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DNA-binding and Cleavage studies of Imidazole Cobalt (III) Ethylenediamine Complexes

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ABSTRACT

Four imidazole Cobalt(III) complexes of the type $[Co(en)_2(L)_2]^{3+}$ *(en = ethane 1, 2, diamine, L = imidazole)* ***(1),*** $[Co(en)_2(L)_2]^{3+}$ *(L = methylimidazole)* ***(2),*** $[Co(en)_2(L)_2]^{3+}$*(L = 1, 2, dimethylimidazole)* ***(3),*** $[Co(en)_2(L)_2]^{3+}$ *(L = ethylimidazole)* ***(4)*** *have been isolated and characterized by Elemental analysis, IR, and* ^1H,^{13}C *NMR spectral methods. The binding of the complexes with calf thymus DNA has been investigated by absorption, emission spectroscopy, viscosity measurements, DNA melting, and DNA photocleavage. The spectroscopic studies together with viscosity measurements and DNA melting studies support that all the complexes bind to CT DNA(=calf thymus DNA) by groove mode. Complexes 3 & 4 bind more avidly to CT DNA than 2 & 1. Noticeably, these complexes were found to promote cleavage of plasmid DNA pBR322 with incubation of complexes.*

Keywords: Co (III) complexes, ethylenediamine, imidazoles, DNA-binding, photocleavage.

INTRODUCTION

There is substantial and continuing interest in redox and spectroscopically active metal complexes that bind and interact with DNA [1, 2]. The interaction of polypyridyl ruthenium complexes with DNA has attracted considerable attention in recent decades, in the hope of developing novel probes of DNA structure or new therapeutic agents [3, 4]. It is at the interface between medicine and inorganic chemistry, and includes metal-based drugs, metal sequestering

or mobilizing agents, metal-containing diagnostic aids, and the medicinal recruitment of endogenous metal ions. Medicinal application of metals can be traced back almost 5000 years [5]. The development of modern medicinal inorganic chemistry, stimulated by the discovery of cisplatin, has been facilitated by the inorganic chemist's extensive knowledge of the coordination and redox properties of metal ions. Metal centers, being positively charged, are favored to bind to negatively charged biomolecules; In particular, Co(III) polypyridyl complexes, were found to possess some excellent DNA binding and DNA-photocleavage properties under light irradiation, and thus they have received attentions of many chemists. Recently, many Co(III) polypyridyl complexes have been synthesized and their DNA-binding and DNA-photocleavage properties were detailedly investigated in experiment [6 & 7]. The extensive studies on substitution reactions of amine complexes of Cobalt(III) have mainly dealt with acid hydrolysis, base hydrolysis and substitution by anionic ligands in different solvents. The consensus on the mechanism is that the reactions involve a dissociative activation process [8-11]. Most recently our group has been synthesized some ruthenium(II) and cobalt(III) ethylenediamine mixed-polypyridyl complexes, which bind to DNA through an intercalative and groove mode and promote cleavage of plasmid pBR 322 DNA [12-16]. In this paper, we are reporting the synthesis and characterization of the complexes 1, 2, 3 and 4 in which 4 possesses a greater binding affinity and their DNA-binding properties are revealed by electronic absorption, emission spectra, viscosity measurement and DNA melting curve. These studies are necessary for further comprehension of binding of transition metal complexes to DNA.

EXPERIMENTAL SECTION

All materials were purchased and used without further purification, imidazole, methyl imidazole, 1,2,Dimethyl imidazole, ethylimidazole, ethylenediamine, CT DNA were purchased from *Aldrich*. All the experiments involving the interaction of the complexes with DNA were carried out in tris Buffer made using double distilled water (5 mM tris-HCl, 5 mM NaCl, p^H = 7.2). A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.90 indicating that the DNA was sufficiently free of protein [17]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [18].

