DNA-binding and photocleavage studies of cobalt (III) ethylenediamine complexes: $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$

Penumaka Nagababu[1], J. Naveena Lavanya Latha[2], Y. Prashanthi[1], Satyanarayana. S[1]*

[1]Department of Chemistry, Osmania University, Hyderabad, India
[2] Krishna University Machilipatnam

Abstract

Two complexes of $[Co(en)_2phen]^{3+}$ **(1)** and $[Co(en)_2bpy]^{3+}$ **(2)** have been synthesized and characterized by UV/VIS, IR, ^1H NMR spectral methods. Absorption spectroscopy, emission, viscosity measurements, DNA melting and DNA photocleavage and the binding of the two complexes with calf thymus DNA have investigated. The spectroscopic studies together with viscosity measurements and DNA melting studies support that both of these complexes bind to CT DNA by groove mode, **(1)** binds more avidly to CT DNA than **(2)** which is consistent with the extended planar and π system of 1, 10, phenanthroline. Noticeably, the two complexes have been found to be efficient photosensitisers for strand scissions in plasmid DNA.

Keywords: Bioinorganic chemistry, Cobalt, N-ligands, DNA cleavage.

Introduction

During the past decades, tremendous interest has been attracted to the interactions of transition metal complexes with nucleic acids [1, 2, & 3]. Metal complexes of the type $[M(LL)_3]^{n+}$ where LL is either 1,10,phenanthroline or modified ligands, are particularly attractive species to recognize and cleave DNA [3, 4, 5, & 6]. In the early 1980s, Barton et al. demonstrated that tris phenanthroline complexes of ruthenium(II) display enantiomeric selectivity in binding to DNA, which can be served as spectroscopic probes in solution to distinguish right- and left-handed DNA, helices [7]. Then they found that tris phenanthroline complexes of cobalt (III) could cleave DNA when irradiated at 254 nm. Furthermore, they conducted the cleavage reactions by using the high stereo specificity of the tris diphenyl phenanthroline metal isomers. The cleavage reaction is also stereo specific. These findings underscore the importance of an intimate association of the metal ion with the duplex. The high level of recognition of DNA conformation by these chiral inorganic complexes suggested the powerful application of stereo specificity in DNA drug design [8]**.** The features common to these complexes are that the molecule has a high

affinity for double-stranded DNA and that the molecule also binds a redox-active metal ion cofactor. The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application. All the studies reveal that modification of the metal or ligands would lead to subtle or substantial changes in the binding modes, location and affinity [9, &10] giving changes to explore various valuable conformations of site-specific DNA probes and potential chemotherapeutical agents. Currently much attention has been paid to the complexes of Ru (II) [11, 12, 13, 14, 15 & 16] but the complexes with metal ions other than Ruthenium (II) have attracted much less attention. We choose to concentrate our work on complexes of cobalt(III), which have the same interesting characteristics and DNA cleaving properties, but have not received as much attention as the Ru (II) systems [8, 17, 18, 19 & 20]. Clearly further studies using various metals to evaluate the effect of intercalated ligand on the DNA binding and cleavage mechanisms are necessary. In this paper, we report the synthesis, characterization of the two complexes $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$, in which phen possesses a greater planar area and extended π system than that of bpy, and their DNA-binding properties are revealed by electronic absorption, emission, viscosity measurement and DNA melting curve. The photochemical DNA cleavage of the two complexes is also demonstrated. These studies are necessary for the further comprehension of binding of transition metal complexes to DNA. They also can be served as complementary studies for the corresponding complexes of ruthenium.

Experimental Section

Materials

All materials were purchased and used without further purification unless otherwise noted. *cis*-$[Co(en)_2Cl_2]Cl.3H_2O$ was prepared by the methods described previously[21] and $[Co(en)_2L]Cl_3$ was prepared by the procedure given below. The structure of complexes have shown in Fig 1. All experiments involving the interaction of the complexes with DNA was carried out in tris buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.0). In solution this DNA had a ratio of UV absorbance at 260 and 280 nm of about 1.90 indicating its purity [22]. DNA concentration per nucleotide was determined by absorbance at 260 nm using the molar absorption coefficient (6600 M^{-1} cm^{-1}) [23].

Synthesis of [Co(en)$_2$phen]Br$_3$ complex

A mixture of cis - $[Co(en)_2Cl_2]Cl$ (1.43g) and 1,10, phenanthroline (1g) was dissolved in ethanol (6ml) and added sodium bromide (3.0g) in water (5ml) the mixture was heated on a water bath until a dark yellow solution was formed. It was then cooled in ice the thick crystalline precipitation of $[Co(en)_2phen]Br_3$ was collected and recrystallised from water (30ml). The yield by this method was about (80%). ^{1}H NMR (ppm., D_2O): 2.71 (dd, 4H, en-CH_2 3.02 (m, 4H en-CH_2) 8.12 (dd, 2H, phen-CH, 8.20 (s, 2H, phen-CH).8.88 (d, 2H, phen-CH) 8.97 (d 2H, phen-CH). IR (C=C): 1453, (C=N):1483, (Co-N (en)) =578, (Co-N (ligand)):498.

