of the free cobalt complex, ϵ_0 is the extinction coefficient of the complex when fully bound to DNA and K_b is the intrinsic binding constant. The ratio of slope to intercept in the plot of $[\text{DNA}]/(\epsilon_A - \epsilon_F)$ vs. $[\text{DNA}]$ gives the values of K_b as $6.0 \pm 0.1 \times 10^3 \text{ M}^{-1}$ (**1**) and $8.8 \pm 0.1 \times 10^3 \text{ M}^{-1}$ (**2**), respectively (Table 3). The DNA-binding affinity of **1** is lower than those of the above metallointercalators (K_b , $[\text{Co}(\text{phen})_2(\text{imp})]^{3+}$, 1.3×10^5 ; $[\text{Co}(\text{phen})_2(\text{PIP})]^{3+}$, 2.2×10^5 ; $[\text{Co}(\text{phen})_2(\text{HPIP})]^{3+}$, $4.1 \times 10^5 \text{ M}^{-1}$) and $[\text{Co}(\text{bzimpy})_2]$ [bzimpy = 2,6-bis(benzimidazol-2-yl)pyridine, $1.6 \times 10^5 \text{ M}^{-1}$] [52]. The weaker DNA binding of the complex may arise from the decreased positive charge on it and the relatively small surface area of the aromatic chromophore involved in partial intercalation in between the DNA base pairs. Further, in addition to the twisting of the two pyridine rings of the L^- ligand in the complex with respect to the isoindoline ring (cf. above), the steric clash involving the second L^- ligand and the DNA polymer would hinder the partial insertion of the isoindoline ring in between the DNA

Table 3
Change in ligand-based spectral features^a of the cobalt(III) complexes on interaction with CT DNA

Complex	Change in absorptivity	$\Delta\epsilon$ (%) ($\text{M}^{-1} \text{ cm}^{-1}$)	Blue shift		
			λ_{max} (nm)	$\Delta\lambda_{\text{max}}$ (nm)	K_b (M^{-1})
$[\text{CoL}_2](\text{ClO}_4)$ (1)	Hypochromism	18	253	3	$6.0 \pm 0.1 \times 10^3$
$[\text{Co}_2]\text{Cl}_3$ (2)	Hypochromism	10	225	0	$8.8 \pm 0.1 \times 10^3$

^a Measurements were made at $R = 25$, where $R = [\text{DNA}]/[\text{Co}]$ in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.1.

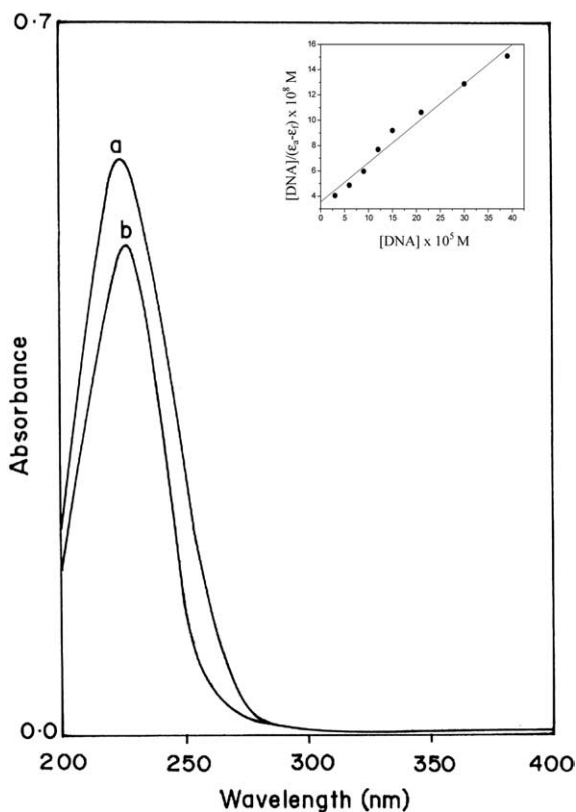


Fig. 4. Absorption spectra of $[\text{Co}(\text{tacn})_2]^{3+}$ (30 μM) in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.1 in the absence (a) and in the presence (b) of CT DNA ($R = 25$). Inset: Plot of $[\text{DNA}]/(\epsilon_A - \epsilon_F) \times 10^8 \text{ M}$ vs. $[\text{DNA}] \times 10^5 \text{ M}$.

base pairs. This type of steric clash has been also suggested for the binding of $[\text{Cu}(\text{phen})_2]^+$ and $[\text{Ru}(\text{phen})_3]^{2+}$ complexes, where intercalation of a phen ligand is inhibited by steric interactions involving the other phen ligands with DNA surface. Thus we propose that the bulky complex **1** binds to DNA most probably through surface binding. On the other hand, the binding constant of complex **2** is slightly higher than that of **1** in spite of the fact that it does not possess a planar aromatic moiety capable of intercalating in between the base pairs of DNA. Obviously, complex **2** with a higher overall charge than **1** would lead to stronger DNA interaction with the polyanionic DNA. It may be noted that no hydrogen bonding interactions involving the $-\text{NH}-$ group of tacn and the DNA bases are possible as they will be hindered by ethylene bridges of tacn.

