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Synthesis, DNA Binding And Photocleavage Studies Of Mixed Ligand Co(III) And Ni(II) Complexes Of Phenanthroline And Naphthyridine

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ABSTRACT

New Co(III) and Ni(II) mixed ligand complexes of the type $[M(phen)_2(napy)](PF_6)_n \times H_2O$ where M=Co(III) or Ni(II), napy=4-methyl-1,8 naphthyridin-2-ol, and phen=1,10-phenanthrolines n=3 or 2, x=3 or 2 have been synthesized and characterized by employing analytical and spectral methods. The DNA binding property of the complexes **(1)** and **(2)** with Calf thymus-DNA have been studied by using absorption spectra, viscosity measurements as well as thermal denaturation studies. The absorption spectral results indicate that the Co(III) and Ni(II) complexes intercalate between the base pairs of the DNA tightly with intrinsic DNA binding constant of 2.2×10^6 M^{-1} and 2.0×10^6 M^{-1} in 5mM Tris-HCl/50mM NaCl buffer at pH 7.2, respectively. The large enhancement in the relative viscosity of DNA on binding to the napy supports the proposed DNA binding modes. The oxidative cleavage and photocleavage activity of the complexes (1) and (2) were studied at the concentration of 20-40μM. The Co(III) and Ni(II) complexes induced DNA photocleavage at the concentration of 40μM after irradiation for UV light at 365nm.

KEYWORDS

Mixed ligand complexes;
Co(III) and Ni(II);
Phenanthroline;
Naphthyridine;
DNA binding;
Photo cleavage.

INTRODUCTION

Current burgeoning interest in small molecules that are capable of binding and cleaving DNA is related to their utility in the design and development synthetic restriction enzymes, new drugs, DNA foot printing agents etc., and to their ability to probe the structure of DNA itself[1-5]. In this regard, metal complexes have been found to be particularly useful because of their potential to bind DNA *via* a multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities[6–13].

Recently, efforts have been directed towards the design of various mixed ligand-metal complexes containing planar, aromatic, fused heterocyclic compounds which have proved to be very useful as structural and functional probes for nucleic acids due to the extensively π conjugated and planar structure. An added

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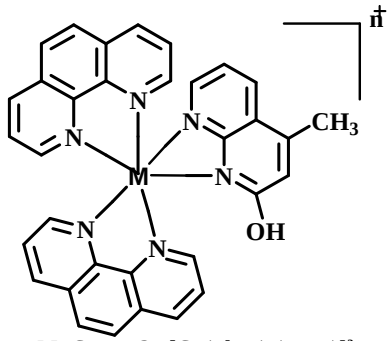
advantage of this class of DNA-binding species is that the metal ion can also be varied such that a series of complexes having the same set of ligands but with varying properties can be generated to facilitate an individual application. Most of the studies have focused on transition metal complexes with heterocyclic ligands such as phen (1,10, phenanthroline), bpy(2,2,bipyridine) and dppz (dipyrido [3,2-a:2':3'-C] phenazine[14-18].

On the otherhand napy (pyrido-pyridine) is one of the novel biologically active ligand, which plays an important role in medicinal field[19]. Antibiotics of this group are being widely used in chemotherapy of infectious diseases. Some of new naphthyridine derivatives used as plant growth regulators, fungicides, bactericides, herbicides, insecticides and nematocides of new generation[20].

With these studies in mind, we recently designed and synthesized mixed ligand metal complexes containing phenanthroline and naphthyridine. This paper discusses the synthesis, spectral characterization, DNA binding and oxidative as well as photocleavage properties of Co(III) and Ni(II) complexes that incorporate phenan throline and naphthyridine ligands mentioned before. The structure of the mixed ligand complex is shown in figure 1.

