A Novel Cobalt(III) Mixed-polypyridyl Complex: Synthesis, Characterization and DNA Binding

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A novel complex [Co(phen)$_2$HPIP]Cl$_3$ [phen = phenanethroline, HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanethroline] has been synthesized and structurally characterized by elemental analysis, UV, IR and ^{1}H NMR spectroscopies. The interaction of the complex with calf thymus DNA (CT DNA) has been studied using absorption and emission spectroscopy, DNA melting techniques and cyclic voltammetry. The compound shows absorption hypochromicity, fluorescence enhancement and DNA melting temperature increment when binding to CT DNA. CV measurement shows a shift in reduction potential and a change in peak current with addition of DNA. These results prove that the compound inserts into DNA base pairs. The shift of peak potential indicates the ion interaction mode between the complex and DNA. The binding constant of the compound to DNA is 4.37×10^4. The complex also seems to be an efficient photocleavage reagent.

Keywords cobalt(III), mixed-polypyridyl, DNA, binding mode

Introduction

The transition metal polypyridyl coordination compounds as the probe of DNA structure and configuration have been extensively studied in the past few years. They interact effectively with DNA by changing the type of heteroaromatic ligand or metal center. Those with phen (phenanethroline) and bpy (2,2′-bipyridyl) or a modified phen as ligands were particularly attractive for developing new diagnostic and therapeutic agents.[1-3] But the interaction mechanisms between them are still ambiguous.[4,5] "Shape selection" rule was proposed as the base of identifying DNA, that is to say, the compounds identify DNA and RNA by their geometry, size, hydrophobicity and hydrogen-bonding ability in accordance with the peculiar section of nucleic acids. For example, the ligand dppz [dipyrido[3,2-*a*:2′,3′-*c*]phenazine] can insert into normal B-form DNA,[6] while the ligand chrysi (5,6-chrysenequinone diimine) can insert into mismatch DNA.[7]

Most metal polypyridyl coordination compounds are positively charged and may thus bind electrostatically to single or double stranded DNA at low ionic strength. For double stranded DNA, some coordination compounds may also bind to the major or minor groove with one ligand inserting between two base pairs of DNA. In order to make mixed-ligand coordination compounds intercalate into DNA, the intercalated ligand must be flat, and have a large surface area and a special geometry that permit overlapping of aromatic ring of the intercalated ligand and the base pairs of DNA. In this report, a new mixed-ligand coordination complex of cobalt(III) containing HPIP [2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanethroline] and phen was synthesized, then our studies are extended to the complex-DNA binding system by using a variety of physical methods. The ability of [Co(phen)$_2$HPIP]Cl$_3$ to induce DNA cleavage upon photoexcitation is also revealed. To our knowledge, this type of cobalt(III) mixed-ligand complex photocleaving DNA is still unknown. The results are valuable in understanding the mode of the complex binding to DNA, as well as laying the foundation for the rational design of DNA structure probes and antitumor drugs.

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Experimental

Materials and instruments

All the experiments involving the interaction of the complex with calf thymus DNA (CT DNA) were carried out in a buffer (5 mmol/L tris-HCl, pH 7.2, 50 mmol/L NaCl) solution at 298 K. The ratio of UV absorbance of CT DNA in the buffer at 260 nm to 280 nm was *ca.* 1.84 :1, indicating that the DNA was free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6600\ dm^{-3}\cdot mol^{-1}\cdot cm^{-1}$) at 260 nm. [$Co(phen)_2Cl_2$]Cl was prepared and purified according to the reported methods.[8] HPIP and PIP [PIP = 2-imidazo[4,5-*f*][1,10]phenanethroline] were prepared according to Ref. 9 and recrystallized from acetone/water. All other reagents and solvents were of analytical grade and were used as received. Deionized triple distilled water was used for preparing the buffer.

Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were obtained on a Shimadzu FTIR-8300 spectrometer with KBr as disks. ^1H NMR spectra were taken on a Bruker DRX-300 MHz NMR spectrometer. The absorbance spectra were recorded on a Hewlett Packard HP-843 chemstation spectrometer. Fluorescence measurements were made with a Perkin-Elmer LS50B fluorescence spectrophotometer. Excitation and emission slits were 10 nm and 8 nm, respectively.

