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A study of binding of DNA extracted from onion with Ruthunium(II) Polypyridyl Complexes

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Abstract

In an aqueous solution, the binding of the Ru(II) polypyridyl complex $[RuL_3]^{2+}$ (where L=bpy, dmbpy) with onion DNA has been investigated using absorption and emission spectrum methods. The MLCT absorption maxima for both compounds are in the 445–460nm region. Using data on absorption intensity and emission investigations, the binding constant (K_b) for these processes is calculated from the Benesi-Hildebrand equation. The luminophore and DNA molecule interact at the ground state in a hydrophobic way. These complexes bind to DNA through intercalative mode. The K_b value depends on the nature of the DNA and also on the ligand used.

Keywords: Metal complex, binding constant, DNA, electrostatic interaction.

Introduction

Metal complexes due to their usefulness in the biological sphere, are thought to play a vital role in therapies[1,2]. Due to their numerous uses in biotechnology and cancer therapy, the study of interactions between transition metal complexes and DNA has attracted a lot of interest[3-8]. Many anticancer medications target DNA as a key target molecule. To comprehend how the drug molecule interacts with DNA, metal complexes bound to DNA were studied. The DNA replication can be stopped in order to destroy the tumour cells. Cancer treatment frequently targets cancer cells' DNA. By attaching to DNA, several chemotherapeutic drugs have anticancer effects. By altering DNA replication, they prevent the formation of tumour cells. The mechanism and affinity of their binding play a role in the development of novel and more effective anticancer medicines. In order to create new compounds or medications for medical use, the interaction of metal complexes with DNA has long been the focus of much research.

Although cisplatin and carboplatin are used, they have a number of negative side effects. Therefore, preparing chemotherapy medications with no side effects or with minimal side effects is our main goal. Both covalent and non-covalent interactions are known to be used by several transition metal complexes to attach to DNA. A DNA nitrogen base replaces the complexes' labile ligand in covalent binding. On the other hand, non-covalent DNA contacts include groove (surface) binding of cationic metal complexes outside of the DNA helix, major or minor groove, intercalative, electrostatic, and these interactions. Damage to DNA molecules can occur under a variety of circumstances, including interactions with other molecules. Due to their potential use as novel therapeutic agents and their photochemical capabilities, which make them potential probes of DNA structure and conformation, this damage may result in a variety of pathological alterations in living things. Complexes of Ru(II) polypyridyl have strong DNA binding properties. These complexes utilise intercalative method to attach to DNA. In view of the background, this study emphasises the interaction of Ru polypyridyl complexes with DNA obtained from onions.

Materials and methods

Without any purification, the ligands 2,2' bipyridine and 4,4'-dimethyl-2,2' -bipyridine used and were procured from Sigma Aldrich. According to the previously published procedure[9], the two $[Ru(NN)_3]^{2+}$ complexes [where NN=2,2' bipyridine (bpy), 4,4' - dimethyl-2, 2' bipyridine (dmbpy)] were created by reacting $RuCl_3.3H2O$ with the appropriate ligands. Utilizing double distilled water, binding tests were conducted. Local stores sold ground onion, salt, ethanol, detergent, and liquid soap were purchased locally. For the DNA isolation, ethanol in a concentration of 95% was utilised.

Synthesis of Ru(II)-polypyridine complexes: Tris (2,2'-bipyridine) Ruthenium (II) Chloride, $[Ru(bpy)_3]Cl_2$: In 25 mL of ethanol, $RuCl_3$ $3H_2O$ (0.5g) and 2, 2- bipyridine (0.6g) were dissolved. The solution was then refluxed for 20 hours. The ethanol solution still contained the resulting orange-red complex that had developed. Using n-propanol as the eluent, the crude product was purified on a silica gel column. Afterwards, the complex was evaporated to recover the pure form[9].

Tris(4,4'-dimethyl-2,2'-bipyridine)ruthenium(II)tetrafluoro borate, $[Ru(dmbpy)_3]$ $(BF_4)_2$: 20 mL of ethylene glycol were used to dissolve $RuCl_3.3H_2O$ (1 mM) and 4,4'-dimethyl-2,2'-bipyridine (3 mM) before they were refluxed for four hours. After cooling to normal temperature, the solution was filtered to

get rid of any insoluble contaminants. The filtrate was then gradually infused with a saturated sodium tetrafluoroborate solution until an orange precipitate was produced. The last drying step involved a vacuum desiccator after the product had been filtered and rinsed with cold water and diethyl ether. By recrystallizing the product from water, the product was further purified[10].