3.2.2. Viscometric studies

To further explore the binding modes of the complexes **1** and **2**, viscosity measurements were carried out on CT DNA bound to varying concentrations of the complexes. The plot of the relative specific viscosities (η/η_0 , where η and η_0 are the specific viscosities of DNA in the presence and absence of the complex, respectively) vs. $1/R (= [\text{Co}]/[\text{DNA}]) = 0-1.0$ is shown in Fig. 5. Inter-

estingly, for **1** there is an increase in specific viscosity of DNA, which is not as pronounced as those observed for the classical intercalator ethidium bromide [52] and the metallointercalators like $[\text{Co}(\text{phen})_2(\text{PIP})]^{3+}$ [22], $[\text{Co}(\text{phen})_2(\text{HPIP})]^{3+}$ [29] and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ [29]. Interestingly, this observation is in contrast to the decrease in DNA viscosity on binding to $[\text{Co}(\text{bzimpy})_2]$ [52]. As intercalation causes a significant increase in viscosity of DNA solutions due to lengthening of the DNA helix as base pairs are separated to accommodate the aromatic chromophore of the bound molecule, it is tempting to ascribe the observed increase in viscosity to intercalative interaction of the complex. However, as illustrated above, the effective intercalation of the isoindoline ring of **1** is discouraged by several factors. So it is obvious that the complex prefers to engage in DNA surface binding with its large size effecting an increase in DNA viscosity, rather than in intercalative DNA interaction. In contrast to **1**, the relative viscosity of DNA slightly decreases on binding to **2**. It is plausible that the smaller size of the cation **2** leads to bending (kinking) and hence shortening of DNA helix to enable stronger binding [53]. In fact, at higher concentrations of the complex the DNA gets precipitated. Similar lowering of viscosity and precipitation at higher concentrations has been reported previously [54].

3.2.3. Emission studies

Since the complexes **1** and **2** are non-emissive both in the presence and absence of CT DNA, competitive ethidium bromide (EthBr) binding studies were undertaken to gain support for the above spectral and viscometric results. They involve the addition of the present

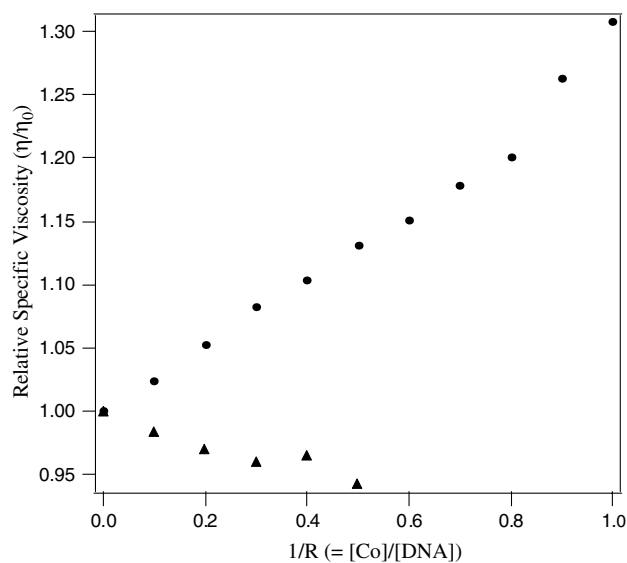


Fig. 5. The effect of $[\text{CoL}_2]^+$ (●) and $[\text{Co}(\text{tacn})_2]^{3+}$ (▲) on the relative specific viscosity of CT DNA.

complexes to DNA pretreated with EthBr ($[DNA]/[EthBr] = 1$) and then measurement of emission intensities of DNA-bound EthBr. The observed enhancement in emission intensity of EthBr in the presence of DNA (Fig. 6) is expected of its strong stacking interaction (intercalation) between the adjacent DNA base pairs [55]. Interestingly, the emission intensity of DNA-bound EthBr is quenched completely on adding complex **1** (Fig. 6A). As complex **1** binds to DNA primarily via surface binding, it cannot displace the strongly DNA-bound EthBr. So the observed quenching is obviously due to the facile intramolecular photo-induced electron transfer [56] to **1** bound to DNA ($E_{1/2}$, 0.051 V, see below). In contrast to **1**, addition of complex **2** quenches the DNA-induced EthBr emission intensity but only to a smaller extent (Fig. 6B). This is expected as the reduction of the DNA-bound complex **2** ($E_{1/2}$, -0.645 V, see below) is more difficult than **1**. Also, it is possible that the low energy π^* orbitals of the coordinated ligand in **1** facilitates the photo-induced electron transfer.

