DNA Cleavage Activity of Ruthenium(II)-Phenanthroline-Phendione Complexon *Escherichia Coli* Genomic DNA

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ABSTRACT

The DNA cleavage activity of $[Ru(phen)_2(phendione)]^{2+}$ (phen = 1,10-phenanthroline and phendione =1,10-phenanthroline-5,6-dione)complex has been investigated on Escherichia coli genomic DNA using Agarose Gel Electrophoresis method. The synthesized complex shows a metal to ligand charge transfer (MLCT) absorption peak at 440 nm in aqueous medium. The DNA cleavage study of the complex is carried out in three different concentrations against an untreated Escherichia coli genomic DNA as control. The extent of cleavage of the DNA is determined by measuring the intensities of the band using UV transilluminator. DNA cleavage studies indicate that the complex shows full cleavageat 50 and 100 µg. The electrophoretic migration examines the pattern for the cleavage by analysing in three lane forms (1,2,3) against the control DNA. The efficiency of the cleavage in the Lane is in the order 2 = 3 > 1. The two complexescontaining phendione ligand have two carbonyl groups attached to the 5th and 6th position which has the ability to cleave the DNA by the formation of semiquinone-type radical. The cleavage occurs due to the proton coupled electron transfer to a photoexcited hydrated Ru(ll)-phenanthroline-phendione complex. Thus, the complex taken in the present investigation inhibits the growth of the pathogenic organism by cleaving the genome.

Keywords: *$[Ru(phen)_2(phendione)]^{2+}$complex, Escherichia coli genomic, DNA cleavage, Semiquinone-type radical, Protoncoupled electron transfer*

1. Introduction

Transition metal complexes have been extensively studied due to their potential applications in biological processes, catalysis, molecular reorganization and host-guest chemistry [1]. Transition metal complexes that are suitable for binding and cleaving the DNA are of considerable interest due to their various applications in nucleic acid chemistry, for modelling the restriction enzymes in genomic research and as structural probes for therapeutic applications in cancer treatment [2]. The metal complexes can bind to DNA through the noncovalent way on the groove, by intercalation and electrostatic binding. Metal complexes can cleave the DNA through three types of mechanisms, which are hydrolytic, oxidative and photolytic cleavages [3]. Metal complexes have been mainly investigated for DNA cleavage, as they offer the advantage of selectivity in DNA cleavage by modulating the nature of both the metals and the ligands respectively. A number of transition metal complexes have attracted attention as potential photodynamic therapy (PDT) agents for cancer treatment, as they often have a high affinity for DNA and accessible photoexcited states in the visible region. Most commonly, the PDT agent works by activation of cellular O_2 to form reactive oxygen species (ROS), which are responsible for inducing apoptosis via DNA cleavage. A large variety of metal complexes have been studied as DNA photocleavage agents [4]. Only a few transition metal complexes are able to cleave DNA upon photoexcitation [5].

DNA plays an important role in the life process since it contains all the genetic information for cellular function. However, DNA molecules are prone to be damaged under various conditions

like interactions with some molecules. This damage may cause various pathological changes in living organisms, which is due to their possible application as new therapeutic agents and their photochemical properties which make them potential probes of DNA structure and conformation [6]. Since DNA is particularly sensitive to oxidative cleavage, the vast majority of the studies on metallonucleases has focused mainly on the molecules that cleave DNA oxidatively. Hence, it is clear that the nature of the ligand and the metal play a pivotal role in their interaction with DNA molecule [7]. DNA is the primary target molecule of many anticancer agents and the binding between DNA and metal complexes are used in understanding the interaction between the drugs and DNA. Cleavage of DNA can be achieved by targeting its basic constituents like base and/or sugar by an oxidative pathway or by hydrolysis of phosphoester linkages. DNA photocleavage can occur by a wide variety of mechanisms such as hydrogen atom abstraction from the sugar ring by photochemically generated radicals, direct electron transfer from the base (usually guanine) to the photoexcited cleaver, singlet oxygen production by transfer of energy from the excited photocleaver and formation of base adducts [8]. Isolation of the genomic DNA (g-DNA) is one of the most important and common experiment that is carried out in molecular biology and includes the transition from cell biology to molecular biology [9]. Most organisms have the same g-DNA in every cell; however, only certain genes are active in each cell to allow for cell function and differentiation within the body.

There is a considerable interest in the interactions of Ru(II) complexes with DNA. Ru(II) complexes are usually used as DNA structural probes, artificial nucleases, DNA molecular light switches and DNA-targeting drugs [10]. Ruthenium complex with 1,10-phenanthroline (phen) or 2,2'-bipyridine (bpy) and their derivatives are also extensively studied due to their interesting physico-chemical and biological properties [11]. Phenanthrolines are a class of compounds with an entirely different mode of action of interest for their potential activity against cancer as well as viral, bacterial and fungal infections. The DNA cleaving ability of phenanthroline has made it a frequently used reagent in DNA foot printing studies. One of the phenanthroline derivative, 1,10-phenanthroline-5,6-dione (phendione), displays significant anticancer activity with and without a coordinated metal [12]. Based on the literature survey, the present study focuses on the DNA cleavage activity of $[Ru(phen)_2(phendione)]^{2+}$ (phen = 1,10-phenanthroline and phendione = 1,10-phenanthroline-5,6-dione) complex on *Escherichia coli* (E. coli) genomic DNA by Agarose gel electrophoresis method.