Extraction of DNA fragments from onion: Small bits of chopped onions were used. A beaker containing about 20 mL of water was used. The water is seasoned with around 1.5 tablespoons of salt, which is then thoroughly dissolved by stirring. Dish soap in the amount of two teaspoons should be added. The table salt, detergent solution, and minced onions were combined. The combined concoction is then transferred to a new beaker. The heated mixture was then filtered out into a new beaker after being swirled over a flame with the aid of a tea strainer. A petri dish was used to hold the ethanol. The filtered solution was pipette out, and the petri dish was filled drop wise with it. After a little while, DNA fragments were seen. After a little while, DNA fragments were seen. Alcohol does not make DNA soluble. All of the mixture's components, with the exception of DNA, remain in solution when ethanol is added. The DNA separates out and condenses in the ethanol layer (Figure-1). Using a glass rod, the produced DNA is spooled on. The extracted DNA was employed for the binding studies and had the appearance of white mucus[11].

Figure-1: Structure of the DNA formed from Onion.

Results and Discussion

Spectral measurements: The SYSTRONICS 2203 Double Beam Spectrophotometer was used to record the absorption spectral observations. With the use of a JASCO/FP 8200 Spectrofluorometer, emission studies were captured. The DNA concentration ranged from $4x10^{-6}$ to $2.8x10^{-7}$M, while the $[Ru(NN)_3]^{2+}$ concentration was set at $2x10^{-5}$M. Both the absorption and emission studies employed the identical sample solutions. To guarantee that the sample solutions' volumes did not vary, all of the emission studies' sample solutions were stored in cold water. At room temperature, all measurements were made. Figure-2 depicts the structures of the two Ruthenium polypyridyl complexes.

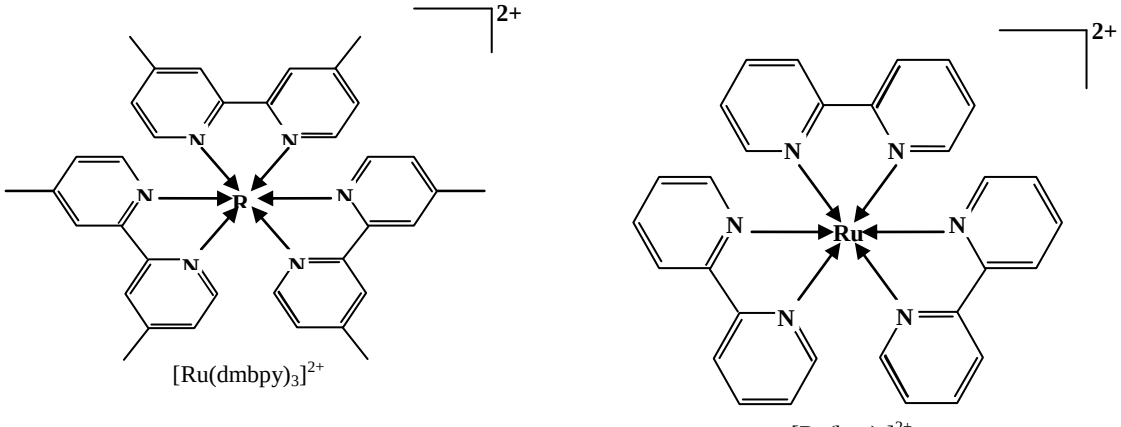
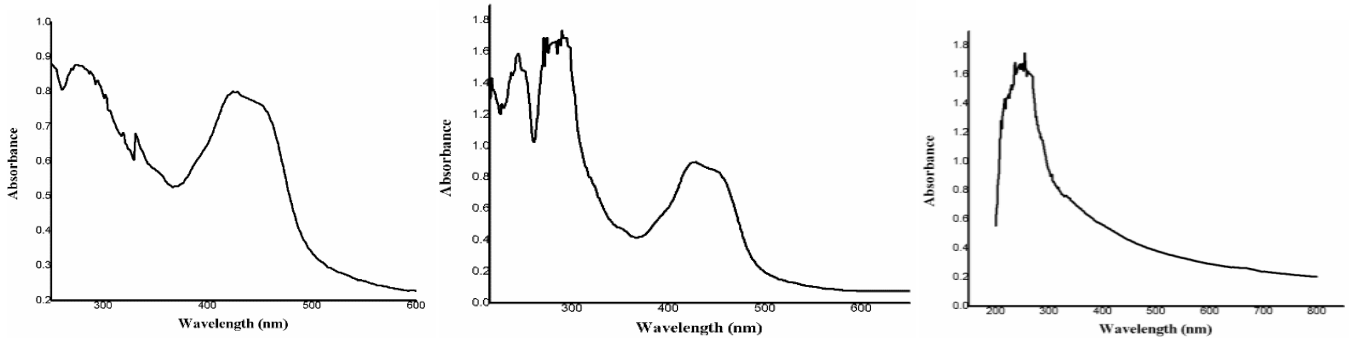
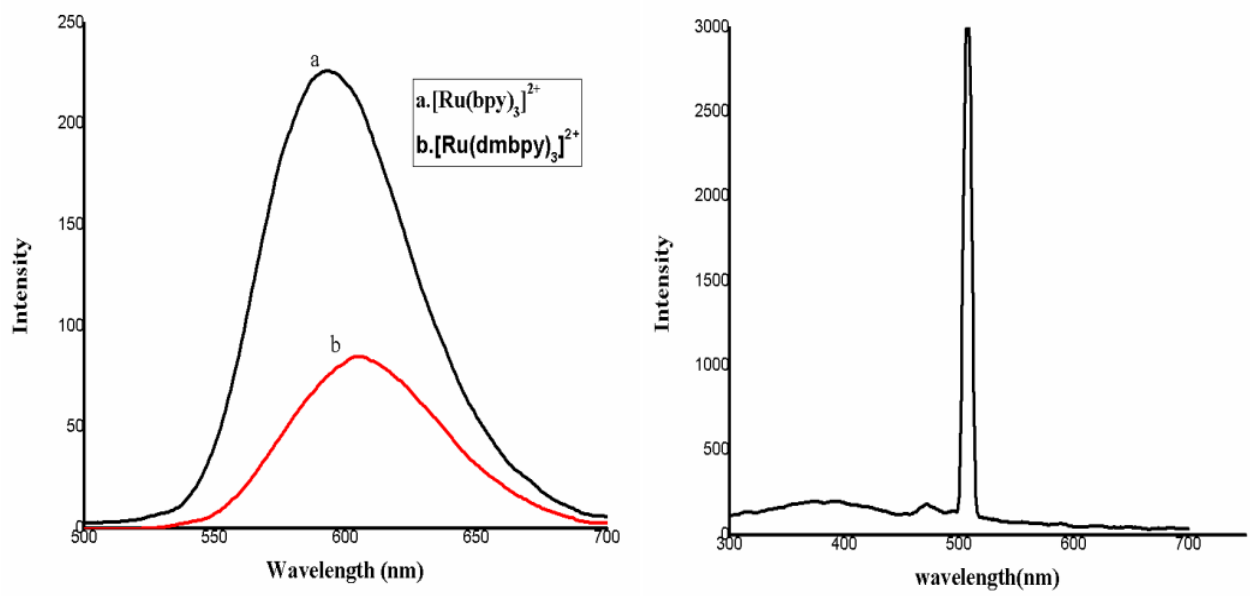


