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Synthesis and Evaluation of 13, 15 and 17 Membered Protonated Outer Anionic Transition Metal Complexes with Polyaza Macrocyclic Complexes for Biomedical Applications

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ABSTRACT

Template synthesis and characterization of series of transition metal complexes of 13, 15 and 17-membered protonated polyaza macrocyclic ligands with metals of Cu, Co, Ni, Mn and Zn were amalgamated and all the newly synthesized complexes have been recently reported by Selvan *et al*. (1). Plasmid Expression and Transcription (PET) system is the most powerful system yet developed for the cloning and expression of recombinant proteins in *Escherichia coli*. In the present study, five metal complexes, namely {(Mac.Cy1.)[CuCl6]}, {(Mac.Cy1.)[Mn Cl6]}, {(Mac.Cy2.)[CuCl6]}, {(Mac.Cy3.)[CuCl6]} and {(Mac.Cy3.)[CoCl6]} have been evaluated for their DNA cleaving ability using PET 30b system.

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INTRODUCTION

Among numerous functional groups, metal ions complexes serve as potential anion binder. Metal ions complexes are small molecules, synthetic or natural have inevitable biological functions and play a vital role in the regulation of biological process for example, bind to DNA to affect protein expression levels, bind to proteins to inhibit their function, interact with lipids to alter the membrane structure and integrity, or become fluorescent in response to a metabolic event. Because metal ions can significantly affect the biochemical function, link between chemistry, biology, pharmacology and medicine has to be worked out for its application especially in the drug industries. While small molecules are usually implied as being organic compounds, inorganic small molecules also have a long history in chemistry, biology and medicine (2 - 5).

Since, time immemorial, people have been using gold and copper for healing purposes. Recent discoveries in bioinorganic chemistry of potential biomedical importance include the use of metal ions as synthetic scaffolds for the preparation of small molecule therapeutics, further, examination of metal-organic frameworks as biological imaging and drug delivery agents by way of arsenic-containing salvarsan was discovered as an anti-syphilis agent the first blockbuster drug. Inorganic compounds should therefore, not be overlooked in the realm of chemical biology. Metal ions bind to ligands (both organic and inorganic) via interactions that are often strong and selective. Metal complexes span a range of coordination geometries that give them unique shapes compared to organic molecules. Bond lengths, bond angles, and number of coordination sites can vary depending on the metal and its oxidation state. Since the distinctive electronic, chemical, and photophysical properties render them particularly useful for a variety of applications (6 - 9).

Amide is a class of ligand that readily participates in hydrogen bonding. Indeed, resonance in the amide group and intermolecular hydrogen bonding between various macrocyclic amide groups (10-13) in polypeptides determine the final protein structure that in turn leads to a specific bioactivity, especially, catalysis of biochemical reactions with high degree of specificity and efficiency. Type II topoisomerases require divalent metal ions in order to cleave DNA. Studies indicate that restriction enzymes utilize variants of canonical two-metal-ion mechanism to promote DNA cleavage. However, the role of these metal ions in the cleavage reaction mediated by restriction enzymes has been not yet been resolved (14).

Unlocking biological information contained within DNA is the pursuit of molecules that can specifically interact with, label, or cleave oligonucleotide sequences. DNA sequences of interest can be specifically targeted by taking advantage of sequence-dependent changes in overall 3-dimensional shape and surface electronic distribution. Metal complexes are especially well suited for DNA interaction because the electropositive nature which innately attract negatively charged phosphate backbone of DNA. Metal complexes with unique 3-dimentional structural scaffolds, can fit into the base stacks and grooves of targeted DNA. Metal complexes useful for structural recognition of DNA must have rigid three-dimensional structure, as fluxional behavior would negate structure based selectivity. The stereochemistry of the complex, if applicable, can provide an element of enantio-selectivity. In addition, unique chemical properties of metal complex can be exploited to label or cleave selected sites, however, in vivo probes must also be highly inert. The complexes that have shown utility as DNA probes have unique properties to serve as DNA foot-printing agents (15).