2. Materials and Methods

2.1 Materials

$RuCl_3.3H_2O$, ligands (1,10-phenanthroline and 1,10-phenanthroline-5,6-dione) and ammonium hexafluorophosphate were procured from Sigma-Aldrich. HPLC grade solvents were used for the synthesis of the complex. The complex $[Ru(phen)_2(phendione)]^{2+}$ was synthesized by

reacting the $[Ru(phen)_2Cl_2]2H_2O$ with phendione according to the procedure previously described [13]. The genomic DNA was isolated from *E. coli* bacteria cells and the isolated genomic DNA was used for the DNA Cleavage studies.

2.2 Determination of DNA Purity

About 10 µL of genomic DNA isolated from the *E. coli* bacterial source was taken in a cuvette and mixed with 990 µL of nuclease free water (Dilution factor 100). For setting blank 1 mL of nuclease free water was taken in another cuvette. The absorbance of DNA was measured at 260 nm and 280 nm and its ratio (A260/A280) was calculated [14].

2.3 DNA Cleavage Study

The *E. coli* genomic DNA was prepared in nuclease free water followed by dilution of DNA stock solution in 0.1 M sodium phosphate buffer (pH 7.4). The DNA (1.5 µg) prepared in nuclease free water was treated with 25, 50 and 100 µg of the test sample prepared in DMSO in a reaction volume of 15 µLand incubated at 37°C for 2 h. After incubation the treated as well as control DNA were subjected to agarose gel electrophoresis in 0.5 % TAE (mixture of tris base, acetic acid and EDTA) buffer for 30 minutes. The DNA cleavage activity of test samples were studied using gel electrophoresis method [15].

2.4 Visualization of DNA by Agarose Gel Electrophoresis

About 1g of agarose was weighed and dissolved in 100 mL TAE buffer by heating and constant stirring in a water bath at 95°C. After cooling (35°C) add 2 µL of (10 mg/mL) Ethidium bromide solution into it and caste the gel with the test sample. After solidifying the gel remove the comb carefully and transfer the gel into the electrophoretic apparatus containing TAE buffer. Appropriate amount of DNA sample was mixed with DNA loading dye and loaded in the wells. The gel was allowed to run at 50 V for half an hour. After the run, the gel was analyzed under UV transilluminator, DNA bands were observed and photographed [16,17].

3. Results and Discussion

The DNA cleavage activity of $[Ru(phen)_2(phendione)]^{2+}$complex has been investigated with *E. coli* genomic DNA using Agarose gel electrophoresis method and the obtained results are discussed in this section. The structure of the complex used in the present study is shown in **Fig.1**.

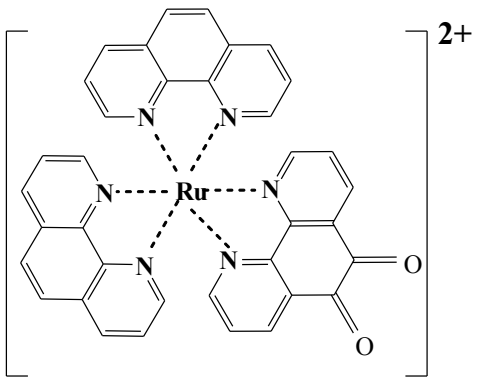

Fig.1 Structure of $[Ru(phen)_2(phendione)]^{2+}$complex

3.1 UV Absorption Analysis of $[Ru(phen)_2(phendione)]^{2+}$ Complex

The absorption spectrum of $[Ru(phen)_2(phendione)]^{2+}$ complex in aqueous medium shows a high energy absorption at 286 nm corresponding to the ligand centered π - π* transition and the low energy absorption at 440 nm assigned to the dπ - π* metal to ligand charge transfer (MLCT) transition (**Fig.2**). The MLCT transition involves electronic excitation from the metal orbital [dπ (Ru)] to the ligand centered acceptor π* orbitals. The absorption bands obtained for $[Ru(phen)_2(phendione)]^{2+}$ complex is in accordance with the reported values [13].

