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DNA binding, DNA cleavage and BSA interaction of Vanadium-Morin complex

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Abstract - **Several oxovanadium(V) complexes have been shown as a potent anticancer agents. In view of the importance of Vanadium complexes a new Vanadium – Morin complex was synthesized and comprehensively characterized. To provide an insight on the mode and affinity of the interaction of these complex with calf thymus (CT) DNA the following experiments were carried out: fluorescence spectroscopy, UV–vis absorption, viscosity and circular dichoroic measurements. The cleavage properties of these complex with super coiled pBR322 DNA have been studied using the gel electrophoresis method, where the complex displayed chemical nuclease activity in presence of H_2O_2, presumably *via* an oxidative mechanism. These experimental results indicate that the complex interact through stacking between the base pairs of double helix DNA and the binding constants (K_b) of the synthesized complex was found to be 6.6 x 10^4 M^{-1}. In addition, the DNA cleavage activity of the complex was determined using agarose gel electrophoresis and the results show that complexes have potent nuclease activity.**

Keywords: Vanadium – Morin; DNA binding and cleavage; oxidative cleavage; Intercalation mode;

I. Introduction

The deoxyribonucleic acid (DNA) is a biomacromolecule that encodes the genetic information necessary for the development and functioning of all known living organism. A plethora of chemotherapeutic anticancer agents currently used is reversibly targeted to DNA [1]. In order to develop a new anti-cancer drug, the binding mechanism of the complex with DNA should be studied. The intercalative mode is the most important mode in which transition metal complexes can intercalate between the base pairs of double helix DNA, forming a π-π overlapping interaction. It is the interaction that generally affects or changes the DNA conventional behavior and so the transition metal complexes possess a very broad application background in the field of bio-inorganic chemistry [2]. The developments of compounds cleaving DNA under physiological conditions is of current interest, due to their potential applications in genomic research and as foot printing and therapeutic agents [3].

On the other hand, serum proteins play an essential role in the transport and metabolism of drug. Therefore, the interactions of metallo-drug with serum albumins have caught more and more attention in the scientific community by studying antitumoral metallo-pharmaceutical pharmacokinetics and structure−activity relationships pharmaceutical pharmacokinetics and structure−activity relationships [4]. Among various serum albumin, bovine serum albumin (BSA) is the most extensively studied owing to its structural homology with human serum albumin (HSA) [5]. Studies on the binding of small molecules to albumins may provide useful structural information that determines the therapeutic effect of drugs. Therefore, the investigation on the binding of such molecules with BSA is of imperative and great importance in life sciences, chemistry, and clinical medicine [6].

Platinum (II) complexes are used as anticancer drugs since long and among them cisplatin as proven to be a highly effective chemotherapeutic agent for treating various types of cancers like ovarian, testicular, head and neck carcinomas[7] with more acceptable toxicity profiles because the clinical applications of cisplatin and its analogs are hindered by some major problems such as drug resistance and systemic toxicity [8]. Therefore, several attempts have been devoted to develop alternative strategies, based on different metals, with improved pharmacological properties and aimed at different targets. Among the various transition metal ions used in pharmacological studies, Vanadium is reported to have biological response in lowering of cholesterol, triglycerides, contraction of blood vessels, enhancement of oxygen-affinity of hemoglobin and myoglobin [9]. Researches on the vanadium complexes have received much attention owing to their ability of binding and cleaving DNA under physiological conditions, and they can be potentially applied as identifying cytotoxic agents with potent activity against cancer cells and probes for genomic research as well as for conformational studies of nucleic acid [10].