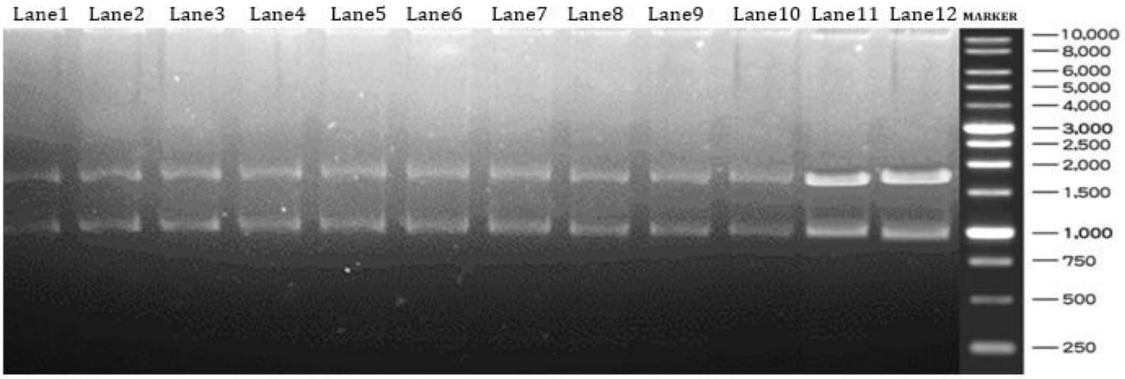
PET (Plasmid Expression and Transcription) System is the most powerful system yet developed for cloning and expression of recombinant proteins in *Escherichia coli*. Target genes are cloned in PET under control of strong transcription and translation signals and expression is induced by T7 RNA polymerase of host cell and all of the cell's resources are channelized for target gene expression to an extent that more than 50% of the total cell protein after a few hours of induction. Importantly, PET system maintains target genes transcriptionally silent in the un-induced state.

MATERIALS AND METHODS

For DNA studies five metal complexes, namely {(Mac.Cy1.)[$CuCl_6$]}, {(Mac.Cy1.)[$MnCl_6$]}, {(Mac.Cy2.)[$CuCl_6$]}, {(Mac.Cy3.)[$CuCl_6$]} and {(Mac.Cy3.)[$CoCl_6$]} were selected. The compounds were evaluated against their cleaving ability towards PET30b vector. The ability to cleave the vector was confirmed using agarose gel electrophoresis system according to the method described by Sambrook *et al.* (16) The supercoiled PET 30b DNA was treated by metal complexes at various concentration in the presence and absence of oxidant hydrogen peroxide followed by dilution of the sample with deionized water to a final volume of 15 µl. The sample solutions are incubated at 37º C for 1 h and electrophoresed, visualized using gel documentation system and photographed.

RESULTS AND DISCUSSION

Recently, two structures of the covalent *Streptococcus pneumoniae* topoisomerase IV-cleaved DNA complex were reported. One of the structures contained no metal ions (17), while the other contained a single Mg^{2+} at each active site. Although it could be due to the participation of a more weakly bound Mg^{2+}, it is also suggested possibility that topoisomerase IV might utilize a single dynamic metal ion for DNA cleavage (18). In the present study, DNA cleavage and stability studies using five metal complexes {(Mac.Cy1.)[$CuCl_6$]}, {(Mac.Cy1.)[$MnCl_6$]}, {(Mac.Cy2.)[$CuCl_6$]}, {(Mac.Cy3.)[$CuCl_6$]} and {(Mac.Cy3.)[$CoCl_6$]} were evaluated using pET 30b. Presence and absence of hydrogen peroxide along with the metal complexes (concentration 0.05 µg, 0.5 µg and 5 µg) significantly the affected the DNA cleavage activity. DNA cleaving activity for all the selected five metal complexes are evaluated without and in the presence of hydrogen peroxide for the metal complex (Fig. 1) at a concentration of 0.05 µg of metal complexes (30 min at 37^{o}C), in all the treatments insignificant DNA cleavage was observed, indicating almost a similar behavior among all of the selected metal ion complex.