MATERIALS AND METHOD

All reagents and solvents were of AR grade, purchased commercially. All the solvents were purified before use. $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, 1,10-phenan throline monohydrate and ammonium hexaflurophos phate (NH_4PF_6), were purchased from Qualigens Fine Chemicals(India). Tris-HCl buffer(5mM Tris-HCl, 50mM NaCl, pH-7.2, Tris=Tris(hydroxymethyl) amino methane) solution was prepared using deionized double distilled water. Agarose, ethidium bromide, and bromophenol blue were purchased from Himedia. Calf thymus DNA (CT-DNA) and pUC19 DNA were purchased from Bangalore Genie(India).



M=Co, n=3: $[Co(phen)_2(napy)]^{3+}$
M=Ni, n=2: $[Ni(phen)_2(napy)]^{2+}$

Figure 1 : Molecular structure of the complex

Melting points were determined in open capillaries and are uncorrected. Micro analyses (C, H and N) were performed in Carlo-Erba 1106-model 240 Perkin-Elmer analyzer. IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. ^{1}H-NMR spectra were recorded on a Bruker AC-P500 spectrometer(300MHz) at 25^{0}C in DMSO-d_6 with tetramethylsilane as the internal reference.

Synthesis of ligand

Synthesis of 4-methyl-1,8-naphthyridin –2-ol (napy)

A mixture of 2-aminopyridine(1g, 0.01mol) and ethylacetoacetate(1.3g, 0.013mol) was irradiated in a microwave oven for 8min and 2 to 4 drops of H_2SO_4 was added and again irradiated for 3min. The solid product obtained was filtered off and recrystallized using ethanol. Yield: 78%, m.p.=205-210^{0}C. Anal. Calc. for $[C_9H_8N_2O]$: C, 67.56; H, 5.00; N, 17.50. Found: C, 67.34; H, 40.86; N, 17.37. IR KBr pellets(cm^{-1}): 3455, 2957,1658, 1588 cm^{-1}. ^{1}H NMR: 6.73(1 H d, J-10.0 Hz) 7.1-7.4(1Hm). 7.75(1Hd, J-10.0Hz) 7.8-8.1 (1Hm), 8.7-8.9 (1H m).

Synthesis of Co(III) and Ni(II) complexes

The complexes $[Co(phen)_2Cl_2]Cl.3H_2O$ and $[Ni(phen)_2Cl_2]$ were prepared as reported previously[21, 22].

Synthesis of $[Co(phen)_2(napy)](PF_6)_3.5H_2O$ (1)

To a 50ml ethanolic solution of$[Co(phen)_2Cl_2]Cl.3H_2O$ (0.57g, 1mM) was added to a ethanolic solution of napy(4-methyl-1,8-naphthyridin–2-ol) (0.162g, 1mM). The mixture was refluxed for 4hr with constant stirring under nitrogen. It was then filtered, and the complex was precipitated upon addition of a saturated ethanolic solution of ammonium hexafluorophosphate. The complex was filtered and further dried under vacuum before being recrystallized (acetone-ether). Yield 74%, Anal.Calc.for $[CoC_{33}H_{24}N_6O](PF_6)_3$: C, 39.09; H, 2.36; N, 8.28; Co, 5.81: Found: C, 39.00; H, 2.41; N, 8.40, Co, 5.30. IR, KBr pellets(cm^{-1}): 421, 839, 1321, 1431, 1581, 1658. ^{1}H-NMR, δppm DMSO-d_6, TMS]: 9.92(d, 2H), 9.18(m, 4H), 8.92(d, 2H), 8.60(d, 6H m), 8.29(d, 2H), 8.00(m, 8H), 7.70(d, 4H)

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Synthesis of $[Ni(phen)_2(napy)](PF_6)_2.2H_2O$ (2)

A solution containing $[Ni(phen)_2Cl_2]$ (0.49g, 1mM) and napy(4-methyl-1,8-naphthyridin–2-ol) (0.162g, 1mM) in ethanol was refluxed for 5 hr under nitrogen, then it was filtered and the crude complex was precipitated upon addition of saturated ethanolic solution of ammonium hexaflurophosphate. The complex was filtered, recrystallized (acetone-ether) and further dried under vacuum. Yield 70%, Anal. Calc. for $[NiC_{33}H_{24}N_6O](PF_6)_2$: C, 45.62; H, 2.76; N, 9.66; Ni, 6.75: Found: C, 45.54; H, 2.13; N, 9.81; Ni, 6.10. IR, KBr pellets(cm^{-1}): 425, 839, 1333, 1420, 1587, 1658. μ_{eff} =2.76±0.02 B.M.