Synthesis of $[Co(en)_2(imd)_2]Br_3$

All these complexes were prepared by literature methods [14, 15, 19, 20, 21] as follows**,** a mixture of *cis* $[Co(en)_2Cl_2]Cl$ (1.43g) and imidazole (1g) was dissolved in ethanol (6ml) and added sodium bromide (3.0 g) in water (5 ml) the mixture was heated on a water bath until a dark yellow solution was formed. It was then cooled in ice the thick crystalline precipitate of $[Co(en)_2(imd)_2]^{3+}$ was collected and recrystallised from water (30 ml).The yield by this method was about 65%. Elemental analysis for $CoC_{10}N_8H_{24}Br_3$, H 5.74 C 28.49 N 26.58 Found: H 5.67 C 28.66 N 26.75, IR: 1441 (C=C), 1570 (C=N), 576 (Co–N (en)), 461.8 (Co–N (L)). ^{1}H-NMR (D_2O) 3.1, (dd, $2CH_2$ $(en)_2$, 2.05(m,$2CH_2(en)_2$, 6.86(C_4-H), 6.76(C_5-H)7.40(C_2-H). ^{13}C [^{1}H] NMR (D_2O) 140, 130.5, 120.2, 45.61, 44.8.

Synthesis of $[Co(en)_2(1\text{-}Me\text{-}imd)_2]Br_3$

Prepared as above with methylimidazole (1g) Yield: 50%. Elemental analysis for $CoC_{12}N_8H_{28}Br_3$ anal. calcd, (%): C 32.05; H 6.28; N 24.92. Found, (%): C 32.01; H 6.18; N

24.71. IR: 1451 (C=C), 1572 (C=N), 568(Co–N (en)), 568.2(Co–N (L)). ^{1}H-NMR (D_2O) 3.25,(dd, $2CH_2$ $(en)_2$, 2.15(m,$2CH_2(en)_2$, 7.15(C_4-H), 6.69(C_5-H), 7.30(C_2-H). ^{13}C [^{1}H]NMR (D_2O) 141.59, 128.5, 125.18, 45.61, 44.12, 35.52.

Synthesis of $[Co(en)_2(1,2,dme\text{-}imd)_2]Br_3$

Prepared as above with 1, 2 dimethylimidazole (1g).Yield: 55%. Elemental analysis for $CoC_{14}N_8H_{34}Cl_3$, H 7.14 C 35.05 N 23.36 Found: H 7.1 C 35.0 N 23.11 IR: 1458 (C=C), 1578 (C=N), 578(Co–N (en)), 570(Co–N (L)). ^{1}H-NMR (D_2O), 3.35,(dd, $2CH_2$ $(en)_2$, 2.55(m,$2CH_2(en)_2$, 7.00(C_4-H), 6.80(C_5-H), 7.48(C_2-H).

Synthesis of $[Co(en)_2(2\text{-}Et\text{-}imd)_2]Br_3$

Prepared as above with ethylimidazole (1g).Yield: 45%. Elemental analysis for $CoC_{14}N_8H_{34}Br_3$, H 7.14 C 35.05 N 23.36 Found: H 7.1 C 35.0 N 23.12 IR: 1461 (C=C), 1580 (C=N), 578(Co–N (en)), 494(Co–N (L)). ^{1}H-NMR (D_2O), 3.20,(dd, $2CH_2$ $en)_2$, 2.35(m,$2CH_2(en)_2$, 7.10(C_4-H), 6.78(C_5-H), 2.83 q (CH_2) 1.17 t (CH_3).

