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Synthesis, Characterisation And DNA Interactions Of A Mixed Ligand Ruthenium(II) Complex

M. Kiruthika[1]*, R. Elayaperumal[2] and T. Vennila[3]

[1]Department of Chemistry, Kongunadu Arts and Science College, Coimbatore, TamilNadu, India.
[2]Department of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli, TamilNadu, India.
[3]Department of Chemistry, Tagore College of Arts and Science, Chennai, Tamilnadu, India.

***Corres. author: kumarikiruthika@gmail.com**

Abstract: A new mixed ligand ruthenium(II) complex $[Ru(bitpy)(bpy)(H_2O)](ClO4)_2$ where bitpy=benz imidazolyl terpyridine and bpy=bipyridine (1) was isolated and characterized by elemental analysis, ESI-MS, electronic absorption spectroscopy and cyclic and differential pulse voltammetry. The interaction of the complex with Calf Thymus DNA (CT DNA) was studied by using absorption, emission spectral methods and thermal denaturation and viscometry studies. Results showed that ruthenium(II) complex can increase DNA's relative viscosity and quench the fluorescence intensity of EB bound to DNA. The complex also exhibited significant cleavage of SC DNA on irradiation with visible light of wavelength at 440 nm.

Keywords: Ruthenium(II), benzimidazolyl terpyridine, DNA cleavage, CT DNA.

1. Introduction

The chemistry of coordination compounds has become a highly specialized branch of the science. A wide variety of biological systems are affected by the bases themselves and also by their metal complexes. The effects, however, are usually so distinct that it is evident that entirely different principles are involved in the mode of biological action. It has become clear that nucleic acids play active role and diverse roles in nature in the past few decades. Heavy metal interactions with nucleic acids indeed have provided the basis for the successful application of cis-platin and related compounds as anticancer drugs. Metal complexes of phenanthroline, bipyridine, and terpyridine have high biological activity. Ruthenium complexes of various ligands have attracted great interest as alternative drugs to cisplatin in cancer chemotherapy, in search of more effective and less toxic metal-based antitumor agents. The significant structural differences between ruthenium and most platinum-based antitumor drugs give a promise that ruthenium-based drugs could be suitable alternatives to cisplatin and carboplatin. Antitumor ruthenium compounds usually possess octahedral, six-coordinated geometry as opposed to the square-planar arrangement of the ligands of cisplatin or carboplatin. In addition, the two additional coordination sites for ruthenium as opposed to the platinum(I1) center in cisplatin or carboplatin may allow for new modes of binding to intracellular targets and, with some ligands, provide for chirality in the complexes and in their interactions with the target structure. However, octahedral $Ru(diimine)_3^{2+/3+}$ DNA-intercalators experienced considerable drug resistance and, as a consequence, could be used essentially as diagnostic agents for cancer [1].

NAMI-A [ImH][trans-Ru(III)Cl_4(DMSO)(Im)] (Im=imidazole), a selective antimetastatic agent, and [IndH] [trans-Ru(III)$Cl_4(Ind)_2$] (Ind = indazole), useful in colorectal tumors, have already entered phase I clinical trials as active anticancer drugs [2]. Ru(II) polypyridyl complexes have been recently studied as potential PDT agent [3], DNA binding agents [4] as well as being implicated in the oxidative damage of DNA [5]. The coordination environment around ruthenium plays the key role in stabilizing its different oxidation states and hence dictates the redox properties of the control atoms [6-7]. Therefore, polypyridyl ruthenium complexes are easily available for a systematic model in which to study the relationships between structure and biorelated reactivity. The first systematic investigation of ruthenium compounds and their antitumor property was done in beginning of 1980s with the compounds fac-[$RuCl_3(NH_3)_3$] and cis-[$RuCl_2(NH_3)_4$]Cl [8] preceded by the discovery that ruthenium red possesses antitumor properties made in the 1970s [9-10]. Since then compounds such as trans-(IndH)[$Ru(ind)_2Cl_4$] (Ind = indazole), mer-[Ru(terpy)Cl_3(terpy = 2,2'-terpyridine),[11-13] [$Ru(dmso)_4Cl_2$] (dmso =dimethyl sulfoxide) [14], ImH[Ru(im)Cl_5],[15] and ImH[$Ru(im)_2$-Cl_4], [16] are also well-known antitumor agents.