DNA-binding experiments

UV Visible absorption spectra of the complexes were recorded on a Shimadzu model UV spectrophotometer at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280nm about 1.8-1.9:1, indicating that the DNA was sufficiently free from protein[23]. The concentration of CT-DNA was determined spectrophotometrically using the molar extinction coefficient at $6000M^{-1}cm^{-1}$ at 260nm[24]. Stock solutions were stored at 4^0C and used within 48 hours of preparation. The complex and DNA solutions were allowed to incubate for 10min before the absorption spectra were recorded. The electronic absorption spectra of complexes in buffer(5mM Tris-HCl, 50mM NaCl pH 7.2) were performed by using a fixed complex concentration to which increasing amounts of DNA stock solution were added. To enable quantitative comparison of the DNA binding affinities the intrinsic binding constant K_b of the complexes for binding with Ct-DNA were obtained by using eq. (1)[25]

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/k_b(\varepsilon_a-\varepsilon_f) \rightarrow \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, ε_a corresponds to the apparent absorption coefficient $A_{abs}/[M]$, ε_f corresponds to the extinction coefficient for the free metal [M] complex and ε_b corresponds to the extinction coefficient for the metal [M] complex in the fully bound form. In plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$vs[DNA]. K_b is given by the ratio of slope to the intercept.

Viscosity measurements were carried out using a semimicro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S maintained at a constant temperature 25^0C±0.1^0C. Each sample was measured three times and an average flow time was calculated. Data were presented as(η/η_0) vs binding ratio. Where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1^0C). The absorbance at 260nm was continuously monitored for solutions of Ct-DNA (0.1μM) in the absence and presence of the complexes (0.5μM). The temperature of the solution was increased by 1^0Cmin^{-1}.

DNA photo cleavage studies

The extent of cleavage of super coiled(SC) pUC19 DNA(0.5μL, 0.5μg) to its nicked circular(NC) form was determined by agarose gel electrophoresis in Tris-HCl buffer(50mM, pH 7.2) containing NaCl(50mM). In the cleavage reactions, the 30μM and 20μM complexes in 18μL buffer were photo-irradiated using monochromatic UV or visible light. The samples were then incubated for 1h at 37^0C followed by addition to the loading buffer containing 25% bromophenolblue, 0.25% xylene cyanol, 30% glycerol(3μL) and finally loaded on 0.8% agarose gel containing 1.0μg/ml ethidium bromide. Electrophoresis was carried out at 50 V for 2h in Tris-borate EDTA(TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using UVITEC Gel Documentation System. Due corrections were made for the trace of NC DNA present in the SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form. The wavelength used for the photo-induced DNA cleavage experiments were 365nm.

RESULTS AND DISCUSSION

Characterization of metal complexes

The elemental analyses of the complexes shows that the metal to ligand ratio is 1:2:1 (metal: phen: napy). The IR, ^{1}H-NMR and magnetic moment data of the new complexes are summarized in the experimental section. Because of comparable -bonding ability of phen and napy, the IR spectra of the complexes shows bands approximately in the region, 1600 and 3300cm^{-1} for phen and napy, respectively. The IR spectra of the com-