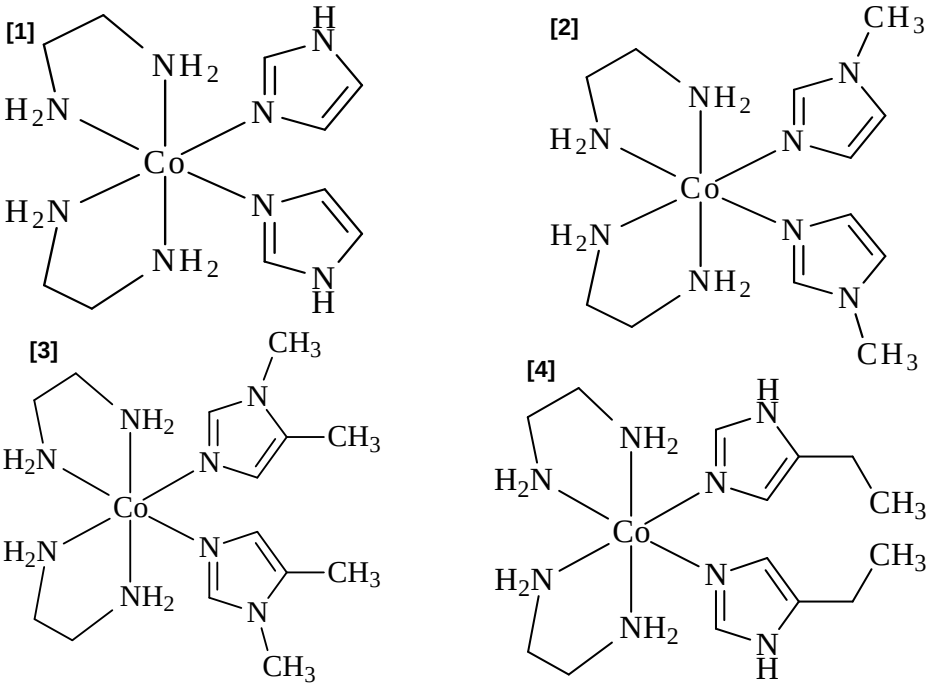

Fig (1) Structure of complexes [1] $[Co(en)_2(imd)_2]$, [2] $[Co(en)_2(1\text{-}me\text{-}imd)_2]$, [3] $[Co(en)_2(1, 2,dme\ imd)_2]$, [4] $[Co(en)_2(2\text{-}et\text{-}imd)_2]$

Physical Measurements.

UV-VIS spectra were recorded on *Elico Bio*-spectrophotometer model *BL198*, emission spectra were recorded on a *Shimadzu Rf-2000* luminescence spectrometer at room temperature. IR spectra were recorded, in KBr phase on *Perkin-Elmer FTIR-1605* spectrophotometer; ^{1}H-NMR spectra were measured on a *Varian XL-300* MHz spectrometer with D_2O as a solvent at room temperature and tetramethylsilane (TMS) as the internal standard, Microanalyses (C, H, N) were carried out on a *Perkin-Elmer* 240 elemental analyzer.

Absorption spectroscopic titrations were carried out at room temperature to determine the binding affinity between DNA and complex. Initially, 3.2 ml of blank solutions containing tris buffer and the cobalt complex sample (25 μM) were placed in the reference and sample cuvettes (1 cm path length), respectively, and then first spectrum was recorded in the range of 200-800 nm. During the titration, aliquot (1-10 μL) of buffered DNA solution (concentration of ~5 to 10mM in base pairs) was added to each cuvette to eliminate the absorbance of DNA itself, and the solutions were mixed for ~5 min then absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra indicating binding saturation had been achieved. The changes in the metal complex concentration due to dilution at the end of each titration were negligible. Absorption spectroscopic titrations were repeated three times at least.

Emission measurements were carried out by using a *HitachiF 4500* spectrophometer. Tris-buffer was used as a blank to make preliminary adjustments. The excitation wavelength was fixed and the emission range was adjusted before measurements. All measurements were made at 25°C in a thermostated cuvette holder with 5 nm entrance slit and 5 nm exit slit. For emission spectral titrations1.0×10^{-5} M concentration of cobalt solutions were used and CT DNA was added in steps till $R = 10$. The emission enhancement factors were measured by comparing the intensities at the emission spectral maxima in the absence and presence of DNA.