The absorption titration was performed by keeping the concentration of the complex constant (10 μmol/L) while varying nucleic acid concentration (10—100 μmol/L), and were recorded with subtraction of the DNA absorbance after equilibrating for 30 min at 298 K. The intrinsic binding constant K was determined according to Eq. (1),[10] where [DNA] is the total concentration of base pairs, ε_A, ε_F and ε_B corresponding to the extinction coefficients for the absolutely bound cobalt complex, free cobalt complex, and the actually bound cobalt complex, respectively. K was obtained from the ratio of the slope to the Y-intercept.

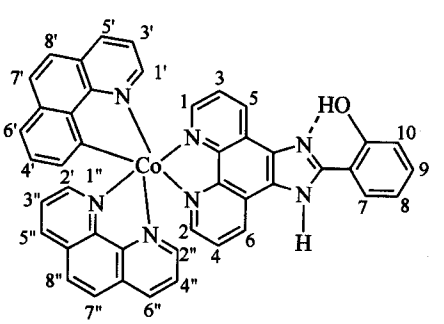
$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K(\varepsilon_B - \varepsilon_F) \quad (1)$$

The DNA melting experiment was done by controlling the temperature of the sample cell with a Shimadzu circulating bath while monitoring the absorbance at 260 nm. Cyclic voltammetry (CV) was performed using a BAS-100A electrochemical analytical instrument (U. S. A.) with saturated Ag/AgCl electrode as the reference electrode. A saturated calomel electrode (SCE) was used in all experiments. DNA samples were electrophoresed through 1% agarose gel containing Tris acetate (50 mmol/L), sodium acetate (20 mmol/L) and sodium chloride (18 mmol/L), pH = 7.2, then stained with ethidium bromide. The photographs were taken under UV light. Simultaneously, the cleavage of copper-phenanthroline was carried out for comparison with the cobalt complex. The concentration of the complex and DNA was varied in individual experiments as indicated in the text and figure legands.

Synthesis of the compound [$Co(phen)_2HPIP$]Cl_3

A mixture of *cis*-[$Co(phen)_2Cl_2$]Cl·$3H_2O$ and HPIP in water-methanol (1:2, $V:V$) was refluxed for 5 h to give a clear red solution. After most of the methanol solvents were removed under reduced pressure, an orange precipitation was obtained by dropwise addition of a saturated aqueous $NaClO_4$ solution. The product was recrystallized in acetonitrile/ether and dried (Scheme 1). Yield 85%; UV-vis (EtOH) λ_{max}: 220, 275, 281, 331 nm; ^1H NMR (D_2O, 300 MHz) δ: 9.30 (t, J = 8.23 Hz, 2H, H-1, H-2), 8.93 (t, J = 8.23 Hz, 4H, H-1′, H-2′, H-1″, H-2″), 8.33 (s, 4H, H-7′, H-8′, H-7″, H-8″), 8.04 (d, J = 7.68 Hz, 1H, H-10), 7.74—7.83 (m, 6H, H-3, H-4, H-3′, H-4′, H-3″, H-4″), 7.58 (d, J = 5.67 Hz, 2H, H-5″, H-6″), 7.51 (d, J = 5.67 Hz, 2H, H-5′, H-6′), 7.44 (d, J = 5.67 Hz, 2H, H-5, H-6), 7.36 (t, 1H, H-8), 6.98—7.06

Scheme 1 Structure of [$Co(Phen)_2HPIP$]$(ClO_4)_3\cdot H_2O$



(m, 2H, H-7, H-9); IR (KBr) ν: 3421, 3101, 1471, 1363, 1089, 623, 405 cm^{-1}. Anal. calcd for $Co(C_{43}H_{30}N_8O_{14}Cl_3)$: C 48.41, H 3.00, N 10.88; found C 48.84, H 3.13, N 11.20.

Because the perchlorate salt is water-insoluble, we transformed it into the water-soluble chloride salt through precipitation from a solution of the perchlorate in acetone by the addition of an solution of tetra-*n*-butylammonium chloride. The solid was filtered, washed with acetone and diethyl ether, and dried.