Even though, we had an interest in metal complexes having homoleptic ligands, our target is to synthesize new mixed ligand complexes with high oxidizing power for DNA cleavage. In the present communication, we describe the synthesis and characterization of a new ruthenium(II) complex [Ru(bitpy)(bpy)(H_2O)](ClO_4)$_2$, (where bitpy is 4′-(1H-benzimidazol-2-yl)-2,2':6',2"-terpyridine and bpy is 2,2'-bipyridyl) having mixed ligand system and its binding and cleavage efficiency in the presence of DNA.

2. Experimental Section

Materials and methods

Ruthenium chloride trihydrate was purchased from Aldrich. Benzimidazole-2-carboxaldehyde was prepared by following a reported procedure [17]. 4′-(1H-benzimidazol-2-yl)-2,2′:6′,2′′-terpyridine was prepared by adopting the procedure available in the literature [18]. Ru^{III}(bitpy)Cl_3 was prepared as per the reported procedure [19]. Acetonitrile, dimethyl sulfoxide, dichloromethane and methanol were of chromatographic grade and were used without further purification. Microanalyses were performed at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. UV–visible spectra of the complex was recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25^oC. The IR spectrum of the complex was recorded using a Perkin Elmer Spectrum RXI FTIR spectrophotometer and the samples were prepared by KBr mull sampling technique. Positive ion electrospray ionization mass spectra of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer. Buffers were prepared using deionised and sonicated triple distilled water. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer (pH, 7.2) was used for DNA binding and cleavage studies. A solution of CT DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8-1.9, indicating that the DNA was sufficiently free from protein [20]. The DNA concentration per nucleotide was determined by absorption spectroscopy using molar absorption coefficient 6600 M^{-1} cm^{-1} at 260 nm [21]. Thermal melting curves were obtained on a Perkin Elmer Lamda 35 UV-Vis spectrophotometer equipped with a peltier temperature controller. Viscometric experiments were carried out using an Ostwald-type viscometer of 3 mL capacity, thermo stated in a water bath maintained at 25^oC. All the DNA gel images were taken using UVITEC gel documentation system and fragments were analysed using UVIchem and UVI-band software.

2.1 Synthesis of [Ru(bitpy)(bpy)(H_2O)](ClO_4)$_2$, 1

Ru^{III}(bitpy)Cl_3 (0.557 g, 1 mmol), 2,2'-bipyridyl (0.156 g, 1mmol) and LiCl (0.5 g, 12.5 mmol) were suspended in EtOH-H_2O solvent mixture (3:1 v/v). Et_3N (0.3 mL) was added as reductant and the mixture was refluxed under inert atmosphere for 4 h while vigorous stirring was maintained. The reaction mixture was cooled to room temperature; the solvent was removed under vacuum to one-third of its initial volume. Saturated aqueous solution of $NaClO_4$ was added to precipitate [Ru(bitpy)(bpy)Cl]$^+$ as perchlorate salt. The product was filtered, washed with water (3 x 10 mL) and dried. The product obtained (0.450 g, 0.5 mmol) and silver perchlorate (0.104 g, 0.5 mmol) were suspended in acetone-water solvent mixture (1:1 v/v) and the mixture was refluxed under nitrogen atmosphere for 2 h. The reaction mixture was cooled to room temperature; the solvent was removed under vacuum to one-third of its initial volume. Saturated aqueous solution of $NaClO_4$ was added to precipitate [Ru(bitpy)(bpy)(H_2O]$^{2+}$ as perchlorate salt. The product was filtered, washed with water (3 x 10

mL) and dried. Yield: 0.409 g (72 %); ESI-MS: m/z 323.93 $(M\text{-}(ClO_4)_2)^{2+}$. IR, cm^{-1} (KBr pellet) 3396, 3064, 1602, 1444, 1081.