As an important class of natural products, Flavonoids have received considerable attention because of their health benefits and chemopreventive properties [11]. Flavonoids are phytochemicals that exist either as free aglycones or as glycosidic conjugates. Chemically, they are polyphenolic and possess a phenyl benzopyrone structure (C6–C3–C6) categorized mainly into flavones, flavanols, isoflavones, flavonols,flavanones, flavanonols and chalcones based on the saturation level, C-ring substitution pattern and opening of the central pyran ring [12]. Polyphenolic compounds, mainly flavonols are possesses metal chelating properties. Morin (3,5,7,20,40-pentahydroxyflavone) is a flavonol that has been identified in fruits, vegetables, tea, wine, and many Chinese herbs which exhibits a variety of pharmacological activities such as antioxidant activity, antinociceptive activity and anti-tumor activity [13].

The present study is focused on an endeavor to explore the important and pertinent issue of binding interaction of Vanadium-morin complex [14] with DNA. DNA interactions of the complex with calf-thymus DNA (CT-DNA) were investigated using absorption and emission spectral titrations, circular dichroic, viscosity experiments and the cleavage experiments with pBR322 supercoiled plasmid DNA have been assayed using agarose gel electrophoresis. Furthermore, UV–Vis and fluorescence spectroscopy have been used to evaluate the binding behavior of the complex with BSA. Systematic studies of the complex may facilitate the molecular design of DNA inhibitors and eventually lead to the development of an anticancer agent.

II. Materials and methods

All chemicals were purchased from commercial sources, and used as received without further purification. UV–Visible spectra were recorded using Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200–500 nm with quartz cells and ε values are expressed in M-1 cm-1. The emission spectra were recorded on a Perkin Elmer LS-45 fluorescence spectrometer. Viscosity measurements were recorded using a Brookfield Programmable LV DVII+ viscometer. Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared using deionized and sonicated triple distilled water. The supercoiled pBR322 DNA and Calf thymus (CT) DNA were procured from Bangalore Genie (India). Circular dichoric spectra of CT-DNA were obtained using a JASCO J-715 spectropolarimeter equipped with a Peltier temperature control device at 25 ± 0.1°C with 0.1 cm path length cuvette.

A. DNA-binding experiments

Absorption spectrophotometric studies

Absorption spectra titrations were performed at room temperature in Tris–HCl/NaCl buffer (50 mM Tris–HCl/1 mM NaCl buffer, pH 7.5) to investigate the binding affinity between CT-DNA and complex. The absorption titration experiments were conducted by keeping the concentration of the complex constant (10 μM) while varying the DNA concentration from 0 – 200 μM. The complex-DNA mixing solutions were incubated for 1 h before the absorption spectra were recorded [15]. The intrinsic binding constant Kb was calculated from the spectroscopic titration data using the following equation:

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

Where ε_a is the extinction coefficient observed for the charge transfer absorption at a given DNA concentration, ε_f the extinction coefficient at the complex free in solution, ε_b the extinction coefficient of the complex when fully bound to DNA, K_b the equilibrium binding constant, and [DNA] the concentration in nucleotides. A plot of [DNA]/ (ε_a - ε_f) versus [DNA] gives K_b as the ratio of the slope to the intercept. The non-linear least square analysis was performed using Origin lab, version 6.1.

B. Emission spectrophotometric studies

The relative binding of complex to CT-DNA was determined with an EB-bound CT-DNA solution in Tris–HCl/ buffer (pH = 7.2, 5 mM Tris–HCl, 50 mM). The quenching constant K_{sv} was deduced from Stern-Volmer method where the ratio of fluorescence of the compound alone (I_0) over the fluorescence of the compound in the presence of CT-DNA (I) is presented as a function of CT-DNA concentration.

$$I_o/I = 1 + K_{sv} [r]. \quad (2)$$

Where I_0, is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K_{sv} is a linear Stern – Volmer quenching constant and r is the ratio of the total concentration of quencher to that of DNA, [M] / [DNA]. A plot of I_0 / I vs. [complex]/ [DNA], K_{sv} is given by the ratio of the slope to the intercept. The apparent binding constant (K_{app}) was calculated using the equation K_{EB}[EB] / K_{app}[complex], where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and $K_{EB} = 1.0 \times 10^7$ M^{-1} ([EB] = 3.3 μM) [16].