Results and discussion

Spectral characteristic of ligand and complex

^1H NMR spectrum of ligand HPIP indicates the hydrogen atom on the imidazole nitrogen may be fast exchanged between the two nitrogens on the imidazole ring, which leads to a narrow peak at low field (δ 13—14). So the whole ligand molecule is symmetric in solution. The complex shows a narrow peak at the lower field (δ 14.51), which indicates that the electron cloud density around the hydrogen on the imidazole is lowered with the ligand coordination. The decrease of the electron cloud density reduces the repulsion between the complex and the DNA, and is advantageous to their interaction.

In the UV region, the intense absorption bands observed for the Co(III) complex from 200 nm to 300 nm are attributed to the intraligand π-π^* transition of the ligand HPIP by comparison with the spectrum of $[Co(phen)_2dppz]^{3+}$. Comparing the structure of the two complexes, we can see clearly that the intense absorption of the complex $[Co(phen)_2HPIP]^{3+}$ at 281 nm is caused by the hydrogen-bond effect of HPIP, which increases the conjugated plane, lowers the energy on π orbital, and makes the absorption band red shift. The band near 331 nm is assigned to metal to ligand charge transfer (MLCT) transition, indicating that the energetic interval from d to π^* orbital is so great that the electrons can not flip easily.

Compared with the IR spectrum of the ligand, all the vibrations of $\nu_{C=C}$, ν_{CCH} and $\delta_{C=C}$ of the complex have red shifts to some extent, which shows that the ligand participates in the coordination.

Study on absorption

The interaction between the complex and CT DNA was investigated using absorption spectrometry. The electronic spectra of the complex in the absence and presence of CT DNA (with subtraction of DNA absorbance for the latter) are illustrated in Fig. 1. Addition increasing quantities of CT DNA results in decrease in the peak intensities. Hypochromism was suggested that there is a strong stacking interaction between the electronic state of the intercalated chromophore and that of the DNA base. These features are similar to those observed with $Ru(phen)_2dppz^{2+}$ and indicate that the complex binds to DNA by intercalation in a manner similar to that for $Ru(phen)_2dppz^{2+}$.[11] The intrinsic binding constant is 4.37×10^4.

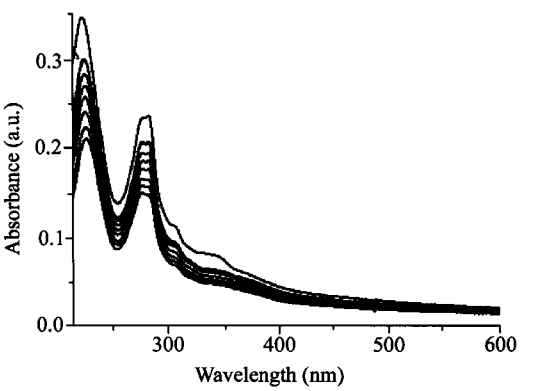


Fig. 1 Electronic spectra traces of $[Co(phen)_2HPIP]^{3+}$ in the tris-HCl buffer upon addition of CT DNA. [Co] = 10 μmol/L, [DNA]/[Co] = 0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 (from above to below).

Study on fluorescence

The results of the emission titration for $[Co(phen)_2HPIP]Cl_3$ with DNA are illustrated with the titration curves in Fig. 2. Binding of the complex to DNA was found to increase the fluorescence intensities to a large extent. The result indicates that the complex gets into a hydrophobic environment inside the DNA and avoids the effect of solvent water molecules. Therefore we infer that the complex can be inserted into the base pairs of DNA. This suggestion is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher.

In this experiment, $[Co(phen)_2PIP]Cl_3$ was used to compare the interactions. The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differently bound ruthenium(II) species, and positively charged free complex ions

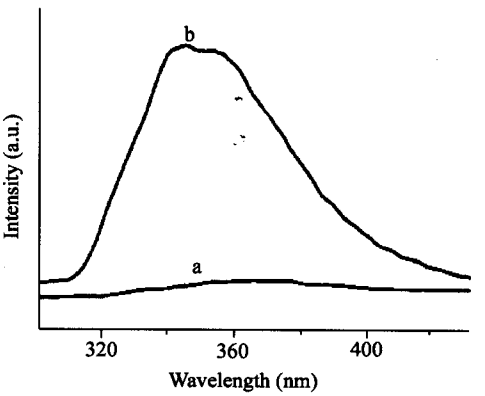


Fig. 2 Fluorescence spectra of $[Co(phen)_2HPIP]^{3+}$ (2 μmol/L) in the absence (a) and presence (b) of DNA ([DNA]/[Co] = 100).