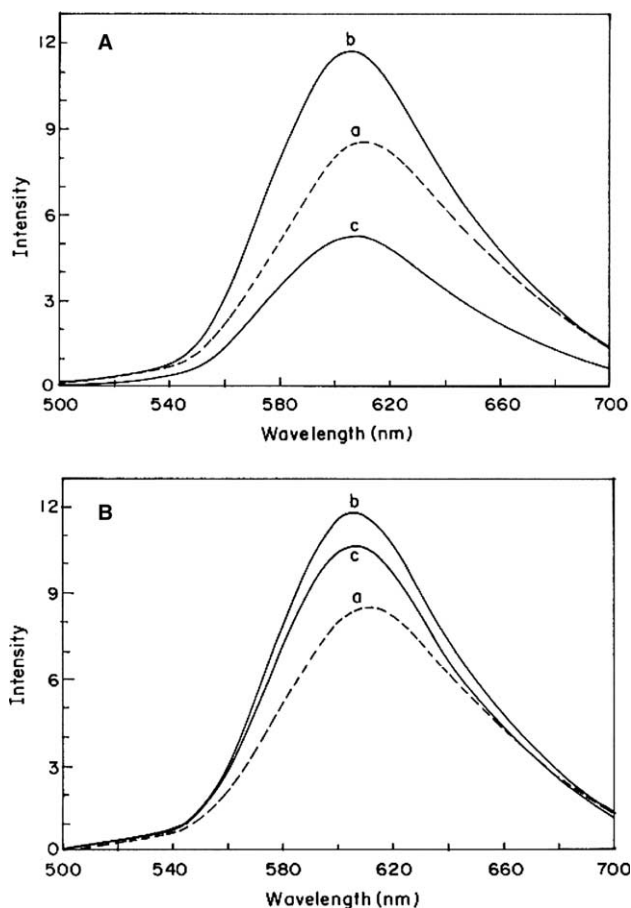


Fig. 6. The emission spectra of ethidium bromide in the absence (a) and presence of DNA (b) and DNA-EthBr + $[CoL_2]^+$ (A) and DNA-EthBr + $[Co(tacn)_2]^{3+}$ (B) (c).

3.2.4. Electrochemical studies

Voltammetric methods have been used to probe the interaction (electrostatic or intercalative) of metal complexes with calf-thymus DNA [57]. We have successfully employed [58] these techniques to discriminate the enantioselective interaction of rac - $[Ru(phen)_3]^{2+}$ with calf-thymus DNA. The application of these methods to the study of interaction of redox-active metal complexes with DNA provides a useful complement to the above spectral and viscometric studies. The cyclic voltammetric (CV) responses of complex **1** in the absence and presence of DNA in 5 mM Tris-HCl/50 mM NaCl buffer are ill-defined. However, a differential pulse voltammetric (DPV) response is observed ($E_{1/2}$, 0.085 V). On the addition of CT DNA ($R = 5$) the peak current is decreased significantly and the redox potential of Co(III)/Co(II) couple is shifted to more negative values ($E_{1/2}$, 0.051 V). In contrast to **1**, complex **2** shows a diffusion-controlled (D , $1.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) [59] and quasireversible CV response (ΔE_p , 100 mV) typical of Co(III)/Co(II) redox couple in the absence of DNA (Table 4). On adding CT DNA the peak currents of DPV and CV responses of **2** are significantly decreased, the Co(III)/Co(II) redox couple becomes less reversible (ΔE_p , 156 mV) and the $E_{1/2}$ value (-0.617 V) is shifted to more negative potentials (-0.645 V). The apparent reduction in peak currents of **1** and **2** on the addition of DNA is attributed to the diffusion of the complexes bound to the large, slowly diffusing DNA molecule (Fig. 7). Further, the shifts in $E_{1/2}$ (DPV) to more negative values indicate that the binding of the complexes in the higher Co(III) oxidation state is favoured supporting the binding of **1** and **2** on the DNA surface with significant contributions from electrostatic forces of interaction. The isoindoline and tacn ligands are expected to reside primarily at the outer, hydrophilic coat of the DNA helix with no significant contribution from the intercalative binding interaction. These results are in accordance with those observed for $[Co(bipy)_3]^{3+}$ [57], $[Co(bipy)_2(phen)]^{3+}$ and $[Co(phen)_2(bipy)]^{3+}$ [27] bound to CT DNA through stronger electrostatic interaction involving the higher Co(III) oxidation state. It is inter-