Lane 1: DNA incubated with 0.05µg {(Mac.Cy3.)[$CuCl_6$]}solution

Lane 2: DNA incubated with 0.05µg {(Mac.Cy3.)[$CuCl_6$]}solution + H_2O_2

Lane 3: DNA incubated with 0.05µg {(Mac.Cy1.)[$CuCl_6$]}solution

Lane 4: DNA incubated with 0.05µg {(Mac.Cy1.)[$CuCl_6$]}solution + H_2O_2

Lane 5: DNA incubated with 0.05µg {(Mac.Cy1.)[$MnCl_6$]}solution

Lane 6: DNA incubated with 0.05µg {(Mac.Cy1.)[$MnCl_6$]}solution + H_2O_2

Lane 7: DNA incubated with 0.05µg {(Mac.Cy2.)[$CuCl_6$]}solution

Lane 8: DNA incubated with 0.05µg {(Mac.Cy2.)[$CuCl_6$]}solution + H_2O_2

Lane 9: DNA incubated with 0.05µg {(Mac.Cy3.)[$CoCl_6$]}solution

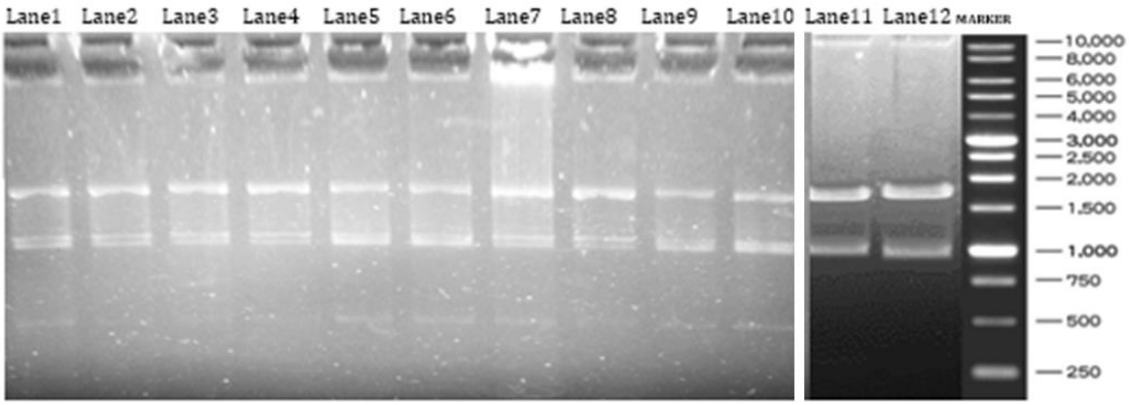
Lane 10: DNA incubated with 0.05µg {(Mac.Cy3.)[$CoCl_6$]}solution + H_2O_2

Lane 11: Control Plasmid pET 30b DNA

Lane 12: Plasmid pET 30b + H_2O_2

Lane 13: Marker

Figure No. 1: DNA incubated with 0.05µg five metal complex solution / presence or absence of H_2O_2