should be readily quenched by $[Fe(CN)_6]^{4-}$. The complex 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 complex. 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 $[Co(phen)_2PIP]Cl_3$ and $[Co(phen)_2HPIP]Cl_3$ in the presence and absence of CT DNA are shown in Fig. 3. The curvature reflects different degrees of protection or relative accessibility of bound cations. The slope can therefore be taken as a measure of the total binding affinity. The large slope is corresponding to poor protection and low binding. Obviously $[Co(phen)_2HPIP]Cl_3$ inserts into DNA much deeper than $[Co(phen)_2PIP]Cl_3$ does. According to the theory of quenching, we can obtain a straight line for whether static or dynamic quenching. So the curves show the binding mode is neither static nor dynamic quenching, and may be electrostatic binding besides intercalation.

DNA *melting experiments*

Other strong evidence for the intercalation of the complexes into helix was obtained from the DNA melting studies.[12] Intercalation 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. Thus, the transition temperature from helix to coil can be determined by monitoring the absorbance of the DNA bases at 260 nm as a function of temperature. The melting curves of CT DNA in the absence and presence of $[Co(phen)_2HPIP]Cl_3$ are presented in Fig. 4. The melting temperature of CT DNA was increased from 78 ℃ to 83 ℃. The increase in the melting temperature is comparable to the value observed with the classical intercalator ethidium and lend strong support for the intercalation of $[Co(phen)_2HPIP]Cl_3$ into the helix.

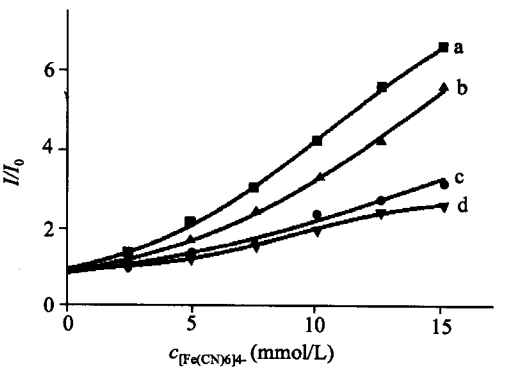


Fig. 3 Emission quenching of $Co(phen)_2PIP^{3+}$ and $Co(phen)_2HPIP^{3+}$ with increasing $[Fe(CN)_6]^{4-}$ in the presence and absence of DNA. [Co] = 2 μmol/L, [DNA]:[Co] = 40:1 (a: $Co(phen)_2PIP^{3+}$, b: $Co(phen)_2HPIP^{3+}$, c: $Co(phen)_2PIP^{3+}$ + DNA, d: $Co(phen)_2HPIP^{3+}$ + DNA).

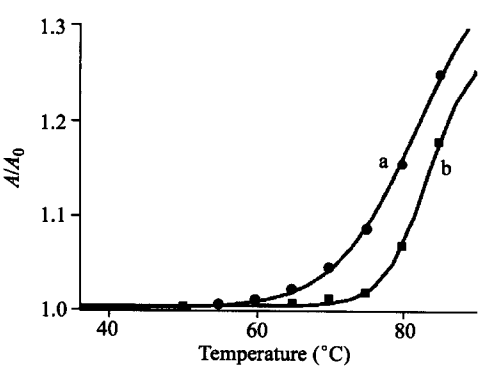


Fig. 4 Plots of A/A_0 vs. temperature of CT DNA (90 μmol/L) (a) and CT DNA in the presence of $[Co(phen)_2HPIP]Cl_3$ (b) with a 9 : 1 ratio of DNA to complex.

