CHAPTER - 4

DNA CLEAVAGE STUDY
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INTRODUCTION

Due to their diverse applications in bioinorganic chemists’ metal complexes that can bind to DNA are gaining considerable attention viz. diagnostic agents for medical applications, development of cleavage agents for probing nucleic acid structure and identifiers of transcription start sites [1-15]. DNA is an important target of antitumor drugs and interactions of DNA with transition metal complexes are important for the design of efficacious drug entities which exhibit different properties than the mainstream protocol drugs viz. cisplatin, etc. which are currently in use. Copper have been used since antiquity in metal-based therapies. Copper is a bioessential element which plays a key role in biological processes, and its complexes are preferred molecules for cancer inhibition by chemotherapy [16-25].

Among the copper complexes explored so far, considerable attention has been focused on 1,10- phenanthroline Cu(II) complexes due to their high nucleolytic efficiency and numerous biological activities such as antitumor, antimicrobial, etc. Several copper complexes have been described as DNA cleaving agents and the best studied example is Sigman’s complex. This complex is reduced in situ and subsequently binds to minor groove of DNA, combines with molecular oxygen, to generate a non-diffusible oxidant and finally induces strand scission by oxidation of the ribose backbone. Literature supports that Co(II) ions specifically bind to the N-7
guanine residue of DNA and cause strand breakage. The kinetic analysis of copper DNA interaction and its site-specific binding with DNA have been well documented. On the contrary, Sn(IV) complexes prefer to bind to the phosphate backbone of the DNA helix have a hard Lewis acid nature, neutralize the negative charge of phosphate sugar, and bring conformational changes in DNA [26-31].

Previous studies of Cu(II) and Cu(II)-Sn(IV) complexes have shown interesting results against various cancerous cell lines (HeLa cells, T47D, HT29). It has been demonstrated that these complexes induce apoptosis via mitochondrial pathway. Thus, heterobimetallic complexes containing Cu(II) and Sn(IV) ions enhance the chemotherapeutic action many-fold as they provide a dual mode of binding at the molecular target site and also exhibit novelty due to preferential selectivity. Furthermore, some additional favorable non-covalent interactions such as hydrogen bonding, van der Waals forces within the groove of DNA can enhance DNA binding multi-fold and enforce specificity [32]. In this section we have tried to find the DNA cleavage activity using CTDNA in order to interpret the cleavage activity of the compounds against microbial DNA.

**EXPERIMENTAL**

**In vitro DNA cleavage analysis protocol**

CT DNA cleavage activity studies of N-hydroxyamidine and their metal ion complexes. Gel electrophoresis was performed by taking 30 µM CT-DNA, 50-70 µM metal complex and 50 µM hydrogen peroxide (H₂O₂) in Tris–HCl/NaCl buffer (pH 7.2) at 37 °C for 2 hr. After incubation, the samples were electrophoresed for 2 hr at 50V on 1% agarose gel using Tris–acetic acid–EDTA buffer (pH 7.2). The gel was then stained using 1 µg cm⁻³ ethidium bromide
(EB) and photographed under ultraviolet light at 360 nm. The compounds were taken to an appropriate amount to study the nucleolytic behavior of these compounds. DNA was uncoiled interacting with the different amount of the sample. The results shows that are able to convert supercoiled to nicked circular thus revealed that metal complexes and ligand behave as efficient chemical nuclease for double strand cleavage of DNA [33, 34].

RESULTS AND DISCUSSION

1. 2-amino-5-nitro-N-hydroxybenzamidine (ANHB) and their metal ion complexes

2-amino-5-nitro-N-hydroxybenzamidine (ANHB) Ligand and their metal(II) complexes were studied for their DNA cleavage activity, the results showed that H2O2 alone shows no cleavage. DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) under anaerobic conditions. No DNA cleavage was observed for the control in which metal complex was absent (lane 1). All the complexes can induce the obvious cleavage of the plasmid DNA at the concentration of 50 µM.

![Gel electrophoresis diagram showing the cleavage of CT DNA by Cr(II), Zn(II) and Co(II) complexes in a buffer containing 50 µM Tris–HCl and 50 µM NaCl in the presence of H2O2 at 37 °C. Lane 1, DNA control; lane 2, DNA + H2O2; lane 3, DNA + [ligand(L)] + H2O2; lane 4, DNA + H2O2 + [CrL2]; lane 5, DNA + H2O2 + [CoL2]; and lane 6, DNA + H2O2 + [ZnL2].](image-url)
At these concentrations, complexes can almost promote 75-90% conversion of supercoiled DNA to nicked and linear DNA (Form I-III). It was observed that at lower concentrations, DNA cleavage was not effective.

The studies revealed that Cr (II) and Zn(II) complexes exhibit much higher cleaving efficiency for CTDNA. The nucleolytic efficiency is shown in the (Fig.1), the supercoiled CTDNA was completely converted to form II and form III. All the compounds except ligand are able to convert supercoiled (Form I) to nicked circular (Form II and Form III) leading to the conclusion that metal complexes behave as efficient chemical nuclease for double strand cleavage of DNA. The Co(II) complexes cleaves the CTDNA less actively than Zn(II), Lane 6. These phenomena imply that metal complexes induced intensively the cleavage of CT DNA in the presence of H$_2$O$_2$. It was found that Zn(II) complex was more potent nucleolytic agent as compared to ANHB ligand, Cr(II) and Co(II) complexes. Chemical environment and their geometric structures may also affect the nucleolytic efficiency of Cr(II) complexes. The different DNA-cleavage efficiency of the complexes may be considered due to the different binding affinity of the complexes to DNA.