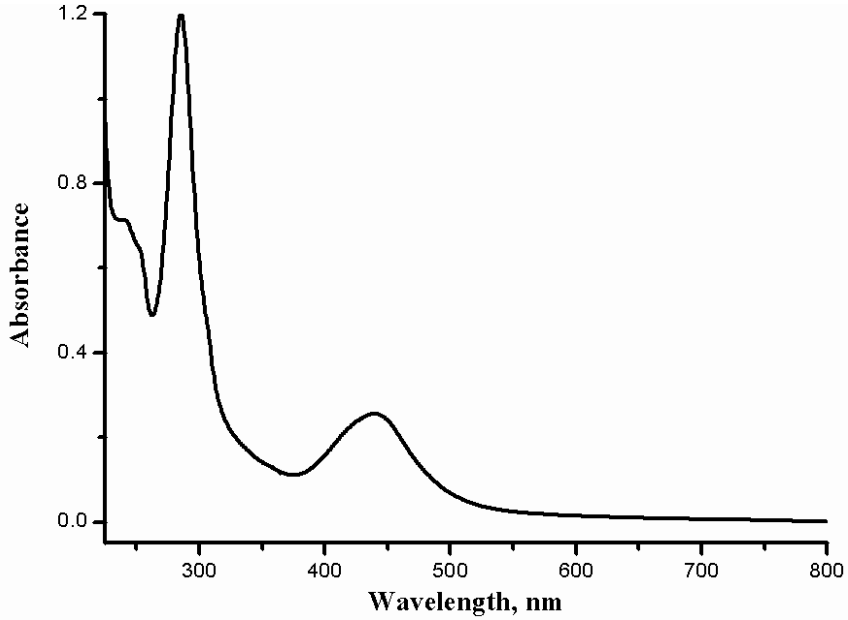

Fig. 2 Absorption spectrum of $[Ru(phen)_2(phendione)]^{2+}$ complex in aqueous medium

3.2 Determination of DNA Purity

The purity of genomic DNA isolated from *E. coli* bacteria cells is determined by taking the ratio of absorbance at 260 nm to the absorbance at 280 nm. The absorbance of DNA measured at 260 nm and 280 nm are 0.17 and 0.1 respectively. The ratio of A260/A280 of the genomic DNA isolated from *E. coli* bacteria cells is 1.7. The obtained result shows that the DNA isolated from E. coli is of good quality, good for downstream studies and used for further DNA cleavage experiments. If the A260/A280 for any DNA goes below 1.6 and above 2.0 the DNA is not suitable for further molecular biology experiments [14].

3.3 DNA Cleavage Activity

The DNA cleavage activity of the $[Ru(phen)_2(phendione)]^{2+}$ complex on *E. coli* genomic DNA is performed in three different concentrations (25, 50and 100μg)against the control DNA (1.5 μg) by agarose gel electrophoresis method. The cleavage of genomic DNA in the presence of this complex is determined from the band intensity. The $[Ru(phen)_2(phendione)]^{2+}$ complex shows no band intensity in lane 2 and 3 indicates full cleavage, whereas in lane 1 the band intensity is not

disappeared and remains the same when compared with the control DNA, this indicates no cleavage (**Fig. 3**).

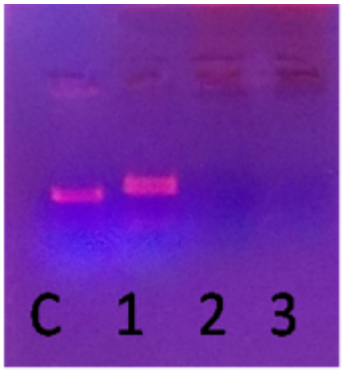

Fig.3 Ethidium bromide stained photocleavage products of $[Ru(phen)_2(phendione)]^{2+}$ complex with genomic DNA of *E.coli*. C- control DNA, 1 - sample (25 µg) + *E. coli* genomic DNA, 2 - sample (50 µg) + E. coli genomic DNA and 3 - sample (100 µg) + E. coli genomic DNA

The cleavage activity of the synthesized complex on *E.coli* genomic DNA in the lane is in the order 2 = 3 > 1. Hence the synthesized complex inhibits the growthof *E.coli* by cleavingits genomic DNA at 50 and 100µg concentrations. The phendione ligand in the $[Ru(phen)_2(phendione)]^{2+}$ complex have two carbonyl groups attached to the 5^{th} and 6^{th} position which has the ability to cleave the *E.coli* genomic DNA by the formation of semiquinone-type radical. The complex abstracts a proton from the DNA base pair and converted into its hydrated form. The cleavage occurs due the proton coupled electron transfer to a photoexcited hydrated $[Ru(phen)_2(phendione)]^{2+}$complex. This result is in accordance with the previously reported study on the photocleavage of Ru(II) complex in plasmic DNA [5]. Thus, the complex taken in the present investigation inhibits the growth of the E. coliby cleaving the genome.

Conclusion

The DNA cleavage activity of the synthesised $[Ru(phen)_2(phendione)]^{2+}$complex is investigated in *E.coli* genomic DNA by Agarose gel electrophoresis method. The cleavage of genomic DNA in the presence of this complex is determined from the band intensity. DNA cleavage studies indicate that 50 and 100 µg concentrations of the complex show full cleavage whereas 25 µg shows no cleavage. The formation of semiquinone-type radical from the two carbonyl groups attached to the 5^{th} and 6^{th} position of phendione ligand cleaves the *E.coli* genomic DNA. The cleavage occurs due to the proton coupled electron transfer to a photoexcited hydrated $[Ru(phen)_2(phendione)]^{2+}$ complex. Thus, the complex taken in the present investigation cleaves the E.coli genomic DNA and it inhibits the growth of the pathogenic organism by cleaving its genome.

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