Viscosity experiments were carried out using an ostwald viscometer maintained at a constant temperature at 30.0 ± 0.1° in a thermostatic water-bath. Calf thymus DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility [22]. Data were presented as $[\eta/\eta_0]^{1/3}$ *vs.* the concentration of Co(III) complex. Viscosity values were calculated from the observed flow time of DNA and DNA-metal complexes containing solution (t >100 s) corrected for the flow time of buffer alone (t_o).

DNA melting experiments were carried out by monitoring the absorption (260 nm) of CT DNA (160 μM, per nucleotide), using a *Schimadzu* model UV-160A spectrophotometer coupled with a temperature controller circulating bath while monitoring the absorbance at various temperatures in the absence and in the presence of a complex.

For the gel electrophoresis experiments, super coiled pBR322 DNA (10 μM) was treated with Co(III) complexes in tris buffer, $p^H = 7.2$ and the solution were incubated for 1 h. The samples were analyzed by electrophoresis for 2.5h at 40 V on a 0.8% agarose gel in Tris-acetic acid-EDTA buffer, $p^H = 8.5$. The gel was stained with 1μg/ml ethidium bromide and then photographed under UV light.

Spectroscopic Characterization

All the complexes exhibits a band at 1458 cm^{-1} and 1578 – 1590 cm^{-1} corresponding to C=C and C=N stretching frequency. A band at around 589 cm^{-1} and 590 cm^{-1} corresponding to Co–N(en) and Co–N of NH_2(en). In UV-Vis spectra the bands between 400-500 nm corresponds to MLCT charge transfer bands. In the ^{1}H-NMR spectra of the Co(III) complex the peaks due to various protons of imidazoles shifted downfield compared to the free ligand suggesting complexation.

As expected the signal for imidazoles appeared in the range between 6.5 to 9.2, and CH_2 of ethylenediamine gave peaks at 2.2 (br., 4 H, CH_2(en)), and 3.1 (br, 4 H, CH_2(en)).

RESULTS AND DISCUSSION

Absorption studies

The electronic spectra of the Cobalt complexes titrated with CT-DNA (at constant concentration of complexes, [Co] = 25 μM) are shown in Fig. 1. With increasing DNA concentration, the hypochromism increases. In order to quantitatively compare the binding strength of the four complexes, the intrinsic binding constants K_b of the four complexes with CT-DNA were determined according to the following equation [23] through a plot of [DNA]/(ε_a-ε_f) versus [DNA].

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/(K\,(\varepsilon_b-\varepsilon_f))$$

Where ε_a is the extinction coefficient observed for the MLCT absorption band at a given DNA concentration, ε_f is the extinction coefficient of the complex in the absence of DNA, ε_b is the extinction coefficient of the complex fully bound to DNA. In plots [DNA]/(ε_a-ε_f) vs [DNA], K_b is given by the ratio of slope to intercept. Intrinsic binding constants K_b of 1, 2, 3 and 4 are given in Table 1 respectively. The binding constants indicate that complex 4 is binding stronger than remaining three. Hypochromism was observed in the complexes in the following order 4>3>2>1.