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plexes shows that the napy ligand act as bidentate by coordination of both pyridine and nitrogen with the metal ions. The sharp ligand band at 1658 cm^{-1} for C=O group appeared almost in the same region in the metal complexes, suggesting that the oxygen atom of napy ligand is not involved in bonding. In addition, the IR spectrum of the PF_6 salt of each complex showed a strong band in the 837-839 cm^{-1} region ascribable to the counter anion and this band was absent for the corresponding chloride salts[26]. In the ^{1}H-NMR spectra of the Co(III) complex, the peaks due to various protons of phen and napy are seen to be shifted in comparison with the corresponding free ligands suggesting complexation. Unlike the Co(III) complex was diamagnetic, Ni(II) complex was found to be paramagnetic with a μ_{eff} value of 2.76±0.02BM as expected for typical d^8 systems[27].

Absorption spectroscopic studies

The application of electronic absorption spectroscopy in DNA binding studies is one of the most useful technique. Complex binding with DNA through interaction usually results in hypohromism and bathochromism due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The absorption spectra of complexes **(1)** and **(2)** in the absence and presence of CT-DNA are given in figure 2 and 3. As the concentration of DNA increased for complex **(1)** the hypochromism in the interligand band reaches as high as 26% at 278nm with a red shift of 4nm. For complex **(2)** upon addition of DNA, the interligand band at 273nm exhibits hypochromism of about 21% with a 3nm red shift. These spectral characteristics indicating the binding of the complexes to DNA in different modes and to different extents. The binding of an intercalative molecule to DNA has been well characterized by large hypochromism and significant red-shift due to strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs with the extent of hypochromism and red-shift commonly consistent with the strength of intercalative interaction.

In order to compare quantitatively the strength of the two complexes the intrinsic binding constants, K_b of the two complexes with DNA was determined by monitoring the change in absorbance 278nm for complex **(1)**and 273 nm for complex**(2)**with increasing concentration of DNA using the equation**(1)**. In plots of [DNA]/(ε_a-ε_f) vs

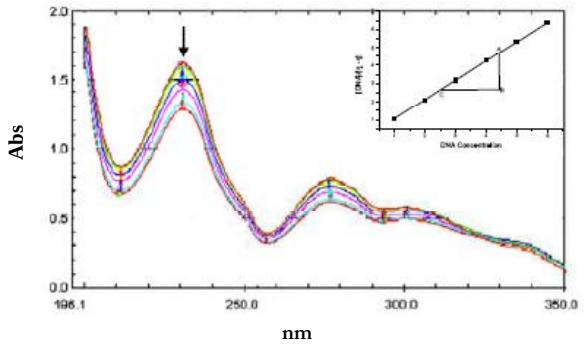

Figure 2 : Absorption spectra of complex (1) in Tris-HCl buffer upon addition of DNA.

[Co]=0.5μM,[DNA]=0.1μM. The absorbance changing upon the increaseof DNA concentration, Inner plot of [DNA]/(ε_a-ε_f)vs[DNA] for the titration of DNA with Co(III) complex

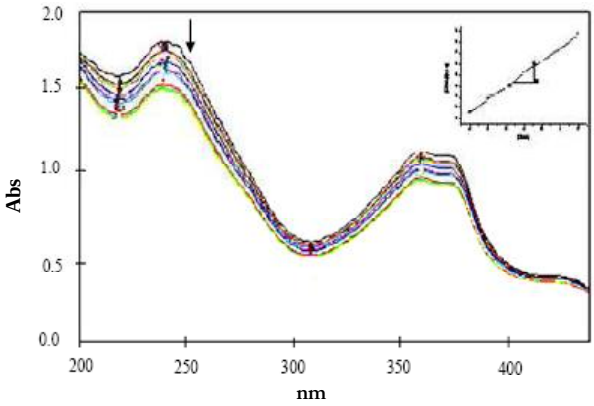

Figure 3 : Absorption spectra of complex (2) in tris-HCL buffer upon addition of CT DNA

[Ni]=0.5mM, [DNA]=0.1mM. arrow shows the absorbance changing upon theincrease of DNA concentration. Inner plot of [DNA]/(ε_a-ε_f)vs[DNA] for the titration of DNA with Ni(II) complex

[DNA], K_b is given by the ratio of slope to the intercept. The interensic binding constants K_b of complex **(1)**and**(2)** were $2.2\times10^6M^{-1}$ and $2.0\times10^6M^{-1}$, respectively. These values are comparable to that of those so-called DNA-intercalative complexes. The results of absorption spectroscopic studies indicate that complex**(1)**binds strongly to DNA by intercalation.