2.2 DNA Binding Studies

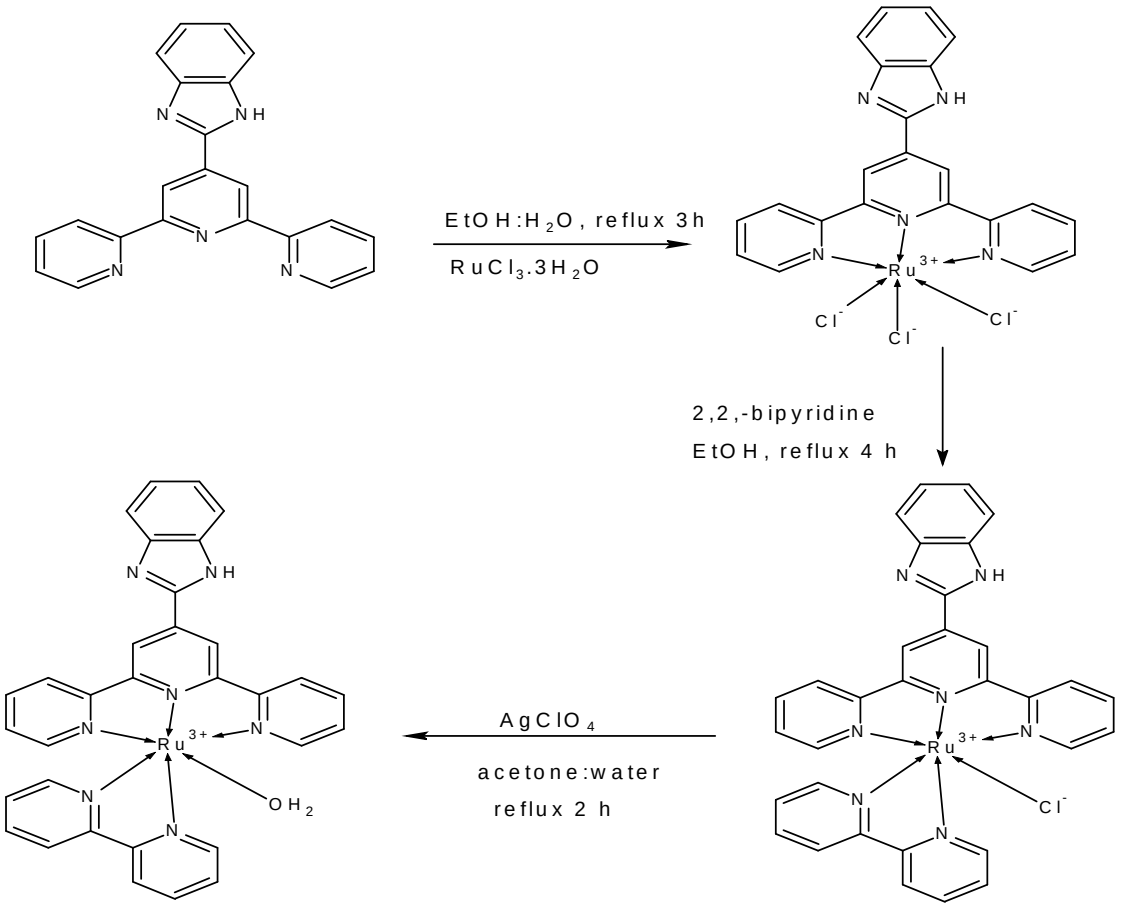
All the experiments involving the interaction of the complexes with DNA were carried out in Tris buffer. Absorption titration experiment was performed by maintaining the metal complex concentration constant (20 µM) and varying the concentration of nucleic acid from 20 to 200 µM. While measuring the absorption spectra, equal amount of DNA was added to both complex solution and the reference solution to eliminate the absorbance of DNA itself. From the absorption data, the intrinsic binding constant K_b was determined using the following equation through a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA],

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

where [DNA] is the concentration of DNA, the apparent absorption coefficient ε_a, ε_f and ε_b corresponds to A_{obsd}/[Ru], the extinction coefficient for free ruthenium complex and extinction coefficient for ruthenium complex in the fully bound form, respectively.