Lane 1: DNA incubated with 0.5µg {(Mac.Cy3.)[$CuCl_6$]}solution

Lane 2: DNA incubated with 0.5µg {(Mac.Cy3.)[$CuCl_6$]}solution + H_2O_2

Lane 3: DNA incubated with 0.5µg {(Mac.Cy1.)[$CuCl_6$]}solution

Lane 4: DNA incubated with 0.5µg {(Mac.Cy1.)[$CuCl_6$]}solution + H_2O_2

Lane 5: DNA incubated with 0.5µg {(Mac.Cy1.)[$MnCl_6$]}solution

Lane 6: DNA incubated with 0.5µg {(Mac.Cy1.)[$MnCl_6$]}solution + H_2O_2

Lane 7: DNA incubated with 0.5µg {(Mac.Cy2.)[$CuCl_6$]}solution

Lane 8: DNA incubated with 0.5µg {(Mac.Cy2.)[$CuCl_6$]}solution + H_2O_2

Lane 9: DNA incubated with 0.5µg {(Mac.Cy3.)[$CoCl_6$]}solution

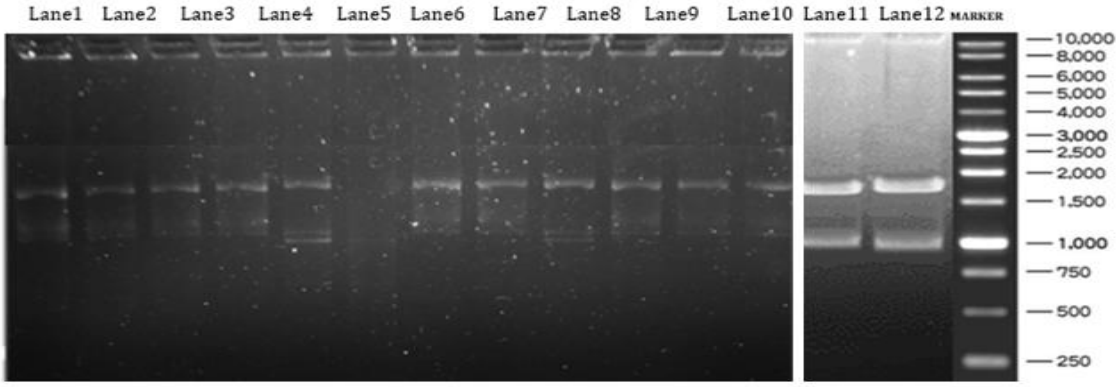
Lane 10: DNA incubated with 0.5µg {(Mac.Cy3.)[$CoCl_6$]}solution + H_2O_2

Lane 11: Control Plasmid PET 30b DNA

Lane 12: Plasmid PET 30b + H_2O_2

Lane 13: Marker

Figure No. 2: DNA incubated with 0.5µg five metal complex solution / presence or absence of H_2O_2



Lane 1: DNA incubated with 5µg {(Mac.Cy3.)[$CuCl_6$]}solution

Lane 2: DNA incubated with 5µg {(Mac.Cy3.)[$CuCl_6$]}solution + H_2O_2

Lane 3: DNA incubated with 5µg {(Mac.Cy1.)[$CuCl_6$]}solution

Lane 4: DNA incubated with 5µg {(Mac.Cy1.)[$CuCl_6$]}solution + H_2O_2

Lane 5: DNA incubated with 5µg {(Mac.Cy1.)[$MnCl_6$]}solution

Lane 6: DNA incubated with 5µg{(Mac.Cy1.)[$MnCl_6$]}solution + H_2O_2

Lane 7: DNA incubated with 5µg{(Mac.Cy2.)[$CuCl_6$]}solution

Lane 8: DNA incubated with 5µg{(Mac.Cy2.)[$CuCl_6$]}solution + H_2O_2

Lane 9: DNA incubated with 5µg{(Mac.Cy3.)[$CoCl_6$]}solution

Lane 10: DNA incubated with 5µg{(Mac.Cy3.)[$CoCl_6$]}solution + H_2O_2

Lane 11: Control Plasmid PET 30b DNA

Lane 12: Plasmid PET 30b + H_2O_2

Lane 13: Marker

Figure No. 3: DNA incubated with 5µg five metal complex solution / presence or absence of H_2O_2

When analyzed for its cleavage activity at a concentration of 0.5 µg for all the selected metal complexes (Fig. 2) with and without H_2O_2, selected metal ion complexes exhibited a moderate DNA cleaving activity. Results indicate that elevated concentration, the complex is able to exhibit DNA cleaving activity. However, DNA cleaving activity of the selected metal ion complexes were not uniform for all the treatments.

Since, there was an appreciable increase in the DNA cleavage activity for the metal ion complexes, the concentration was further increased to 5 µg. At this elevated concentration the metal ion complexes (Fig. 3) exhibited considerable DNA cleaving activity. Results of the present study indicate that the extent of DNA damage was positively correlated to the concentration of the metal ion complex in the solution. However, addition of hydrogen peroxide to the metal complex solution further increases the DNA cleaving activity of the metal ions complex. Results indicate that probable autocatalytic activity of the DNA was induced in the presence of metal ions coupled with hydrogen peroxide. Likewise, Noble and Maxwell (19) suggested that mutant gyrase proteins with substitutions at residues believed to complex with metal ions displayed greater DNA cleavage in the presence of Mg^{2+} and Ca^{2+} than did either divalent cation alone. Furthermore, increase in DNA catalytic activity could be attributed to the formation of free hydroxyl radical in the presence of hydrogen peroxide which could cause significant damage to the DNA molecule.

CONCLUSION

DNA cleavage and stability studies above mentioned five metal complexes are evaluated against their cleaving ability towards PET 30b vector. Since there is an appreciable increase in the DNA cleavage activity for the metal ion complexes from 0.05 to 0.5 µg, the concentration was further increased to 5 µg. At this elevated concentration, the metal ion complexes exhibited considerable DNA cleaving activity. Results of the present study

indicate that the extent of DNA damage was significant and positively correlated to the concentration of metal complex in the solution has prospective biomedical application as potential anticancer agent.

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