Study on CV

Native DNA is not reducible at the mercury electrode, because the stability of the intact double helix makes the reducible bases inaccessible to the electrode. The free complex shows no oxidative peak, which indicates that $[Co(phen)_2HPIP]Cl_3$ is not easily oxidized. In the tris-HCl buffer (pH 7.2), the addition of DNA causes considerable decrease of the peak current of $[Co(phen)_2HPIP]Cl_3$, and the peak potential has a small shift vs. a solution without DNA shown in Fig. 5. We think that $[Co(phen)_2HPIP]Cl_3$ is intercalated into the base pairs of DNA by the HPIP ligand. Because of the intercalation, $[Co(phen)_2HPIP]Cl_3$ is not readily accessible to the electrode, thus causing the peak currents of the CV waves to decrease. Moreover, the obvious shift of peak potential indicates the ion interaction mode between the complex and DNA. Here this mode may be the interaction between $[Co(phen)_2HPIP]Cl_3$ and DNA via the phosphate group of DNA.[13]

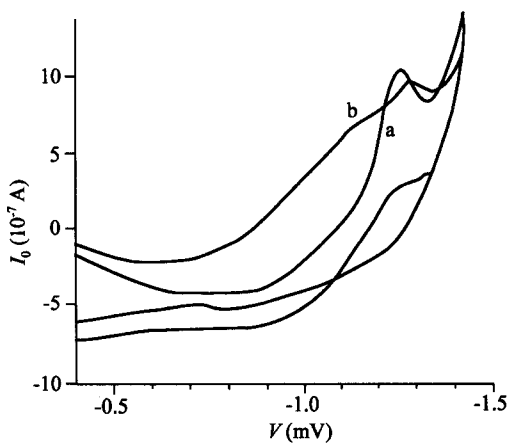


Fig. 5 Cyclic voltammograms of $[Co(phen)_2HPIP]Cl_3$ (10 μmol/L) (a) and $[Co(phen)_2HPIP]Cl_3$ + DNA (b).

Photochemistry Reaction of $[Co(phen)_2HPIP]Cl_3$ *with* CT DNA

Some transition metal polypyridyl coordination compounds can cleave DNA when irradiated with 254 nm light.[14] The irradiation of CT DNA in the presence of $[Co(phen)_2HPIP]Cl_3$ was studied so as to determine the efficiency, with which it sensitizes DNA cleavage. This can be achieved by monitoring the absorption spectra of the complex-DNA system with 254 nm light. Fig. 6 shows the change of absorption spectra of the complex-DNA system (the background spectra of involved buffer was deducted automatically by the instrument). With no irradiation , the decrease in the absorbance at 260 nm of the complex-DNA system compared to the total sum of absorbance of the complex and DNA alone suggested the reaction between the cobalt and DNA. The hyperchromism effect resulted from the damage of the DNA double-helix structure. In the presence of light, the absorbance at 260 nm of the system increased obviously. Meanwhile, we tried to observe the absorbance change of $[Co(phen)_2HPIP]Cl_3$ or DNA with identical concentration during the same time under 254 nm light, and found that DNA did not change, while the $[Co(phen)_2HPIP]Cl_3$ had a small enhancement in absorption. The net spectral change is "hyperchromic effect". This spectral change process reflected the corresponding change. Therefore, the above process reflected the secondary structural damage to DNA. This result will be further supported by the electrophoresis experiment.

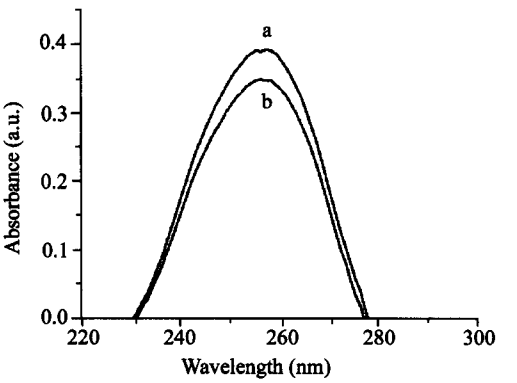


Fig. 6 Absorption spectra of the $[Co(phen)_2HPIP]Cl_3$-DNA system ($[Co] = 10$ μmol/L, $[DNA]/[Co] = 5$) without light (b) and after irradiation (a) at 254.7 nm for 30 min.