Aqueous solution of the ruthenium(II) complex **1** was excited at 440 nm and its emission was recorded in the absence and presence of 20–200 µM CT-DNA. Viscosity experiment was carried out on an Ostwald's viscometer, immersed in a thermostated water bath maintained at 25 ± 1°C. DNA concentration was kept constant (100 µM) and the concentration of metal complex was varied from 0 to 40 µM. Data are presented as $(\eta/\eta_o)^{1/3}$ Vs 1/R, where R = [DNA]/[Ru] and η is the viscosity of DNA in the presence of the ruthenium(II) complex and η_o is the relative viscosity of DNA alone. Relative viscosity values were calculated from the observed flow time of DNA solution (t) and corrected for the flow time of buffer alone (t_o), using the expression $\eta_o = (t - t_o)/t_o$.

Scheme. 1 Synthetic scheme for mixed ligand Ru(II) complex 1



2.3 Photonuclease activity

Photonuclease activity of the complexes was monitored using gel electrophoresis of plasmid DNA (pUC19). The solutions were prepared for the photolysis experiment containing 3 μL of 100 μg mL^{-1} plasmid DNA in Tris buffer and varying amounts of complex **1** (0–48 μM). Each solution was incubated for 1 hour and then irradiated at 440 nm for various time intervals varying from 10 min to 60 min. The samples were then subjected to electrophoresis in 0.8% agarose gel (tris-boric acid-EDTA buffer, pH 8.0) at 50 V for 2 h. The gel was stained with 0.5 μg mL^{-1} of ethidium bromide. The stained gel was illuminated under UV lamp and gel documented. In a separate experiment the DNA was incubated with 48 μM of the metal complex and 10 mM of histidine and irradiated at 440 nm. The photolysed solution was subsequently subjected to electrophoresis.

3. Results And Discussion

3.1 Synthesis and spectral characterization

The complex $[Ru(bitpy)(bpy)(H_2O)](ClO_4)_2$, where bitpy is the tridentate ligand 4′-(1H-benzimidazol-2-yl)-2,2':6',2"-terpyridine and bpy is the bidentate ligand 2,2'-bipyridine, have been isolated from ethanolic solution containing $Ru^{III}(bitpy)Cl_3$ as the starting material.

Table. 3.1. Microanalytical data of Ru(II) complex

Compound	Colour	Empirical formula	Molecular weight	Elemental analysis Calculated (found) (%)		
				C	H	N
$Ru^{III}(bitpy)Cl_3$	Reddish brown	$C_{22}H_{15}Cl_3N_5Ru$	556.82	47.45 (47.46)	2.72 (2.68)	12.57 (12.54)
$Ru(bitpy)(bpy)(H_2O)](ClO_4)_2$	Reddish brown	$C_{32}H_{25}Cl_2N_7O_9Ru$	823.56	46.67 (46.68)	3.06 (3.04)	11.90 (11.88)

The complex was obtained in good yield and characterized by using elemental analysis, ESI-MS and UV-Vis spectral techniques. The synthetic route for the present complex is shown in Scheme.1. Microanalytical data for the present complex is given in Table.3.1. The stoichiometry of the complex was determined as $[Ru(bitpy)(bpy)(H_2O)](ClO4)_2$ based on elemental analysis. The ESI-MS data revealed that the complex retains its identity in solution. The ESI mass spectra of the complex shows base peak assignable to the parent ion $(M-(ClO4)_2)^{2+}$. FT-IR spectrum of the present compound shows peak in the region of 1085 cm^{-1} corresponding to ClO_4^- stretching frequency. The absorption spectrum of the present complex is typical of the ruthenium polypyridyl complexes with intense UV bands assignable to ligand-centered π-π^* transitions at 284.8, 318.4 and 332.8 nm. Metal to ligand charge transfer (MLCT) transition is observed as a slightly broad band in the visible region at 476.8 nm for complex **1**. The electrochemical character of the present complex was investigated in acetonitrile solution by employing cyclic (CV) and differential pulse voltammetry (DPV). From DPV of complex **1**, Ru^{2+}/Ru^{3+} redox couple shows redox peak at +0.66 V. Also the present complex show quasi reversible electrochemical wave for Ru^{2+}/Ru^{3+} couple in acetonitrile and its $E_{1/2}$ value has been found to be +0.841 V.