Synthesis of [Co(en)$_2$bpy]Br$_3$ complex

The complex $[Co(en)_2bpy]Cl_3$ was prepared as above procedure from the mixture of cis - $[Co(en)_2Cl_2]Cl$ (1.43g) and 2,2,bipyridine(1g). The yield by this method was about (80%) ^{1}H NMR (ppm.D_2O): 2.8 (s, 4H, en-CH_2w) 3.12, (m, 4H en-CH_2) 7.97, (dd, 2H, bpy-CH) 8.55 (dd, 2H, bpy-CH) 8.6, (d, 2H, bpy-CH) 8.8 (d, 2H, bpy-CH). IR (C=C): 1453, (C=N): 1483, (Co-N (en)) =578, (Co-N(ligand)): 498.

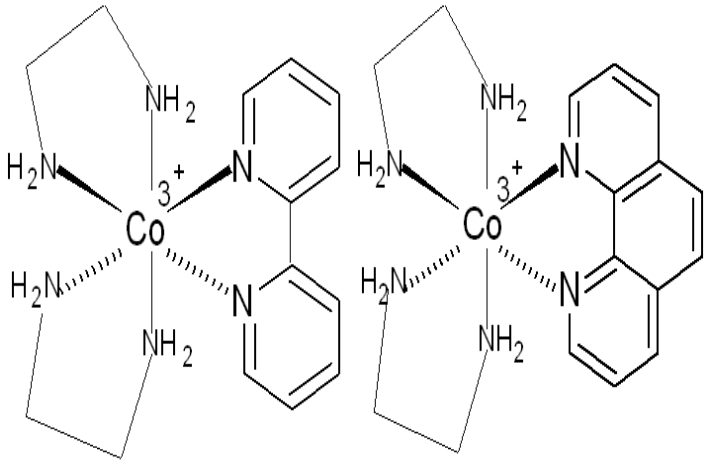

Fig (1) Chemical structure of complexes 1 and 2

Physical measurements

UV-Visible 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 in a *Perkin-Elmer FTIR-1605* spectrophotometer. ^{1}H-NMR spectra were measured with a *Varian XL-300* MHz spectrometer with DMSO as a solvent at room temperature and tetramethylsilane (TMS) as the internal standard. Spectrophotometric titrations were carried out at room temperature to determine the binding affinity between DNA and complex. Solution of a complex 3.0 ml (20 μM) was taken in a cuvette placed in the sample compartment, and its spectrum recorded in the range of 200-800 nm was taken in another cuvette and served as the reference. During the titration, small, identical aliquot (1-10 μL) of buffered DNA solution (concentration of~5 to 10 mM in base pairs) were added to each cuvette (reference and sample) to eliminate the absorbance of DNA itself. Solutions were mixed for~5 min absorption spectra were recorded. Titration as above was continued and repeated until there was no further change in the spectra. This ensured that binding had maximized. The changes in metal complex concentration caused by the small volume changes due to titration were negligible. Titrations were repeated three times at least. The cobalt (III) complexes, on other hand, showed additional MLCT bands between 400–500 nm [24].

Emission measurements were carried out by using a *HitachiF 4500* spectroflurometer. Tris-buffer was used as blank to make preliminary adjustments. The excitation wavelength was fixed at and the emission range was adjusted before measurements. All measurements were made at 25°C using, a thermostated cuvette holder with entrance exit slits of 5 nm. Emission titration experiments were performed at a fixed metal complex concentration (20 μM)to which increments of a stock DNA solution (0-160 μM) containing the same concentration of the metal complexes were added The emission enhancement was measured by comparing emission intensities at 578 and 558 nm in the absence and presence of CT DNA.

Viscosity experiments were carried out in an Ostwald viscometer maintained at 30.0 ± 0.1°C in a thermostatic water-bath. To minimize complexities arising from DNA flexibility. Calf thymus DNA samples ~200 base pairs in length were prepared by sonication [25]. Data obtained were

presented as $(\eta/\eta_0)^{1/3}$ *vs* the concentration of cobalt(III) complexes, where ŋ is the viscosity of DNA in presence of complexes and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solution (t >100 s) corrected for flow time of buffer alone (t_o), $\eta=t-t_{o/}\,t_o$ [26].