Viscosity measurements

The DNA binding modes of complex is further investigated by viscosity measurement, which is sensitive to the increase in length of DNA is regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structure data.[28] The viscosity of a DNA solution is sensitive to

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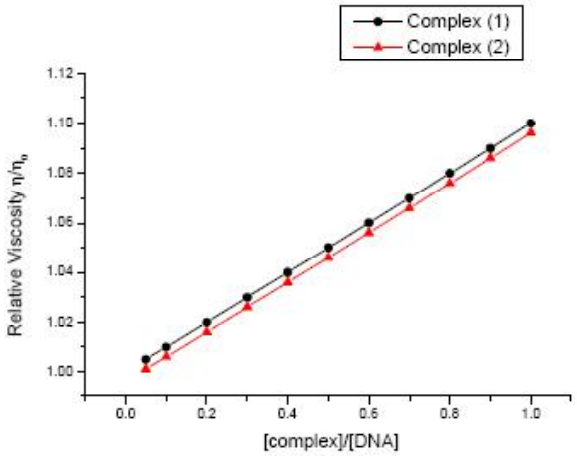

Figure 4 : Effect of increasing amounts of the complex Co(III) [—•—] and Ni(II) [—◂—] on the relative viscosities of CT-DNA at 25 (±0.1)^{0}C

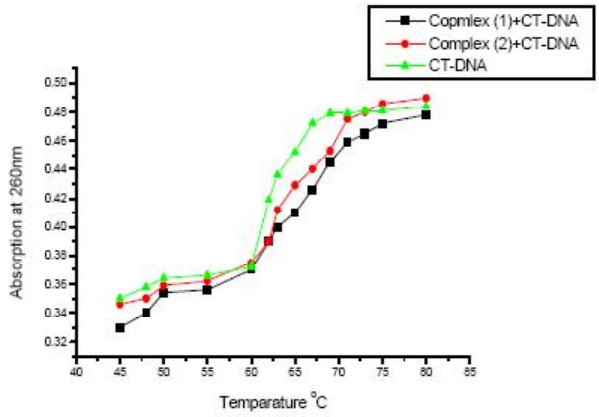

Figure 5 : Melting curves of CT-DNA in the absence and presence of complexes

the addition of organic drugs and metal complexes bound by intercalation. Intercalation is expected to lengthen the DNA helix as the base pairs get apart to accommodate the bound ligand, leading to an increase in the DNA viscosity. In contrast, a partial and /or non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length, and concomitantly, its viscosity[29]. To understand the nature of DNA binding of mixed ligand Co(III) and Ni(II) complexes, viscosity measurements were carried out on Ct-DNA by varying the concentration of the added complex. The values of relative viscosity($\eta/_o$), where η and η_o are the specific viscosities of DNA in the presence and absence of the complexes, respectively are plotted against [Co]/[DNA] and [Ni]/[DNA](Figure 4). As seen in figure 4, the viscosity of the DNA increases upon increasing the concentration of the complexes to DNA. This experimental result suggested that, both the complexes intercalated between two adjacent base pairs of DNA through a classical intercalation mode.