3.2 DNA binding and photocleavage experiments

3.2.1 Electronic absorption study

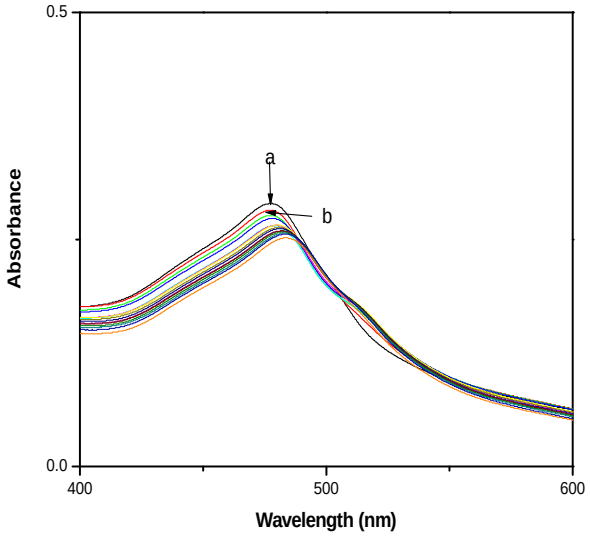
Interaction of metal complexes with DNA can be monitored by absorption spectral titration [22]. In general, metal complex binding to DNA through intercalation, results in hypochromism and red shift (bathochromism), due to the strong stacking interaction between

aromatic chromophore of the complex and the base pairs of DNA. The intercalative binding strength is determined by the extent of hypochromism exhibited by the complex. The absorption

spectra of complex **1** in the absence and presence of calf thymus DNA is shown in Figure. 1. Complex **1** shows strong hypochromism with red shift in their MLCT band with increasing amount of DNA. The ligand centered transitions of the complexes too show hypochromism with red shift in the presence of incremental amount of DNA. The strong hypochromism with red shift in the spectral band of the complexes in the presence of CT-DNA are characteristic of intercalative mode of binding. The DNA binding constant, K_b for the complex determined from the spectral titration is found to be 1.2 $*10^6$, indicating that the molecule binds DNA fairly strongly.

3.2.2 Competitive binding study

Excitation of complex **1** either in aqueous solution or in the presence of CT-DNA did not result in any fluorescence emission at the room temperature. Competitive ethidium bromide(EB) binding studies were undertaken to gain support for the extent of binding of the metal complexes with DNA. The extent of quenching of emission of EB bound to DNA by competitive displacement is a measure of strength of intercalation between the second molecule and DNA [23]. EB emits intense fluorescence in the presence of DNA, due to its strong intercalation between the adjacent DNA base pairs



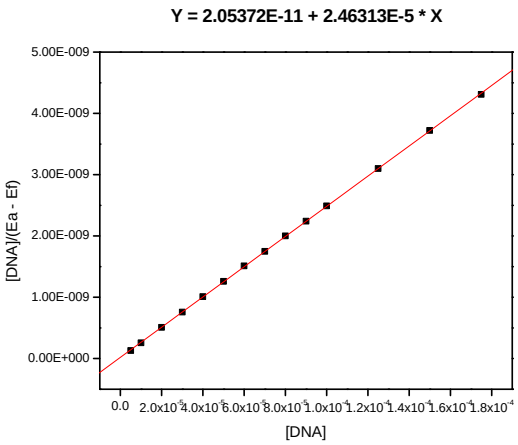


Figure 1. Absorption spectra of complex **1** (20 μM) in the absence (a) and presence (b) of increasing concentrations of CT DNA (20–200 μM).

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Increasing the concentration of complex **1** with respect to DNA pretreated with EB, causes an appreciable quenching in the emission intensity, indicating the displacement of EB fluorophore by the present complex. The fluorescence quenching curve of EB bound to DNA by complex **1** is shown in **Figure. 2.** The quenching plot of EB-DNA by successive addition of the ruthenium(II) complex is linear and the quenching constant, K value obtained is 1.90. This result supports the conclusion derived from the spectral titration of the complex with DNA.