2. 3-methyl-N-hydroxybenzamidine (MHB) and their metal ion complexes

3-methyl-N-hydroxybenzamidine (MHB) ligand and their Zn(II), Co(II) and Cr(II) complexes were studied using the gel electrophoresis. It was found that separation (Fig.2.) of CT DNA by Co(II) complex shows it’s excellent cleavage activity (70 µM) against CT DNA as compared to other metal(II) complexes and ligand. Form 1 denotes the coiled stage of CT DNA, Form 2 exhibits the first uncoiled stage and single fragmentation by the compounds. Form 3 denotes the second uncoiled stage due to the nucleage activity of the metal complexes (Fig.2).
Fig. 2. Gel electrophoresis diagram showing the cleavage of CT DNA (50 µM) by ligand, Co(II), Cr(II) and Zn(II) complexes (70 µM) in a buffer containing 50 mM Tris–HCl and 50 mM NaCl at 37 °C. Lane I, DNA control; lane II, DNA + [ligand(L)]; lane III, DNA + [CoL₂]; lane IV, DNA + [CrL₂]; and lane V, DNA + [ZnL₂].

3. 2-amino-5-nitro-N-hydroxybenzamidine (ANHB) and their metal ion complexes

With 2-amino-5-nitro-N-hydroxybenzamidine (ANHB) ligand and its metal complexes in the presence of H₂O₂ as an oxidant, at micro molar concentrations, Lane 2 exhibits no significant cleavage activity corresponding to ligand. The nuclease activity is greatly enhanced by the incorporation of metal ion in the respective ANHB ligand. It is evident from Fig. 3, that the complexes cleave DNA more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals. These hydroxyl free radicals participate in the oxidation of
the deoxyribose moiety, followed by the hydrolytic cleavage of the sugar phosphate backbone. There is a considerable increase in the intensity of bands for open circular form in the case of Cr(II); lane 4 and Zn(II); lane 6 complexes compared to that of Co(II); lane 5 complexes. Lane 3 indicates ligand activity as compared to lane 1 and lane 2 for DNA control and DNA+H₂O₂. It was concluded that Co(II) complex has better DNA cleavage activity compared to other compounds.

4. N-hydroxy-4-[(hydroxyimino)methyl]benzamidine (HIMB) and their metal ion complexes

DNA cleavage N-hydroxy-4-[(hydroxyimino)methyl]benzamidine (HIMB) and their metal ion complexes was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) under anaerobic conditions. No DNA cleavage was found for the lane 1 and Lane 2 which incorporates with the DNA control and DNA + H₂O₂ respectively (Fig.4). It was observed that ligand at 70 μM concentration can induce almost 80% supercoiled DNA to linear and nicked DNA (lane 3). It was shown that Cr(II) and Co(II) complexes can almost promote 65% cleavage at their maximum concentration of 50 μM. At lower concentration no cleavage was observed for these complexes. No significant conversion od CTDNA was observed for Zn(II) complex.

Fig 4. Gel electrophoresis diagram showing the cleavage of CT DNA by Cr(II),Zn(II) and Co(II) complexes in a buffer containing 50 μM Tris–HCl and 50 μM NaCl in the presence of H₂O₂ at 37 °C. Lane 1, DNA control; lane 2, DNA + H₂O₂; lane 3, DNA + ligand(L) + H₂O₂; lane 4, DNA + H₂O₂ + [CrL]; lane 5, DNA + H₂O₂ + [CoL] and lane 6, DNA + H₂O₂ + [ZnL].
5. *N*-4-(dihydroxy)benzamidine (PHB) ligand and their metal ion complexes

PHB ligand and their Cr(II), Co(II) and Zn(II) complexes were synthesized and screened for their DNA cleavage analysis using Gel electrophoresis experiments. At micro molar concentrations for 2 hr incubation periods, the ligand (Lane 3) exhibits no significant cleavage activity in the presence of H$_2$O$_2$ (Fig.5).

![Gel electrophoresis diagram](image)

Fig 5. Gel electrophoresis diagram showing the cleavage of CT DNA by Cr(II), Zn(II) and Co(II) complexes in a buffer containing 50 µM Tris–HCl and 50 µM NaCl in the presence of H$_2$O$_2$ at 37 °C. Lane 1, DNA control; lane 2, DNA + H$_2$O$_2$; lane 3, DNA + ligand(L) + H$_2$O$_2$; lane 4, DNA + H$_2$O$_2$ + [CrL]; lane 5, DNA + H$_2$O$_2$ + [CoL] and lane 6, DNA + H$_2$O$_2$ + [ZnL].

Nucleolytic activity is greatly enhanced by the incorporation of metal ion in the respective N-4-(dihydroxy)benzamidine ligand. It is evident that the complexes cleave DNA more efficiently in the presence of oxidant. Lane 4 and Lane 5 represents Cr(II) and Co(II) complexes nuclease activity. These complexes are able to induce 90% cleavage of CTDNA at 70 µM and 55 µM concentration. It was observed that Zn(II) can promote 70% conversion of coiled DNA to nicked and linear DNA at its maximum concentration of 70 µM.

**Conclusion**

The DNA cleavage of all the compounds were evaluated using CTDNA, results exhibited positive activity. All the complexes have almost 50-95% activity to cleave the DNA molecules.
Reference