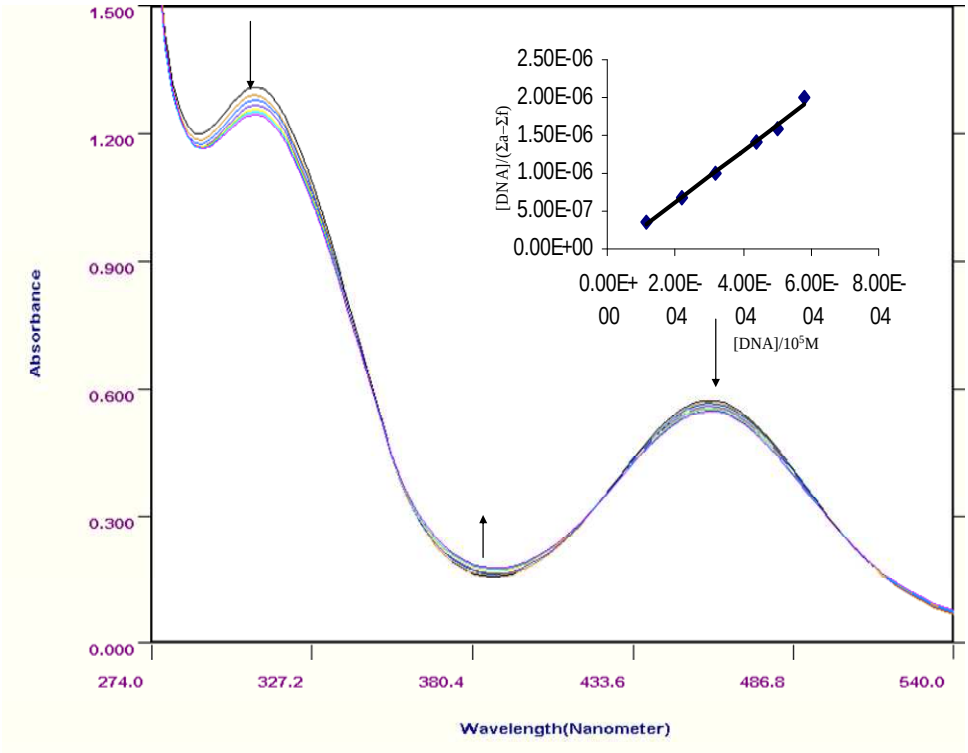

Fig (2) Absorption spectra of [Co(en)$_2$(1, 2,dme imd)$_2$] in absence (top) and presence (lower) of DNA with subtraction of the DNA absorbance. [Complex]=10μM; [DNA]/[Complex]=0, 5, 10, 15

Arrow shows the absorbance changes upon increasing DNA concentrations. Insert plots of [DNA]/(Σ_a–Σ_f) vs [DNA] for the titration of DNA with complexes; (•) experimental data points; solid lines, linear fitting of the data.

Table (1) UV, Emission peaks, absorption binding constant and melting studies of Co(III) complexes

Compound	T_M°C	UV peaks (nm)	Emission peaks	Absorption K_b M^{-1}
CT DNA	60	…..	…..	…..
Co Cl_2 $6H_2O$	….	227,492	….	…..
cis$[Co(en)_2Cl_2]Cl$	.....	247, 592	.....	.....
$[Co(en)_2(imd)_2]Br_3$	62	311, 486	403, 580	$2.0\pm0.2x10^3$
$[Co(en)_2(1\text{-me-imd})_2]Br_3$	62	268, 463	405, 578	$2.6\pm0.3x10^3$
$[Co(en)_2(1, 2, dme\ imd)_2]Br_3$	63	308, 458	401, 579	$4.2\pm0.1x10^3$
$[Co(en)_2(2\text{-et-imd})_2]Br_3$	63	333, 478	405, 580	$5.2\pm0.2x10^3$

Fluorescence Studies

The interaction of the complex with DNA was studied using fluorescence spectroscopy method. The enhancements in the emission intensity of the complexes with increasing DNA concentration are shown in Fig (3). The complexes 1, 2, 3 and 4 can emit luminescence in tris buffer (p^H =7.2) at ambient temperature with maxima at 580, 579, 579 and 580 nm. Binding of four complexes to CT DNA was found to increase the fluorescence intensity. The plots of the relative intensity versus the ratio of [DNA]/[Co] are also inserted in Fig (3). On addition of CT DNA, the emission intensity increases steadily.