3.2.3 Viscosity Measurement

Hydrodynamic measurement which is more sensitive to the length change of nucleic acid is a critical test for a binding model in solution in the absence of crystallographic structural data. Of the hydrodynamic measurements, viscosity measurements provide a tool for the study of metal ion - DNA interaction since optical studies provide necessary but not sufficient clues to support the binding model.

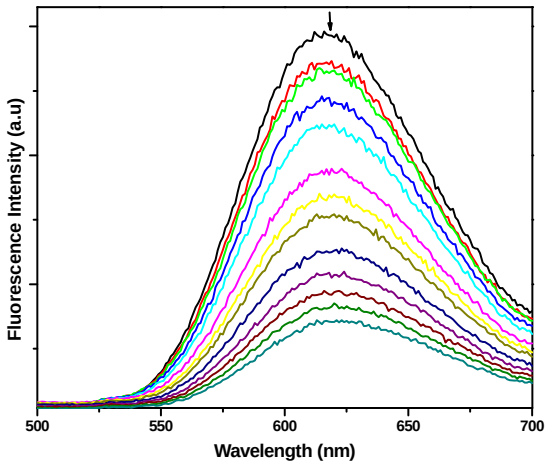

Figure.2. Emission spectra of 20 µM solution of complex **1** in the presence of DNA (20–200 µM).

In general, classical intercalators increase the viscosity of DNA due to the accommodation of the ligand between the base pairs of the helix. In contrast, non-classical intercalators could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity. Here, the viscosity of CT DNA increased with increasing concentration of 1 (Figure. 3). Such a trend is typical of intercalators. However, since complex **1** has been proposed to have an octahedral geometry and the ligands are not strictly coplanar one can expect only partial intercalation of these molecules between the DNA bases.

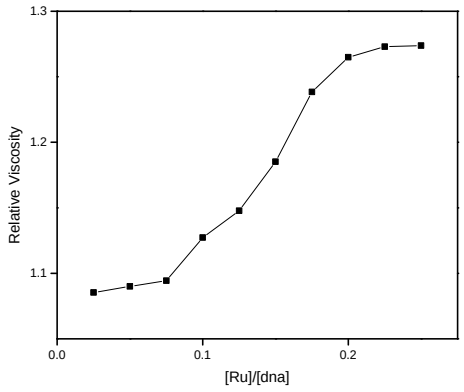

Figure. 3. Effect of complex **1** on the relative viscosity of DNA.

3.2.4. Thermal denaturation studies

The melting of DNA can be used to make a distinction between those molecules which bind via intercalation and those which bind externally. The melting temperature *Tm* is the temperature at which 50% of the DNA has become single-stranded. Also, it can be determined from the thermal denaturation curves of DNA [24]. In the absence of any added complex, the melting transition of CT DNA is sharp. Intercalation of organic dyes or metallointercalators generally results in a considerable stabilization of the DNA duplex with a corresponding large increase in melting temperature (*Tm*).

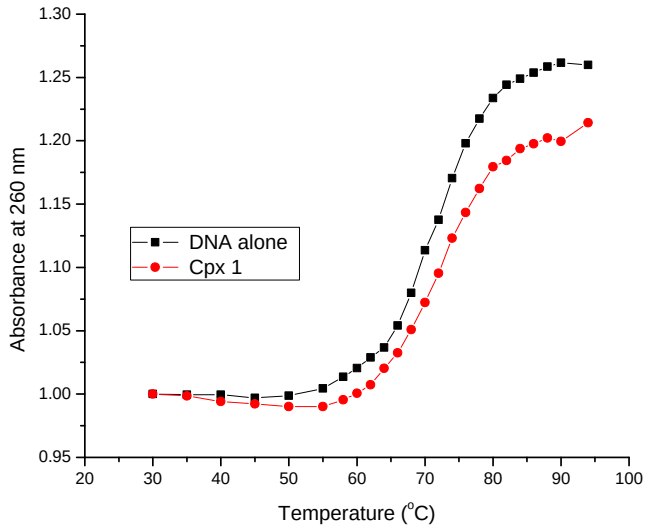

Figure. 4. The melting curves of CT-DNA (100 µM) at 260nm in the absence and presence of complex **1**.