C. Viscosity measurement studies

Titrations were performed for the complex (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM), and each compound was introduced into DNA solution (0.5 mM) present in the viscometer. Data were presented as (η/ηο) versus binding ratio of concentration of complex to that of concentration of CT-DNA, where η is the viscosity of DNA in the presence of complex and ηo is the viscosity of DNA alone.

D. Circular dichoric spectral studies

The conformational changes of CT-DNA induced by complex were monitored by CD spectroscopy in the presence of Tris– HCl buffer at room temperature. The spectrum were recorded in the region of 220-320 nm for 200 μM DNA in the presence of 100 μM of complex.

E. DNA cleavage study

The DNA cleavage experiments were carried out by the agarose gel electrophoresis method, by incubation at 37°C. In the procedure, pBR322 DNA (0.1 lg/lL) in 50 mM Tris–HCl buffer (pH 7.2) was treated with the complex containing 1% DMF. The samples were subjected to electrophoresed for 3 h at 50 V on a 0.8% agarose gel in Tris–acetic acid-EDTA buffer. Bands were visualized by UV Transilluminator and photographed. The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its supercoiled (SC) form. The extent of cleavage of SC DNA was determined by measuring the intensities of the bands using a UVITECH Gel Documentation System.

III. Result and discussion

A. Absorption spectral studies

The interaction of Vanadium-morin complex with calf-thymus DNA was monitored by UV–visible spectroscopy. The absorption spectra of complex in aqueous solutions were compared in the absence and in the presence of CT-DNA are shown in the **Fig (1).** respectively. Binding of the complex to DNA lead to perturbation in their ligand centered band. The extent of the hypochromism in the absorption band is generally consistent with the strength of intercalative binding/interaction [Ref 14]. As expected, we have observed a minor bathochromic shift along with significant hypochromicity. These observations can be rationalized by the following reasons. When the complex intercalate the base pairs of CT-DNA, the π*-orbital of the intercalated ligand in the complexes can couple with the π-orbital of the base pairs of CT-DNA, thus decreasing the π–π * transition energy and resulting in bathochromism. Furthermore, the coupling π-orbital is partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism. The absorption peaks at 246 nm and 378 nm for the complex are attributed to intraligand π–π* transition. The calculated intrinsic binding constant (K_b) for the complex with CT-DNA (insets figures) were determined to be 6.6 x 10^4 M^{-1} respectively using Equation (1). Although the electronic absorption studies have confirmed that the complex can bind to DNA by intercalation, it is necessary to carry out other experiments to prove the binding mode.

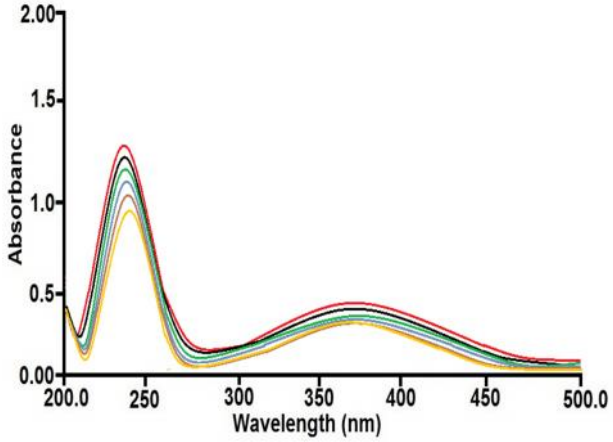

Figure 1. Absorption spectrum of complex (10 μM) in 5 mM Tris-HCl/ 50mM NaCl buffer at pH 7.5 in the absence and presence of increasing amounts of DNA. Inset shows the least-squares fit of [DNA]/ε_a-ε_f vs. [DNA] for the complex.