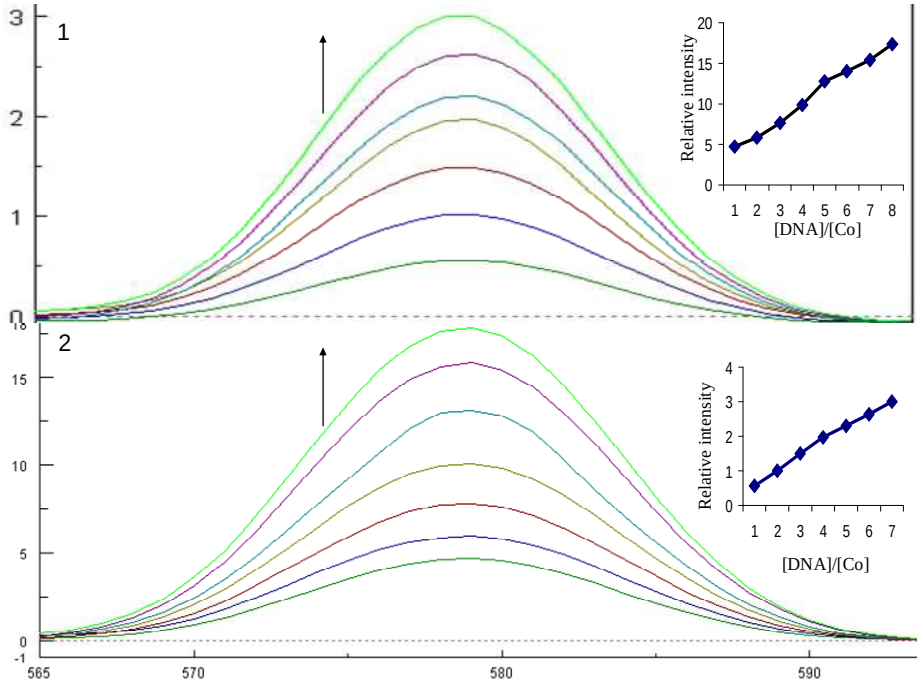

Fig (3) Emission spectra of complexes $[Co(en)_2(1\text{-me-imd})_2$ and $[Co(en)_2(1,2,dme\ imd)_2]$ in tris-HCl buffer in the presence and absence of CT DNA, the emission intensity increase upon addition of CT DNA (0.5 µl, 10 µl, 15 µl --- of DNA)

Arrow shows the intensity change upon increasing DNA concentrations. Insert: plots of relative integrated emission intensity versus [DNA]/ [Co]

The emission intensity difference between absence of CT DNA and presence of CT DNA is greater for complex 4 than other complexes as shown in Fig (3). The extent of enhancement increases on going from 1 to 4 which is consistent with the above absorption spectral results.

Thus increase in fluorescence intensity supports DNA binding to the complex. The platening of fluorescence indicate that the complex saturates all binding sites for it on DNA. The order of increase in emission intensity of complexes are corresponding to increase in binding strength.

This observation is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ distinguish between bound cobalt(III) species and positively charged free complex ions as the ions are readily quenched by$[Fe(CN)_6]^{4-}$. The complexes binding to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$be repelled by the negative DNA phosphate backbone. The method essentially consists of titrating a given amount of DNA-metal complexes with increasing the concentration of $[Fe(CN)_6]^{4-}$ and measuring the change in fluorescence intensity. The method essentially consists of titrating a given amount of DNA-metal complexes with increasing the concentration of $[Fe(CN)_6]^{4-}$ and measuring the change in fluorescence intensity. The ferro-cyanide quenching curves for 1, 2, 3 and 4 complexes in the presence and absence of CT DNA are shown in Fig (4). Obviously complex 4 insert into DNA much deeper than 3, 2 and 1. The absorption and fluorescence spectroscopic studies support the binding of complexes with DNA.