Thermal denaturation studies were carried out with an *Elico Bio*-spectrophotometer model *BL198*, equipped with temperature-controlling programmer (±0.1°C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (100 μM) in the absence and presence of the cobalt(III) complex (10 μM). The temperature of the solution was increased by 1°C min^{-1}. For gel electrophoresis experiments, supercoiled pBR322 and DNA+ complex were irradiated for 1 hr at λmax=302 nm. The both samples were analyzed by electrophoresis for 2.5h at 40 V on a 0.8% agarose gel in buffer, pH 7.2. The gel was stained with 1μg/ml ethidiumbromide and then photographed under UV light.

Spectroscopic Characterization

The IR spectral data for the complexes clearly exhibits a band at (C=C): 1453, (C=N):1483, (Co-N (en)) =578, (Co-N (ligand)):498, and 1453, (C=N): 1483, (Co-N (en)) =578, (Co-N(ligand)):for $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^3$ complexes respectively. In the ^{1}H-NMR spectra of the complexes the peaks due to various protons of ligand shifted downfield compared to the free ligand suggesting complexation. As expected the signal for bipyridine and phenanthroline appeared in the range around 7 to 9.

Results and Discussion

Electronic Absorption Spectral studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques [27]. Electronic spectra indicate the nature of interaction of complexes and DNA, since cobalt complexes often have abundant spectroscopy properties. The absorption spectra of complexes in the absence and presence of CT-DNA are given in Fig 2. Addition of increasing amounts of CT DNA results in hypochromism in the UV spectra of both $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$. According to the data presented in Fig 2, it seems that the spectral perturbation of the two complexes upon addition of DNA follows: $[Co(en)_2phen]^{3+}$>$[Co(en)_2bpy]^{3+}$. These spectral data may suggest a groove mode of binding that involves a stacking interaction between the complex and the base pairs of DNA. In order to quantitatively compare the binding strength of the two complexes, the intrinsic binding constants *K* of the two complexes with CT DNA were determined according to the following equation [28] through a plot of $[DNA]/(\Sigma_a-\Sigma_f)$ versus [DNA].

$$[DNA]/(\Sigma_a-\Sigma_f)=[DNA]/(\Sigma_b-\Sigma_f)+1/K_b(\Sigma_b-\Sigma_f))$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient Σ_a, Σ_f and Σ_b correspond to A_{obsd}/[Co], the extinction coefficient for the cobalt complex in the free and fully bound form, respectively. In plots $[DNA]/(\Sigma_a-\Sigma_f)$ versus [DNA] K_b is given by the ratio of slope to intercept. Intrinsic binding constants K_b of $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ were obtained about $4.34 \pm 0.1 \times 10^3$ and $2.08 \pm 0.2 \times 10^3$ M^{-1} from absorbance data. The binding constants indicate that $[Co(en)_2phen]^{3+}$ binds more strongly than $[Co(en)_2bpy]^{3+}$. This

result is expected, since phen possesses a greater planar area and extended π system than that of bpy, which will lead to phen penetrating more deeply into and makes stacking more strongly.