B. Emission spectral studies

In order to further investigate the interaction mode of complex with DNA, a competitive binding experiment using EB as a probe was carried out. EB does not show any appreciable emission in the buffer solution due to fluorescence quenching of the free EB by solvent molecules, while in the presence of CT DNA, the fluorescence intensity of EB is highly enhanced due to its strong intercalation between the adjacent DNA base pairs [17]. EB shows an increase in the emission intensity due to its intercalative binding to CT-DNA. The changes in the fluorescence intensity at 611 nm (excitation at 545 nm) of EB-CT-DNA were measured with respect to the concentration of the complex. On addition of CT-DNA to complex, the fluorescence intensity decreased with increasing the concentration of CT-DNA without any change in the shape and position of the fluorescence peaks as depicted in **Fig (2)**. Such a decrease in the intensity is called fluorescence quenching. The extent of reduction of the emission intensity gives a measure of the binding propensity of the complexes to CT-DNA. The K_{sv} value of complex was calculated from the quenching plots. The apparent binding constants (K_{app}) at room temperature were calculated to be 6.9 x $10^5 M^{-1}$.

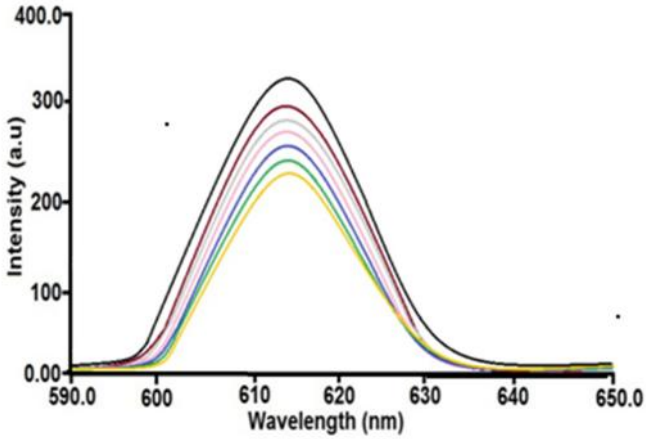

Figure 2. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of I_0/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex.

C. Viscosity studies

To further clarify the nature of the binding interaction between both complexes and DNA, viscosity measurements were carried out on CT-DNA by varying the concentration of the added complexes. Spectroscopic data are necessary, but not sufficient to support a binding mode. Classical intercalative mode causes a significant increase in viscosity of DNA solution due to separation of base pairs at intercalation sites and increase in overall DNA length. In contrast, complex those bind exclusively in the DNA grooves typically cause less positive or negative or no change in DNA viscosity [18]. The relative specific viscosity of DNA increases with increase in the concentration of the complex revealing strong evidence for the interaction of the complex with CT-DNA by intercalation mode as represented in **Fig (3)**.

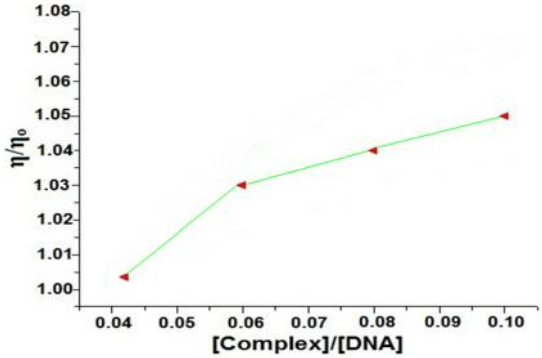

Figure 3. Plot of relative viscosity Vs [complex] /[DNA]

D. Circular dichronism studies

The CD spectral analysis is very useful when it comes to the investigation of morphological changes in DNA double strands due to the interaction with small molecules. The band at 275 nm, due to base stacking, and at 246 nm, due to right handed helicity of the strands, are very sensitive towards the interaction with such small molecules. Any change in the base stacking pattern or the helicity of the strands is manifested by a change in the band position, the intensity, or both. A simple electrostatic interaction or groove binding has nearly no or a very insignificant effect on the band at 275 nm, whereas the same band undergoes considerable change in intensity due to intercalation by small molecules [19]. The intensity increase of the band at 275 nm was less pronounced for the synthesized complex as shown in **Fig (4)**. This indicates that the complex interacts with the DNA double strands by the intercalative mode between the base pairs of DNA strands without any significant change in the right-handed helicity of the DNA.