The present complex and CT DNA alone were incubated with CT DNA, their temperatures raised from 25 to 94°C and the absorbance of the solutions at 260 nm was monitored. The melting curve of CT DNA alone and that of CT DNA in the presence of complex **1** is shown in **figure. 4.** CT-DNA was seen to melt at 66°C in the absence of complex. Interestingly, the melting temperature of CT-DNA even in the presence of complex **1** was found to be 72°C. As said above, this observed behavior is that expected for an intercalative binding mode.

3.2.5 DNA photocleavage study

The gel electrophoretic separation showing the cleavage of supercoiled pUC 19 DNA induced by the complex is shown in **figure. 5.** The cleavage of SC DNA to nicked circular (NC) DNA has been observed in the presence of complex **1** when incubated for 1 h and irradiated at 440 nm for 30 min. However, in the absence of light, no DNA cleavage has been observed. It can be seen that only supercoiled form (form I) of pUC 19 DNA was observed in control group (lane 1). On the other hand, the tested ruthenium(II) complex could cleave DNA from supercoiled form to nicked circular form (formII) upon irradiation. Cleavage of DNA by the complex is also observed even in the presence of a hydroxyl radical and singlet oxygen quencher. This result clearly indicates that DNA damage occurs through guanine base oxidation of DNA by the excited state of the ruthenium complex [25-26].

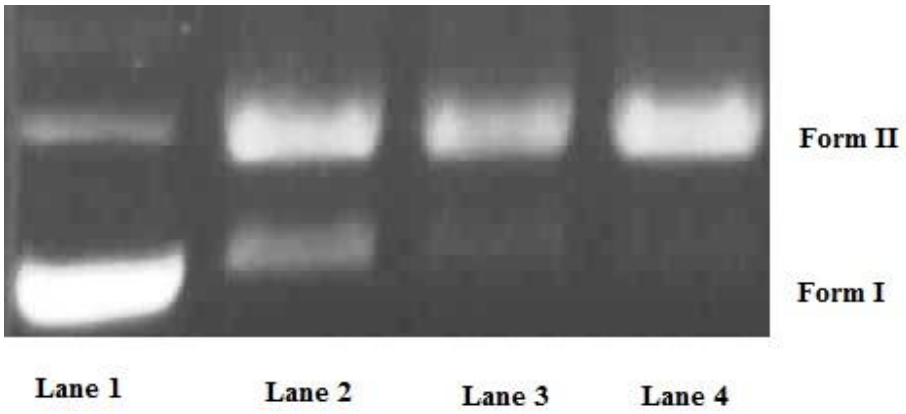

Figure. 5. Cleavage of supercoiled pUC19 by the complex 1, when incubated for 1 h and followed by irradiation at 440 nm for 30 min. Lane 1: pUC19 DNA alone, Lane 2: pUC19 DNA in the presence of 48 µM complex 1, Lane 3: pUC19 DNA in the presence of 48 µM complex 1 + 10 mM histidine. Lanes 4: pUC19 DNA in the presence of 48 µM complex 1 + 10 mM DMSO.

4. Conclusions

In the present work a mixed ligand mononuclear ruthenium(II) complex has been isolated and characterized by various physico-chemical techniques. The DNA binding behavior of the ruthenium(II) complex has been examined by absorption titration and thermal denaturation studies, fluorescence emission spectroscopy and viscosity measurements. Results suggest that the Ru(II) complex binds to DNA with an intercalative mode. And also the Ru(II) complex is extremely efficient in promoting the cleavage of pUC19 DNA in the presence of light.