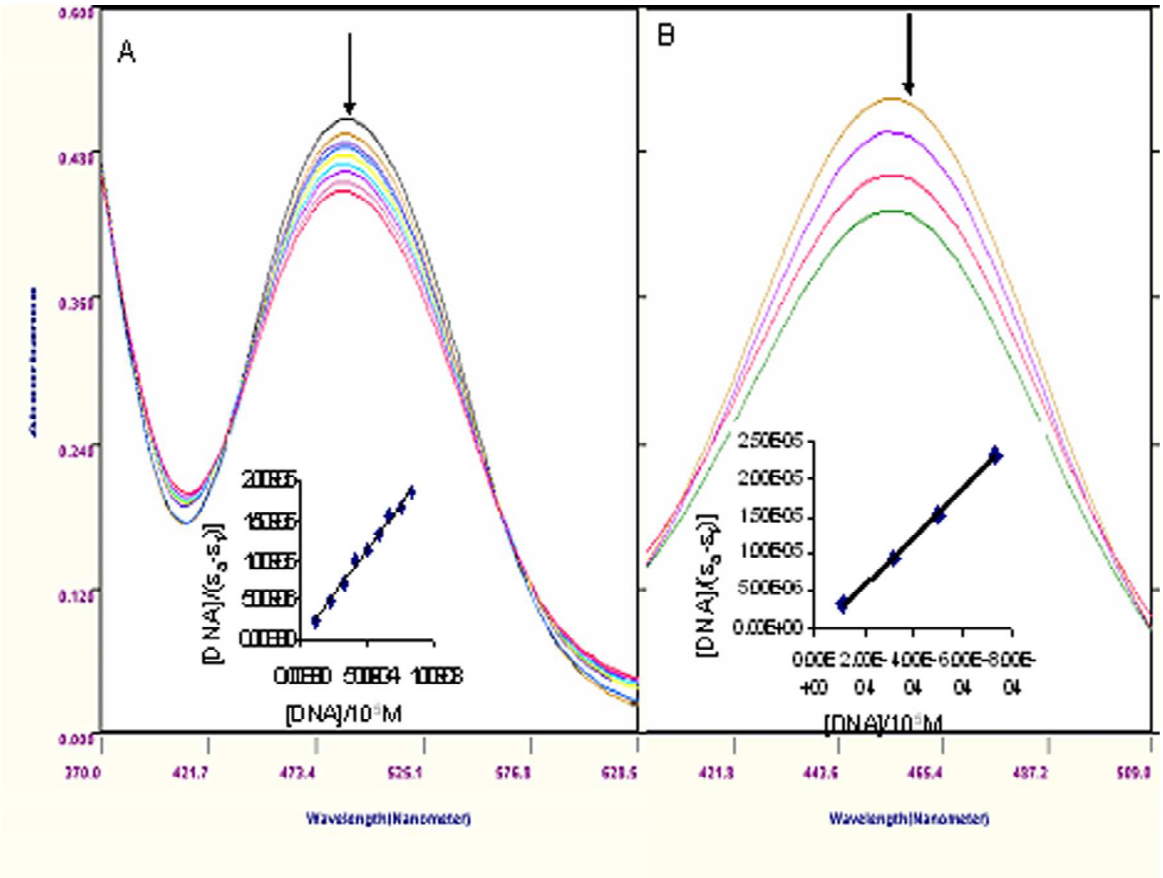

Fig 2 Absorption spectra of $[Co(en)_2phen]^{3+}$ (A) $[Co(en)_2bpy]^{3+}$ (B) (top) in the absence and presence of CT DNA the absorbance changes upon increasing CT DNA concentrations

(10µl, 20µl, 30µl, 40µl of DNA addition,---). The arrow showing the intensity change upon increasing DNA concentration. Insert: plots of [DNA]/(∑a - ∑f) versus [DNA] for the titration of DNA with complexes (.) experimental data points; solid line, linear fitting of the data.

Fluorescence Spectroscopic Studies

The complexes $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ can emit luminescence in Tris buffer (pH 7.0) at ambient temperature. Binding of both complexes to DNA was found to increase the fluorescence intensity. The emission spectra of both complexes in the absence and presence of CT DNA are shown in Fig 3. The emission intensity difference between the complex alone and complex in presence of DNA is greater for phen complex than bpy complex as shown in Fig 3. Upon addition of CT DNA, the emission intensity increases steadily. The extent of enhancement increases on going from $[Co(en)_2bpy]^{3+}$ to $[Co(en)_2phen]^{3+}$ which is consistent with the above absorption spectral results.