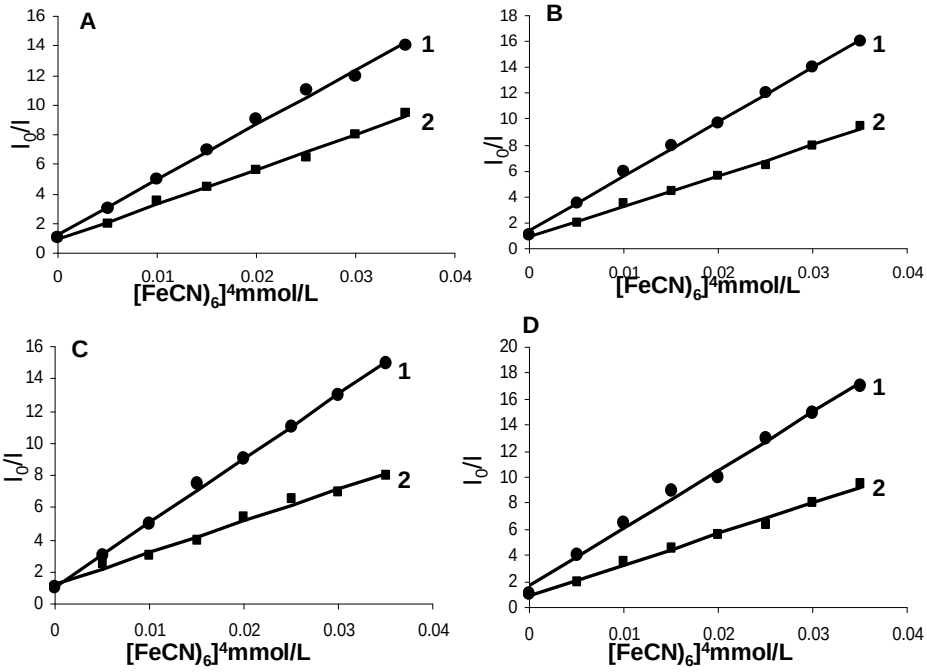

Fig (4) Emission quenching curves of $[Fe(CN)_6]^{4-}$, quenching in A,B,C and D complexes respectively [DNA]/[Co]=40). 1 quenching in absence of DNA 2 quenching in presence of DNA (complex + DNA)

Viscosity Studies

The viscosity studies yield a significant result for intercalation. In the absence of crystallographic data, hydrodynamic methods, which are sensitive to DNA length increases, are regarded as the least ambiguous and the most critical tests of binding in solution. A classical intercalative mode demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity [22, 24]. For complexes 1, 2, 3 and 4 the viscosity of DNA not increases with the increasing of the concentration of complex which is

not similar to that proven intercalator EtBr Fig (5). On the basis of the viscosity results, the complexes binds to DNA may be through major or minor groove.

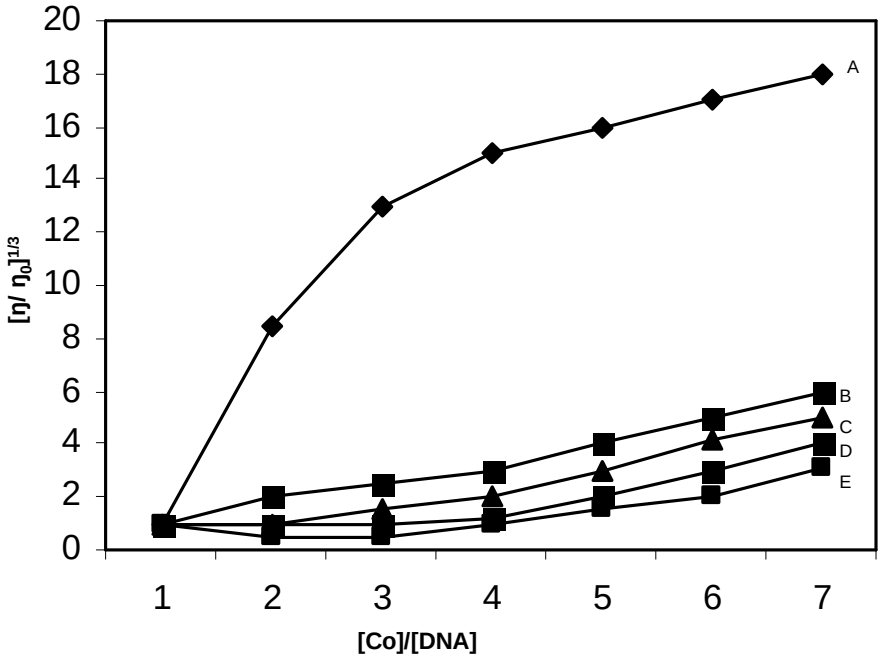

Fig (5) Effect of increasing amount of EtBr (A), 4 (B), 3 (C), 2 (D) and 1(E) complexes on the relative viscosities of CT DNA at 25 ± 0.1°C