Table 4
Electrochemical behaviour^a for the cobalt(III) complexes on interaction with CT DNA at $R (= [DNA]/[Co]) = 5$

Complex	R	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (CV, V)	$E_{1/2}$ (DPV ^b , V)
$[CoL_2]ClO_4$ 1	0	—	—	—	0.085
	5	—	—	—	0.051
$[Co(tacn)_2]Cl_3$ 2	0	-0.666	-0.566	-0.616	-0.617
	5	-0.718	-0.562	-0.640	-0.645

^a Measured vs. sat. Calomel electrode, using glassy carbon electrode; scan rate, 50 mV s^{-1} ; supporting electrolyte 5 mM Tris-HCl/50 mM NaCl; Complex concentration: $5 \times 10^{-4} \text{ M}$.

^b Differential pulse voltammetry (DPV), scan rate 1 mV s^{-1} , pulse height 50 mV.

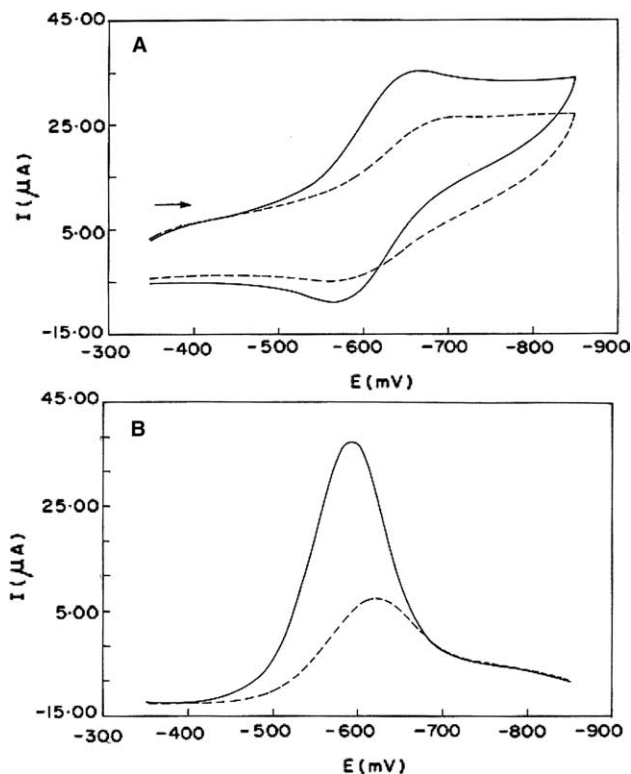


Fig. 7. Cyclic (A) and differential pulse (B) voltammograms of $[\text{Co}(\text{tacn})_2]^{3+}$ (0.5 mM) in the absence (—) and in the presence (---) of CT DNA at $R = 5$ in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.1.

esting to note that the lower Co(II) oxidation state favours intercalation of the phen and imp ligands, respectively, of $[\text{Co}(\text{phen})_3]^{3+}$ [57] and $[\text{Co}(\text{phen})_2(\text{imp})]^{3+}$ on interaction with CT DNA [27]. Thus the electrochemical results are in agreement with the above spectral results reinforcing the proposal that the complexes bind to DNA surface involving electrostatic forces of interaction.

4. Conclusions

The X-ray crystal structure of $[\text{CoL}_2](\text{ClO}_4)$, where HL is the tridentate 3N ligand 1,3-bis(2-pyridylimino)isoindoline, has been successfully determined. The Co(III) complex possesses a distorted octahedral geometry in which the two deprotonated isoindoline anions are coordinated meridionally to cobalt(III) via one deprotonated isoindoline and two pyridine nitrogen atoms. The average dihedral angle between pyridine and isoindoline rings is 25.9° , suggesting that the two pyridine rings are not significantly twisted from planarity. The structure reveals that the effective intercalation of the isoindoline ring would be prevented by the steric clashes from the two pyridine rings of the other isoindoline ring with the DNA surface. In fact, the spectral,

visometric and electrochemical studies of the interaction of $[\text{CoL}_2]^+$ and $[\text{Co}(\text{tacn})_2]^{3+}$ with DNA lead us to conclude that the complexes bind on the DNA surface with no possibility for partial intercalation interaction. The electrochemical results reveal that the complexes bind to DNA more strongly in the higher Co(III) than in Co(II) oxidation state, which illustrates the importance of electrostatic forces of DNA interaction of the complexes.

5. Supporting information

Crystallographic data (excluding structure factors) for the structures **1** and **1a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 256231 (**1**), CCDC 256232 (**1a**). Copies of the data can be obtained free of charge on application to CCDS, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44-1223/336-033; deposit@ccds.cam.ac.uk].

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