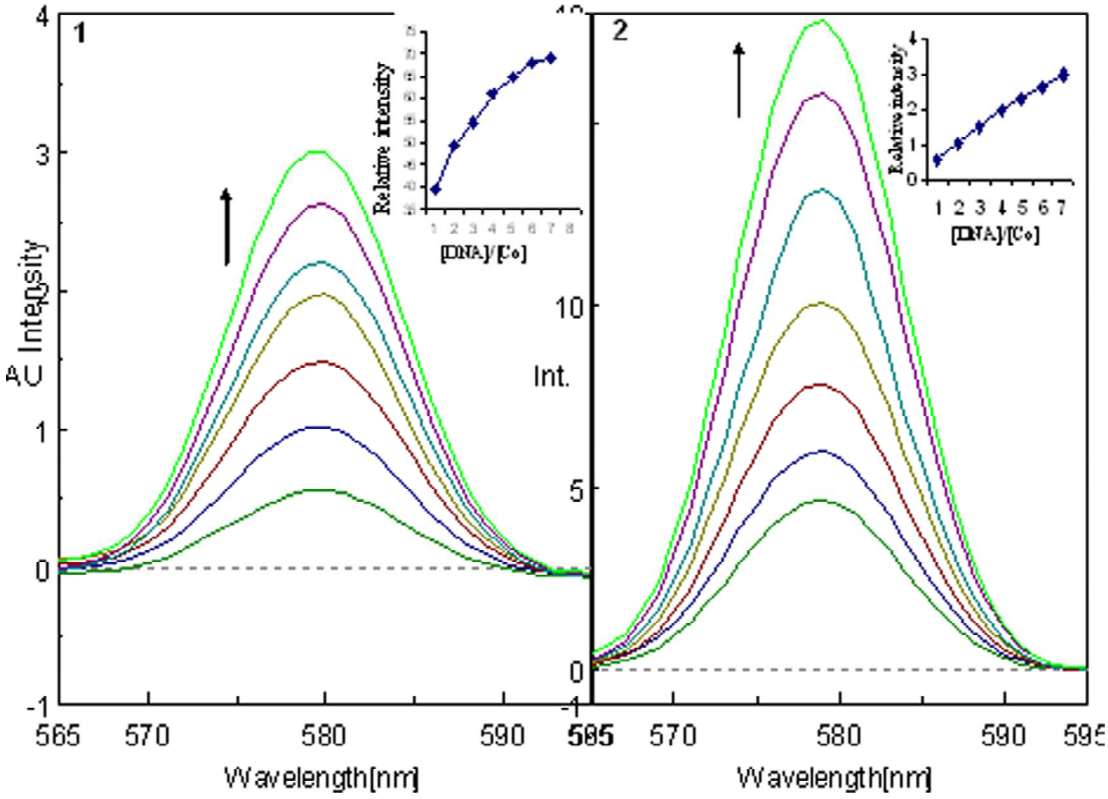

Fig 3 Emission spectra of complexes $[Co(en)_2bpy]^{3+}$ (2) $[Co(en)_2phen]^{3+}$ in BPE buffer in the absence and presence of CT DNA. Arrow shows the intensity change upon increasing DNA concentrations. Insert plots of relative integrated emission intensity versus [DNA]/[Co]

This observation is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differentially bound cobalt(III) species and positively charged free complex ions should be readily quenched by$[Fe(CN)_6]^{4-}$. The complexes binding to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering quenching of the emission of the bound complexes. 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 Fig 4. The ferro-cyanide quenching curves for $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ in the presence and absence of CT DNA are shown in Fig 5. Obviously $[Co(en)_2phen]^{3+}$ inserts into DNA much deeper then $[Co(en)_2bpy]^{3+}$. The absorption and fluorescence spectroscopy studies determine the binding of complexes with DNA.

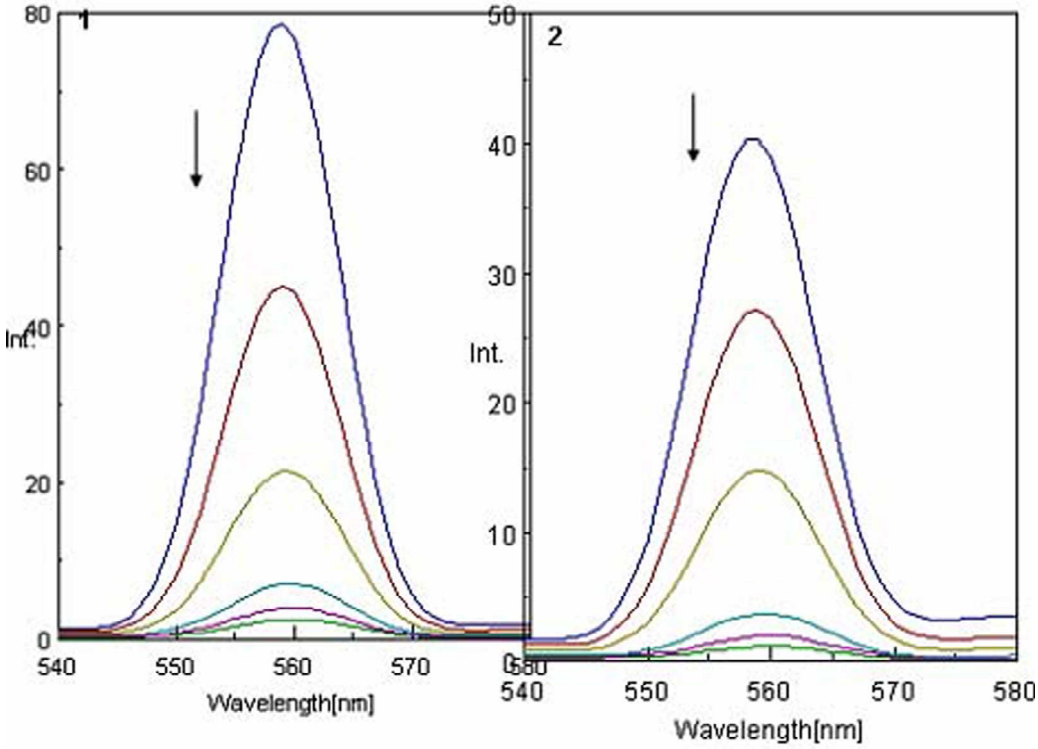

Fig 4 Fluorescence quenching curves of Ferrocyanide bound to DNA by complex. $[Co(en)_2(phen)_2]^{3+}$ and 2 complex $[Co(en)_2(bpy)_2]^{3+}$ + DNA=80ul