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References

1. Wheate, N. J., Brodie, C. R., Collins, J. G., Kemp, S., Aldrich-Wright, J. R.:Mini Rev. Med. Chem. 7 (2007) 627.
2. (a) Sava, G., Alessio, E., Bergamo, A., Mestroni, G., in: M.J. Clarke, P.J. Sadler (Eds.), Topics in Biological Inorganic Chemistry, vol. 1, Springer, Berlin (1999) 143; (b) Arion, V. B., Reisner, E., Fremuth, M., Jakupec, M. A., Keppler, B. K., Kukushkin, V. Y., Pombeiro, A. J. L.: Inorg. Chem. 42 (2003) 6024; (c) Malina, J., Novakova, O., Keppler, B. K., Alessio, E., Brabec, V.: J. Biol. Inorg. Chem. 6 (2001) 435.
3. Swavey, S., Brewer,K.J.: Inorg. Chem. 41 (2002) 6196.
4. Erkkila, K. E., Odom, D. T., Barton, J.K., Chem. Rev. 99 (1999) 2777.
5. (a) Armistead, P. M., Thorp, H. H.: Anal. Chem. 73 (2001) 558; (b) Yang, I. V., Thorp, H. H.: Inorg. Chem. 39 (2000) 4969; (c) Carter, P. J., Cheng, C. C., Thorp, H. H.: J. Am. Chem. Soc. 120 (1998) 632; (d) Vialas, C., Claparols, C., Pratviel, G., Meunier, B.: J. Am. Chem. Soc. 122 (2000) 2157.
6. Chakravarty, J., Bhattacharya, S.: Polyhedron, 15 (1996) 1047.
7. Baitalik, S., Adhikary, B.: Polyhedron, 16 (1997) 4073.
8. Clarke, M. J.: In Metal Ions In Biological Systems., 11 (1980) 231.
9. Rudolph, R.: Arch. Exp. Veterinarmed., 25, (1971) 925.
10. Anghileri, L. J., Krebsforsch, Z.: Klin.Onkol. Cancer Res. Clin. Oncol., 83 (1975) 213.
11. Keppler, B. K., Henn, U.M., Juhl, M. R., Berger, R., Niebi, F., Wagner, E.: Prog. Clin. Biochem. Med.,10 (1989) 41.
12. Novakova, O.,Kasparkova, J., Vrana, O.,Van Vilet, P.M., Reedijk, J., Brabec,V.: Biochemistry, 34 (1995) 12369.
13. Vilaplana, R. A., Gonazalez-Vichez, F., Gutierrez-Puebla, E., Ruiz-Valero, C.: Inorg. Chim. Acta., 224 (1994) 15.
14. Sava, G., Bergamo, A., Zorzet, S., Gava, B., Casarsa, C.: Eur. J. Cancer., 38 (2002) 427.
15. Keppler, B. K., Wehe, D., Enders, H., Rupp, W.: Inorg. Chem., 26 (1987) 844.
16. Keppler, B. K., Rupp, W., Juhl, U. M., Endress, H., Nieu, R., Blazer, W. S.: Inorg.Chem., 26 (1987) 4366.
17. Zakrzewski, A., Janota, H.: Pestycydy (Warsaw), 3 (2003) 1.
18. Pal, I., Basuli, F., Bhattacharya, S.: Proceedings of the Indian Academy of Sciences: Chemical Sciences, 114 (2002) 255.
19. Sathyaraj, G., Kiruthika, M., Weyhermuller, T., Nair, B. U.: Dalton Trans., 2012, 41, 8460.
20. Murmur, J.: J. Mol. Biol. 3 (1961) 208.
21. Reichmann, M. F., Rice, S. A., Thomas, C. A., Doty, P.: J. Am. Chem. Soc. 76 (1954) 3047.
22. Kelly, T. M., Tossi, A.B., McConnell, D. J., Strekas, T. C.: Nucleic Acids Res. 13 (1985) 6017.
23. Baguley, B. C., Lebert, M.: Biochemistry 23 (1984) 937.
24. Anderson, R. A.: Biol. Trace Element Res. 32 (1992), 19.
25. Ivanov, V. I., Minchenkova, L. E., Schyolkina, A. K., Poletayer, A.I.: Biopolymers, 12 (1973) 89.
26. Vicendo, P., Mouysset, S., Paillous, N.: Photochem. Photobiol., 65 (1997) 647.

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