Thermal denaturation study

Thermal behaviors of DNA in the presence of complexes can give insight into their conformational changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hypochromic effect on the absorption spectra of DNA bases (λ_{max}=260nm). In order to identify this transition process, the melting temperature T_m, which is defined as the temperature where half of the total basepairs is bounded, is usually introduced. According to the literature,[30] the intercalation of natural or synthesized organic and metallointercalators generally results in a considerable increase in melting temperature(T_m). The T_m DNA was found to be 60±1^{0}C under experimental conditions. Under the same set of conditions, addition of complexes **(1)** and **(2)** increased T_m (1^{0}C) by 6^{0}C and 4^{0}C, respectively which indicates that these compounds stabilize the double helix of DNA (Figure 5). The increase in T_m of the latter is comparable to that of classical intercalators[31]. So from above data we conclude our metal complexes as a new class of DNA intercalators.

Oxidative DNA cleavage studies

The charecterization of DNA recognition by transition metal complexes has been aided by the DNA cleavage chemistry that is associated with redox-active or photoactivated metal acomplexes. The consequence of DNA cleavage is relaxation of the supercoiled circular form of pUC19 DNA in to a nicked circular form and linear form. When circular plasmid DNA is subjected to electrophoresis, the fastest migration will be observed for the supercoiled(SC) form (Form I). If one strand is cleaved the supercoiled form will relax to produce a slower mixing open circular form of Nicked circular(NC)(FormII).

From the figure 6. It is shown that at the concentrations of 20μM, 30μM and 40μM the complex **(1)** is able to convert 52%, 65% and 71% of the initial super

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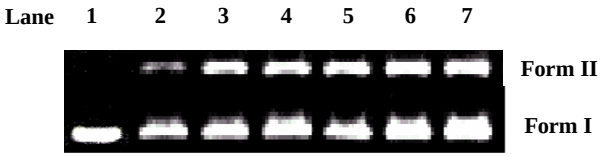

Figure 6 : Cleavage of supercoiled pUC19 DNA (0.5μg) by the cobalt(III) and Nickel(II) complexes in a buffer containing 50 mM Tris-HCl and 50 mM NaCl at 37°C.

Lane 1, DNA alone; Lane 2, DNA+20μM of complex (1); Lane 3, DNA+30μM of complex (1); Lane 4, DNA+40μM of complex (1); Lane 5, DNA+20μM of complex (2); Lane 6, DNA+30μM of complex (2); Lane 7, DNA+40μM of complex (2). Forms I-II are supercoiled, nicked circular DNA, respectively

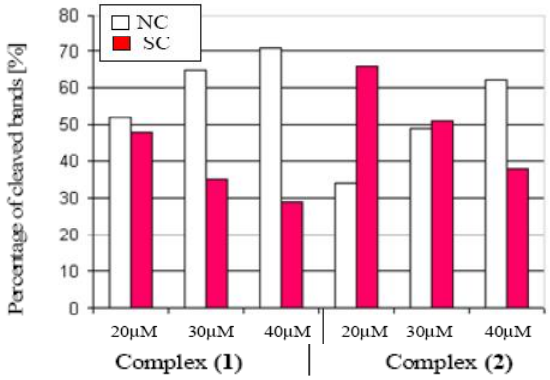

Figure 7 : Quantification of gel electrophoresis bands originating from sc and oc DNA in our cleavage experiments. The sum of intensities of both bands is standardized to 100% for each individual lane. Metal complexes and concentrations are annotated (dd H_2O: doubly distilled water as background). See text and experimental section for details

coiled(SC) form (Form I) to Nicked circular(NC) form(Form II) respectively(Lane 2-4). Whereas complex(2) at the same concentration is able to convert only 34%, 49% and 62% of the initial super coiled(SC) form (Form I) to Nicked circular(NC) form(Form II) respectively. In conclusion it is shown that, both the complexes(1)and(2) at the concentration of 40μM shows more cleavage activity than at the lower concentrations. Hence we choose 40μM complex concentration for the photocleavage experiments. From these results we infer that cobalt(III) complex (1) shows good cleavage activity than the nickle(II) complex (2).