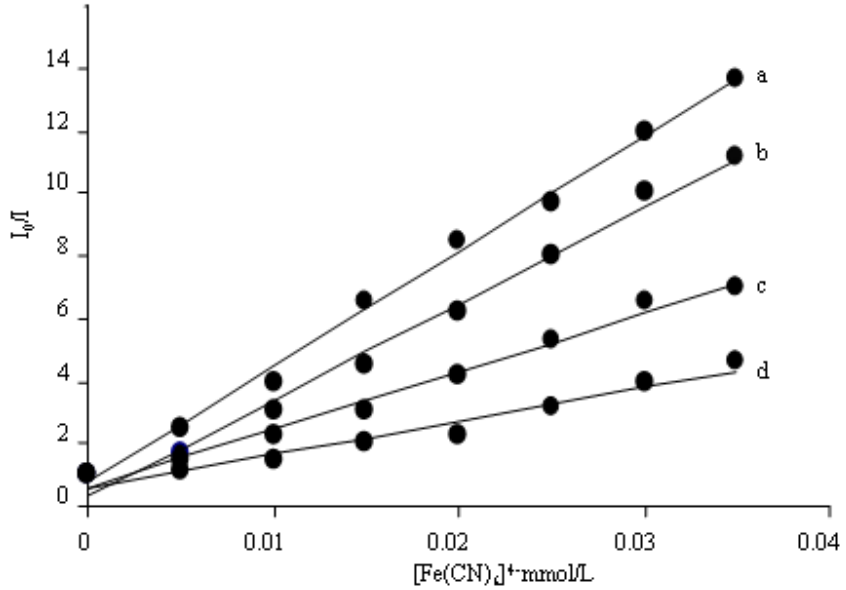

Fig 5 Emission quenching of $[Co(en)_2(bpy)]^{3+}$and $[Co(en)_2(Phen)]^{3+}$ with increasing $[Fe(CN)_6]^{4-}$ In the presence and absence of DNA. [Co]=2µmol/L, [DNA]:[Co]=40:1(a: $[Co(en)_2(bpy)]^{3+}$,b: $[Co(en)_2(phen)]^{3+}$,c: $[Co(en)_2(bpy)]^{3+}$ with CTDNA, d: $[Co(en)_2(phen)]^{3+}$ with CTDNA)

Viscosity Studies

Interaction between the complexes and DNA was also studied by viscosity measurements. In the absence of crystallographic structural data, hydrodynamic methods, which are sensitive to DNA length, are known to be among the some definitive & critical indicator of binding strength. Intercalation was the effect of in increasing DNA viscosity [26]. The effects of both complexes and EtBr on the viscosity of rod–like DNA are shown in Fig 6. For $[Co\ (en)_2\ bpy]^{3+}$ and $[Co(en)_2phen]^{3+}$ complexes the viscosity of DNA increases slightly with the increasing of the concentration of complex which is similar to that of proven $[Co(phen)_3]^{3+}$[29]. Both complexes did not change the relative viscosity of DNA in a manner consistent with binding by electrostatic (or) groove mode. This result also parallels the pronounced hypochromism and spectral red shift and emission enhancement of both complexes, whereas proven classical intercalator EtBr viscosity of DNA increases with the increase of the concentration of complex. So these two complexes do not extend DNA helix length. On the basis of the viscosity results, it seems that these will bind with DNA through groove binding. Although Borton and co-workers have proposed that 1, 10, phenathroline in $[Co(phen)_3]^{3+}$ intercalated into double-stranded DNA [30], this viscosity experiment shows that $[Co(en)_2bpy]^{3+}$ and $[Co(en)_2phen]^{3+}$ are not a DNA intercalating agents.