DNA melting studies

Another strong evidence for binding of the 1, 2, 3 and 4 complexes to the double helix of DNA is that binding of small molecules into the double helix is known to increase the helix melting temperature. Helix melting temperature is the temperature at which the double helix is denatured into single-stranded DNA. Hence melting of the helix leads to an increase in the absorption at this wavelength [25]. Thus the transition temperature from helix to coil can be determined by monitoring the absorbance of the DNA base at 260 nm as a function of temperature. The increase in the melting temperature values of 1, 2, 3 and 4 not comparable to the value observed with the classical intercalator EtBr. The values are present in table (1). It is clear from these figures that they are non-intercalators because the relative absorbance is not so high compared to that of the pure DNA sample. The increase in absorbance of complexes follows the order1< 2< 3< 4.

Photocleavage of pBR 322 DNA by complexes

There has been considerable interest in DNA endonucleolytic cleavage reactions which are activated by metal ions. The delivery of high concentrations of metal ion to the helix, in locally generating oxygen or hydroxide radicals, yields an efficient DNA cleavage reaction. DNA photocleavage was monitored by relation of supercoiled circular pBR 322 (form I) into nicked circular (form II) and linear (form III). When circular plasmid DNA is subjected to electrophoresis, relatively fast migration was observed for the supercoiled form (form I). If scission occurs on one strand (nicking), the supercoils will relax to generate a slower-moving open circular form (form II) [26]. If both strands are cleaved, a linear form (III) will be generated that migrates between forms I and II. Fig (6) shows the gel electrophoresis separations of

plasmid pBR 322 DNA after incubation with complexes 1, 2, 3 & 4. This is the result of single stranded cleavage of pBR322 DNA. The incubation with Co(III) and irradiation with UV light yields significant strand scission. It is most likely that the reduction of Co(III) is the important step leading to DNA photocleavage. Further studies are carried out to find out the path of reaction mechanism.

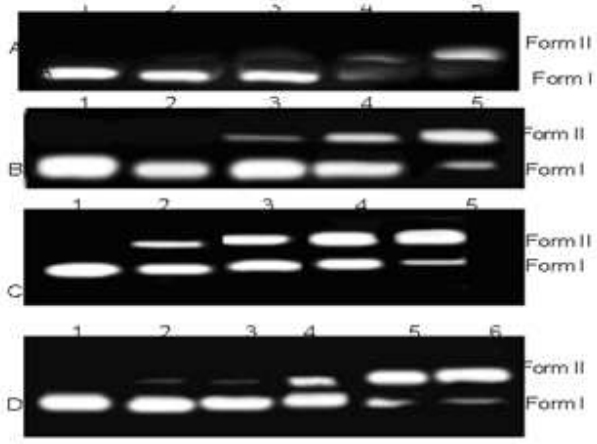

Fig (6) Photocleavage of pBR 322 DNA, Lane 1 control plasmid DNA (untreated pBR 322DNA), lane 2, 3, 4, 5lane +5µm at 0time. [Complexes 1(A), 2(B), 3(C) and 4(D).]

CONCLUSION

Four complexes of the type $[Co(en)_2(L)_2]Br_3$ were synthesis and characterized. The binding behavior of complexes 1, 2, 3 and 4 with DNA were characterized by absorption titration, fluorescence quenching and viscosity measurements. The results showed that the binding constants followed the order: 4>3>2>1 complexes. Indicating that the complex 4 binds stronger than other complexes. The DNA melting and viscosity studies tell these complexes are not intercalators. The photocleavage studies show that these complexes cleave the pBR 322 DNA.

Acknowledgements
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