These qualitative findings could be quantified by densitometric analysis of the bands originating from SC and NC plasmids. Bands from the linear form, although clearly visible on the gels, were difficult to quantify.

Figure 8 (a) : Gel electrophoresis diagram of the control experiments using SC DNA (0.5μg), (1) (30 μM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; lane2, DNA+D_2O(14μl)+(1); lane 3, DNA +DMSO (4μL)+(1); lane 4, DNA+NaN3 (38 μ M+(1);

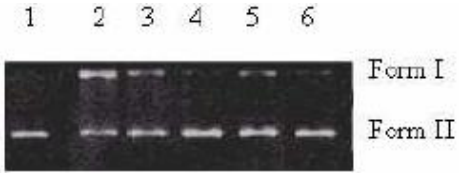

Figure 8(b) : Gel electrophoresis diagram of the control experiments using SC DNA (0.5μg), (2) (30 μM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; lane2, DNA+D_2O(14μl)+(2); lane 3, DNA+ DMSO(4μL)+(2); lane 4, DNA+NaN3(38 μ M)+(2); lane 5, DNA+(2).

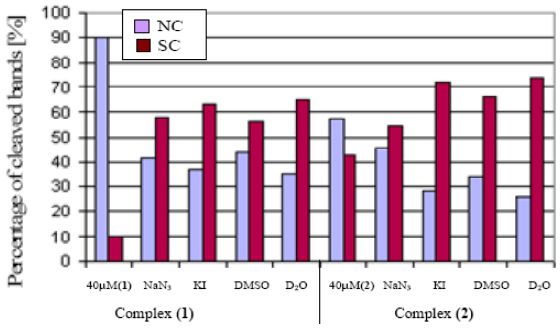

Figure 9 : Quantification of gel electrophoresis bands originating from SC and NC DNA in our photo cleavage experiments. The sum of intensities of both bands is standardized to 100% for each individual lane. Metal complexes and concentrations are annotated (dd H_2O: doubly distilled water as background). See text and experimental section for details

Large errors arise on weaker bands because the definition of the background is somewhat arbitrary in those cases. Therefore, parameters for quantification were chosen such that only the SC and NC bands were included in the procedure. The sum of intensity of both bands was standardized to 100% in all lanes. A plot of relative intensities is presented in figure 7. (for oxidative cleavage) and in figure 9. (for photocleavage).

DNA photo cleavage studies

pUC19 DNA photocleavage by the cobalt(III) com-

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plexes has been investigated in detail in this study. Control experiments have suggested that untreated DNA does not show any cleavage in the dark and even upon irradiation by light. Control experiments have also suggested that phen and napy are not detectably active under the dark and light irradiated conditions. Irradiation of pUC19 DNA samples containing both the complexes**(1)** and **(2)** were carried out in the presence of various 'inhibitors' for 1h exposure at 365nm(Figure 8). The complex **(1)**(40μM in 18 μl volume) convert 89% of SC DNA(Form I) to NC DNA (Form II) showing significant cleavage activity (Figure 8(a), lane 2). On the other hand, DMSO which scavenges $OH^{??}$radical seems to inhibit the photocleavage with 45% conversion (lane 5). Further support for the generation of $OH^{?}$upon photolysis of the complex with NaN_3, KI and D_2O with 40%, 36% and 35% conversion of SC-DNA to NC-DNA respectively (Lane 3, 4, and 6).

The nickel(II) complex **(2)** also exhibit light induced nuclease activity when irradiated to UV light for 1h at 365nm. The complex **(2)** (40μm in 18 μl volume) convert 57% of SC DNA (Form I) to NC DNA (Form II) showing less cleavage activity compared to that of complex **(1)** (Figure 8(b), lane 2). In the presence of inhibitors also the complex shows very less nuclease activity (with NaN_3 46%, with KI 28%, with DMSO 34% and with D_2O solvent 26% respectively), probably because of the paramagnetic nature of these complexes that, in principle, would render the excited states of these molecules less effective.

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