Photoactivated cleavage of pBR322 DNA *by* $[Co(phen)_2HPIP]Cl_3$

The cleavage reaction on plasmid can be monitored by agarose gel electrophoresis, relatively fast migration will be observed for the intact 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).[14] If both strands are cleaved, a linear form

(Form III) will be generated that migrates between Forms I and II. Fig. 7 shows gel electrophoretic seperation of pBR322 after incubation with cobalt complex and irradiation. The result reveals the conversion of Form I to Form II after a irradiation for 120 min in the presence of varying concentrations of $[Co(phen)_2HPIP]Cl_3$. With the concentration of the complex increasing, Form I of pBR322 DNA diminished gradually, whereas the amount of Form II increased. Neither irradiation of the DNA at 254.7 nm without cobalt nor incubation with cobalt complex without light yielded significant scission. It is likely that the reduction of Co(III) is the important step leading to DNA cleavage. Here, the Co(III) complex is photo-deoxidized, with perhaps concomitant hydroxide oxidation of ligand HPIP that is responsible for cleavage. The mechanism of photoactivated cleaving DNA with $[Co(phen)_2HPIP]Cl_3$ needs to be studied further.

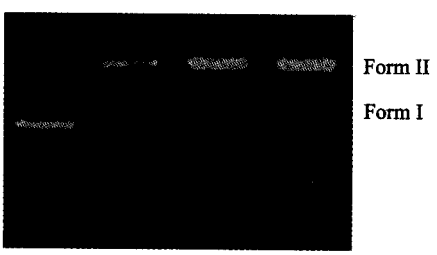


Fig. 7 Cleavage of pBR322 DNA in the absence and presence of $[Co(phen)_2HPIP]Cl_3$ after 120 min irradition with 254.7 nm light. DNA alone (Lane 1). The concentrations of $[Co(phen)_2HPIP]Cl_3$ were 1 μmol/L, 2 μmol/L and 3 μmol/L (Lane 2—4), respectively.

Comparison of DNA *cleavage of* $[Co(phen)_2HPIP]Cl_3$ *with copper phenanthroline*

Copper phenanthroline is a good DNA cleavage reagent and has been used as "footprint" agent. It has been widely assumed that the bis(1,10-phenanthroline)-Cu(I) complex, $[(phen)_2Cu(I)]$, is the active species and is responsible for DNA cleavage when the ratio of the phenanthroline to copper is greater than 2:1. The cleavage experiment were carried out with ascorbate as the reducing agent and the ratio of phenanthroline to copper was 10:1 as literature.[15]

Fig. 8 shows gel electrophoretic seperation of pBR322 after incubation with cobalt complex and irradition and copper complex and reducing agent. The result shows that $[(phen)_2Cu(I)]$ is the more effective cleave reagent than $[Co(phen)_2HPIP]Cl_3$ under the same concentration in our experiment. $[(phen)_2Cu(I)]$ can cleave pBR322 into short fragment or mono-nucleotide acid, while $[Co(phen)_2HPIP]Cl_3$ only causes the relaxation of closed pBR322 supercoiled DNA to nicked circles. In this point, $[Co(phen)_2HPIP]Cl_3$ is not suitable to be used as "footprint" agent.

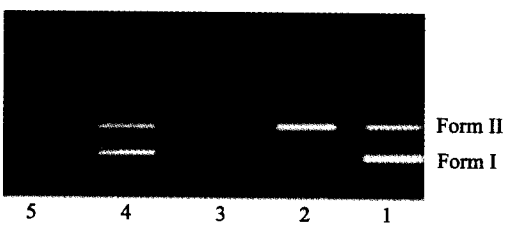


Fig. 8 Cleavage of pBR322 DNA by $[Co(phen)_2HPIP]Cl_3$ (Lane 2: 1 μmol/L, Lane 4: 0.1 μmol/L) after 120 min irradition with 254.7 nm light and $[(phen)_2Cu(I)]$ (Lane 3: 1 μmol/L, Lane 5: 0.1 μmol/L) with 5 mmol/L ascorbate. DNA alone (Lane 1).

Conclusion

We have shown that $Co(phen)_2HPIP^{3+}$ binds to DNA by intercalation of HPIP ligand. Evidence for intercalation is provided by the strong hypochromism by UV-vis spectroscopy and the increment of melting point. This intercalation of the HPIP ligand gives rise to the molecular light switch properties. When irradiated at 254 nm, the complex is capable of inducing single-strand scissions of DNA. Although it can not be used as "footprint" agent, it holds great potential of acting as DNA-targeted new diagnostic and therapeutic agents. The complex is also useful for DNA modification and preparation of bio-functional materials.

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