Figure-2: Structure of the Ruthenium polypy



(a) (b) (c)

Figure-3: Absorption spectrum (a) $[Ru(bpy)_3]^{2+}$ (b)$[Ru(dmbpy)_3]^{2+}$ complexes (c) DNA in aqueous medium.



(a) (b)

Figure-4: Emission maximum of a. Ruthenium polypyridyl complexes b. DNA in aqueous medium.

Figure-3 displays the absorption spectra of the two metal complexes $[Ru(NN)_3]^{2+}$. Due to its photophysical and excited state features, the most researched complex is $[Ru(bpy)_3]^{2+}$. In aqueous media, the complex $[Ru(bpy)_3]^{2+}$ has a maximum absorption at 453nm, while the complex exhibits a maximum emission at 596nm (Figure-4). The onion DNA in aqueous solution exhibits an absorption maximum of 263nm. This solution exhibits an emission maxima of 509nm when excited by light with a wavelength of 263nm. The molarities of the double stranded DNA solution were computed using the formula DNA = 6600mol^{-1}cm^{-1} L, and the DNA concentrations were measured using the absorbance ratio A_{260}/A_{280}, which was in the range of 1.80-1.90. The photophysical properties are shown in Table-1.

Table-1: Spectral properties of Ruthenium polypyridyl complexes and DNA in aqueous medium.

Complex	Absorption maximum(nm)	Emission maximum(nm)
$[Ru(bpy)_3]^{2+}$	448	593
$[Ru(dmbpy)_3]^{2+}$	458	605
DNA	263	509

To investigate how metal complexes interact with DNA, electronic absorption spectroscopy is a crucial tool. Using the information obtained from the emission and absorption spectrum measurements, the DNA binding with different metal complexes were determined. The total volume of the DNA metal complex solution was kept at 5mL and the DNA concentration was adjusted while the metal complex concentration was kept constant. For the measurements of absorbance, the change in absorbance was computed. The change in emission intensity was also computed for the emission spectrum data. Using the Benesi-Hildebrand plot, the binding constant for the DNA-metal interaction is accomplished. The stoichiometry of non-bonding interactions as well as the binding constant K can be calculated mathematically using the Benesi-Hildebrand method. Charge-transfer complexes and guest-host molecular complexation are examples of chemical equilibria that generate one-to-one complexes where this technique has traditionally been used. The theoretical foundation of this method is the assumption that, when either one of the reactants is present in excess amounts over the other reactant, the characteristic electronic absorption spectra of the other reactant are transparent in the collective absorption/emission range of the reactant system. When either one of the reactants is present in excess amounts over the other reactant. Therefore, the association constant of the reaction can be calculated by comparing the absorption spectra of the reaction before and after the creation of the product and its equilibrium. The binding constant (K_b) of the complexes with $[Ru(NN)_3]^{2+}$ complexes with DNA were determined from the Benesi – Hildebrand equation using absorption intensity ratio[1].