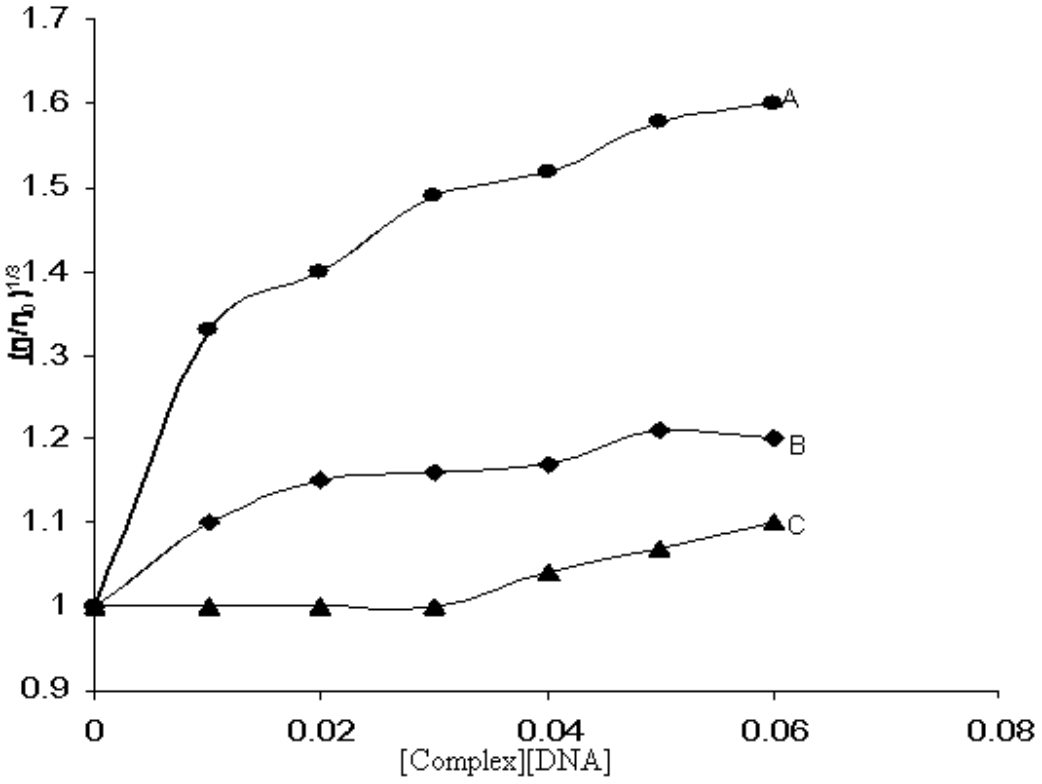

Fig 6 Effect of increasing among of EtBr (A) $[Co(en)_2(phen)]^{+3}$ (B) and $[Co(en)_2(bpy)]^{+3}$ (C) on the relative viscosities of CT DNA at 25± 0.1^{0}C

DNA melting experiments

Other strong evidence for binding of the $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ complexes to the double helix was obtained from the CT DNA melting studies. The binding of small molecules

into the double helix is known to increase the helix melting temperature, at which the double helix is denatured into single-stranded DNA. The extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than in the single stranded form. Hence melting of the helix leads to an increase in the absorption at this wavelength. The complexes were incubated with CT DNA, heated up to 85°C from ambient temperature and the OD at 260 nm was monitored [31]. Binding of complexes does lead to an increase in Δ*Tm* of DNA by 3-4°C, The increase in the melting temperature is not comparable to the value observed with the classical intercalator EtBr. It is clear from this values that the complexes $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ are non-intercalator because the relative absorbance is not so high compared to that of the pure DNA sample. The order of increase in absorbance of complexes is $[Co(en)_2phen]^{3+} > [Co(en)_2bpy]^{3+}$.

Photoactivated cleavage of pBR 322 plasmid DNA

There has been considerable interest in DNA endonucleolytic cleavage reactions that are activated by metal ions [32 & 33]. 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 cleavage was monitored by reaction of supercoiled circular pBR 322 (form I) into nicked circular (form II). When circular plasimd DNA is subjected to electrophoresis, relatively fast migration will be observed for the supercoiled (form I). If scission occurs on one strand (nicking), the supercoils will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (III) will be generated that migrates between forms II and form I, Fig 7 shows the gel electrophoretic separations of plasmid pBR 322 DNA after incubation with Co(III) complexes and irradiation at 302 nm.

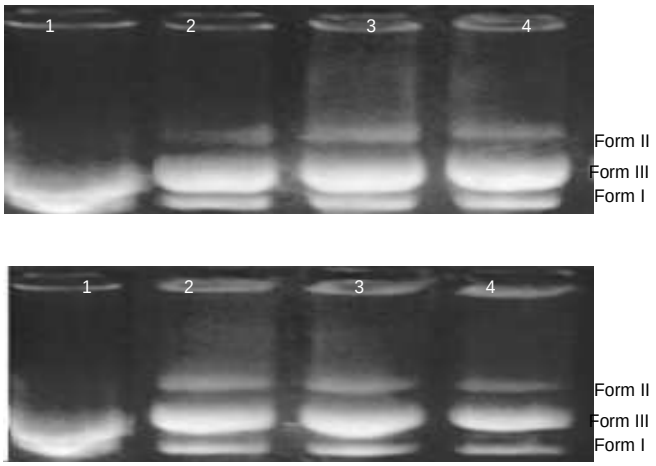

Fig 7 Photoactivated cleavage of pBR 322 DNA, Lane 1 control plamid DNA (untreated pBR 322), lane 2-4 addtion of complex $[Co(en)_2 phen]^{3+}$ 5µl, 10µl, 20µl, (λ_{irrd}=360nm) at 5 min, 10min, 20min, 30min