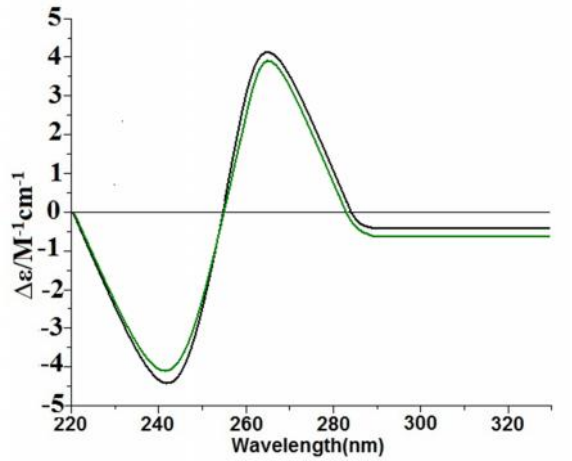

Figure 4. Circular dichoric measurements of the complex.

E. DNA cleavage experiments

The nuclease activity of the complex has been précised by means of the electrophoretic mobility of pBR322 plasmid DNA incubated with complex. The complex was incubated with DNA at identical conditions. The influence of the complex on the tertiary structure of DNA was determined by its ability to modify the electrophoretic mobility of supercoiled form (Form I, SC) to nicked circular form (Form II, NC) or linear open circular form (Form III, LC). Alteration of the DNA structure causes retardation in the migration of supercoiled DNA and a slight increase in the mobility of open circular DNA to a point where both forms comigrate [20].

In the present study, the gel electrophoresis experiment was carried out at room temperature using our synthesized complex in the presence of H_2O_2 as an oxidant. It was found that, at very low concentrations, few complexes exhibit nuclease activity in the presence of H_2O_2. From the results of **Fig (5),** at higher concentration the Vanadium-morin complex showed better nuclease activity. Control experiments using H_2O_2 did not show any significant cleavage of pBR322 DNA (lane 1). At and above the concentration of 40 µM and 50 µM, complex is able to convert the initial SC (Form I) to NC (Form II) (lane 4 and 5).

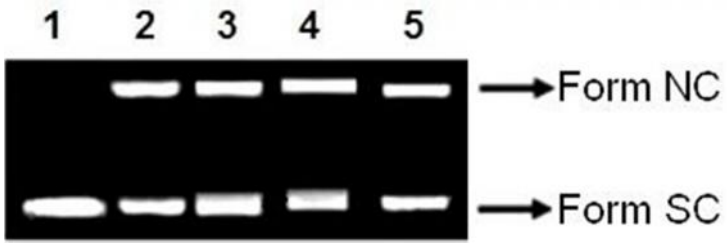

Figure 5. Gel electrophoresis diagram showing the cleavage of pBR322 DNA (0.1 lg/lL) in 50 mM Tris–HCl buffer (pH 7.2) in the presence of H_2O_2 : lane 1. DNA control; lane 2. DNA+ 10 µM (Vanadium-Morin complex); Lane 3. DNA+ 25µM (Vanadium-Morin complex); Lane 4. DNA+ 40 µM (Vanadium-Morin complex); Lane 5. DNA+ 50 µM (Vanadium-Morin complex).

IV. CONCLUSION

In summary, the binding interaction between the Vanadium-Morin complex and CT-DNA has been sustained by using UV-Visible, fluorescence, viscosity and circular dichroic measurements. The obtained results collectively showed that the synthesized complex binds to CT-DNA by an intercalating mode. Noticeably the synthesized complex 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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