$$\frac{1}{\Delta A} = \frac{1}{K_b}\,\Delta\varepsilon\,[H] + \frac{1}{\Delta\epsilon}\,[Q] \qquad (1)$$

Where A represents the variation in absorbance of the complex at various DNA quantities ([Q]). The luminophore's concentration is [H]. The ratio between the y-intercept and the slope of the straight line can be used to determine -K_b from the plot of 1/A of verses 1 /([Q])[12]. Both the adsorption and emission measurements are used to display the Benesi-Hildebrand plot for the metal DNA complex.

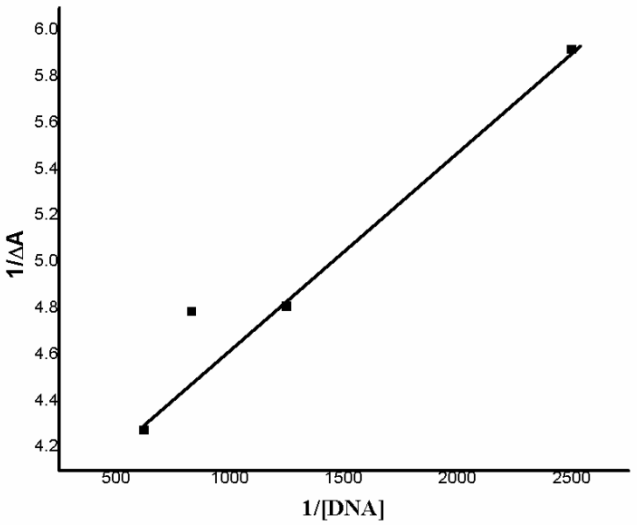


Figure-5: Benesi-Hildebrand plots for the binding of $[Ru(dmbpy)_3]^{2+}$ with incremental addition of Onion DNA in aqueous medium.

Table-2: Binding Constant K_b (M^{-1}) for DNA with $[Ru(NN)_3]^{2+}$ complexes in aqueous medium.

Complex	Binding Constant(M^{-1})	
	Absorption measurement	Emission measurement
$[Ru(bpy)_3]^{2+}$	2.58 x10^3	4.59 x10^3
$[Ru(dmbpy)_3]^{2+}$	3.23 x10^3	4.55 x10^3

The red shift was produced by the addition of onion's extracted DNA. The ground state contact between isolated DNA and the bipyridyl rings of the luminophore is characterised by hydrophobic or stacking interactions. The interaction between stacking and binding makes binding stronger. When going from $[Ru(bpy)_3]^{2+}$ to $[Ru(dtbpy)_3]^{2+}$, the hydrophobicity increases. Because of the bulky alkyl to substituted ligands' presence, this is due to the presence of bulky alkyl group and substituted ligands in this case. With an increase in hydrophobicity, K_b value rises. This finding demonstrates that k_b values depend on how hydrophobic the ligands are. According to the findings, the $[Ru(RR)_3]^{2+}$ complex strongly binds to onion DNA, and the K_b is influenced by the DNA's purity and the ligands in the complex. Using absorption spectral measurement, the binding constants for $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ complexes with the DNA of onions are 2.584 x 10^3 and 3.236x10^3 M^{-1}, respectively. At the LC and MLCT regions, it is discovered that the K_b of the complex with onion DNA is 4.9x10^3 and 1.09x10^3, respectively. This shows that the MLCT region of the DNA is where the complex interacts to it rather than the LC region. There aren't many differences in the binding constant value obtained using emission spectral measurement. In comparison to the bipyridyl complex, $[Ru(dmbpy)_3]^{2+}$ shows better binding effectiveness with onion DNA. The interaction of these DNA molecules with Ru polypyridyl complexes presents a route to treating cancer cells because onions have antitumor properties.

Conclusion

Using absorption and emission spectrum methods, the binding of Ruthenium polypyridyl complexes with DNA isolated from onions has been investigated. The $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ complexes with onion DNA reveal Kb values of 2.584 x 103 and 3.236 x 103 M^{-1}, respectively. The represented values demonstrate an affinity for onion DNA binding in both electrostatic and intercalative modes.

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