Fig 7 reveals the conversion of Form I and II after 60 min irradiation in the presence of varying concentrations of $[Co(en)_2phen]^{3+}$. It can be seen that with increasing the concentration of $[Co(en)_2phen]^{3+}$ complex form (I) decreases form II increase gradually, with increasing irradiation time, form I of pBR 322 DNA diminishes gradually, This is the result of single

stranded cleavage of pBR322 DNA. It can also be seen in Fig 7 that neither irradiation of DNA at 302 nm without $[Co(en)_2phen]^{3+}$ complex nor incubation with $[Co(en)_2phen]^{3+}$ complex without light yields significant strand scission. It is likely that the reduction of $[Co(en)_2phen]^{3+}$ complex is the important step leading to DNA cleavage. Further study is necessary to clarify the reaction mechanism.

DNA cleavage and sequencing studies

All the above results cumulatively indicate that these Co(III) complexes bind to CT-DNA in the groove mode. Therefore to know the exact position where these complexes can break the DNA, before and after the cleavage was sequenced by automated sequencer. The CT-DNA before treatment produced a single band at ~1.5 kb, which is almost relevant to the base length of CT-DNA (1402 bases). Whereas, after the treatment with complexes, it was observed there is breakage of CT-DNA at 3 positions in case of phenanthroline and at 2 positions in case of bipyridine. To further confirm this we have sequenced the CT-DNA before and after the treatment with Co(III) complexes. DNA sequencing results indicated that bipyridine cleaves DNA at 2 positions there by giving three bands in the agarose gel, whereas, phenanthroline cleaves CT-DNA at three positions. These cleaved positions were found to be similar with some well known restriction endonucleases that are being used in genetic engineering to cut the DNA at the specific regions. It can be suggested from the results Fig 8, that bipyridine possesses the restriction endonuclease activity of Sac1 and Acc651 while phenanthroline possess Xho II, Sal I and Kpn 1 activities. The Acc65 activity by bipyridine and KpnI activity by phenanthroline ligands were located at 753 & 749 base positions. Since these positions have no major difference the DNA base from 745 – 755 were suggested to be located in the groove region.

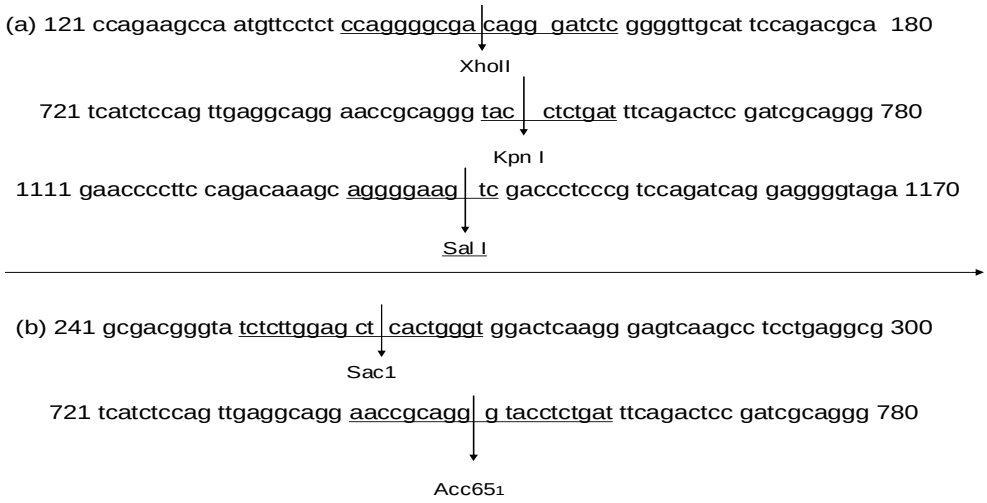

Fig 8 (a) with $[Co(en)_2phen]^{3+}$ (b) with $[Co(en)_2bpy]^{3+}$. CT-DNA with Co (III) complexes were incubated at RT for 30 min and the DNA was ethanol precipitated and processed for sequencing

Conclusion

Two complexes $[Co(en)_2phen]^{3+}$ and $[Co(en)_2bpy]^{3+}$ are synthesized and characterized. Spectroscopic studies together with viscosity and DNA melting experiments support that both of the complexes bind to CT DNA by groove via phen and bpy in to the base pairs of DNA. The intrinsic binding constants indicate that $[Co(en)_2phen]^{3+}$ binds more strongly to CT DNA than $[Co(en)_2bpy]^{3+}$ which is consistent with the extended planar and π system of phen. Noticeably both complexes have been found to promote cleavage of plasmid pBR 322 DNA from the supercoiled form I to the open circular from II upon irradiation, which may be taken as the potential DNA cleavage reagent.

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