



Mononuclear Co(II) and Mn(II) Complexes Framework Based on Schiff Base Ligand: Designing, Synthesis, Structural Elucidation, Efficient DNA Binding and Cleavage Activity

KEYWORDS

o-phenylenediamine, molecular modelling, pBR322 DNA

Raju Ashokan

Rangappan Rajavel

Department of Chemistry, Periyar University, Salem-11, Tamilnadu, India

Department of Chemistry, Periyar University, Salem-11, Tamilnadu, India

ABSTRACT A group of combinations which have more importance in biological studies, biochemistry and also medicinal applications are Schiff base complexes. They have been considered by the chemists. Schiff base ligand was provided by means of condensation of o-phenylenediamine, 2-amino 4-chlorophenol with 1, 10-phenanthroline in absolute ethanol solvent with reflux method. Then the complexes of transition metals Co(II) and Mn(II) with the ligand to being determined synthesized mononuclear Schiff base metal complexes and using elemental analyses, molar conductance, magnetic susceptibility, IR, UV-Vis spectral data and ¹H NMR studies. The elemental analysis of the complexes confine to the stoichiometry of the type ML₁L₂. The possible geometries of metal complexes were evaluated using 3D molecular modelling picture. The binding properties of metal complexes with DNA have been investigated by electronic absorption spectra showing that the complexes have the ability of interaction with DNA by intercalative mode. All the metal complexes cleave the pBR322 DNA in presence of H₂O₂.

INTRODUCTION

Success of cisplatin as an effective anticancer agent has drawn the attention of many bioinorganic chemists towards developing other active transition metal anticancer complexes with better efficiency (Pulimamidi Rabinra Reddy et al., 2011). Although there are some other biological targets in tumor cells, including RNA, protein or enzyme it is generally accepted that DNA is the primary target for many anticancer drugs. Hence interactions between small molecules and DNA among the primary action mechanism of anticancer activity and designing of molecules that bind and cleave DNA have attracted extensive attention. The cleavage of DNA may be considered as an enzymatic reaction which comprises various biological processes as well as the biotechnological manipulation of genetic material. There is a substantial literature supporting the application of artificial DNA cleaving agents in biotechnology; structural studies of nucleic acids or development of new drugs. Transition metal complexes have been widely exploited for metallohydrolases capable of mimicking the function of endonucleases and to develop synthetic binding and cleavage agents for DNA (Saeid Menati et al., 2011).

In view of the diversified roles of Schiff base metal complexes, it was thought important to design new Schiff base complexes and study their role in DNA cleavage and binding. Here, we report the synthesis, characterization, DNA binding and cleavage activities of Co(II) and Mn(II) complexes. Complexes were structurally characterized by various physico chemical techniques. Copper complex bind strongly with DNA and cleave it efficiently as compared to relate other Schiff base systems.

EXPERIMENTAL PROTOCOLS

Reagents and Instruments

Metal salts, o-phenylenediamine, 2-amino 4-chlorophenol and 1, 10-phenanthroline was obtained from Aldrich and used as received. Ethanol, DMSO and DMF were used as solvents purchased from Merck and Loba chemicals. Elemental analysis was carried out on a Carlo Erba Model 1106 elemental analyzer. IR spectra were recorded on a Thermo Nicolet, Avatar 370 model spectrophotometer on KBr disks in the range 4000– 400 cm⁻¹. Molar conductiv-

ity was measured by using an ELICO CM 185 conductivity Bridge using freshly prepared solution of the complexes in DMF solution. Electronic spectra were recorded at 300 K on a Perkin-Elmer Lambda 40(UV-Vis) spectrometer using DMF in the range 200-800 nm. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature. The ¹H NMR spectra were recorded in DMSO-d₆ on a BRUKER ADVANCED III 400 MHz spectrophotometer using TMS as an internal reference.

Synthesis of Schiff base (H₂L¹)

The Schiff base ligand was prepared according to the literature method. To an ethanolic solution of 2-amino 4-chlorophenol (2 mmol) and ethanolic solution of o-phenylenediamine (1 mmol) was added in drop wise. The reaction mixture was kept on water bath for refluxion (Raman et al., 2012). It was stirred for 2 hrs. Pale Yellow colour solid was separated and were filtered off, washed with 5 ml of cold ethanol and then dried in air. **Yield:** 78 %. M.p: 156-158 °C. *Anal. Calc. For C₂₀H₁₄N₂O₂Cl₂:* C, 62.50; H, 3.64; N, 7.29. Found: C, 62.49; H, 3.65; N, 7.28. (%). IR (KBr pellet, cm⁻¹): 3355 ν(-OH); 1612 ν(-CH=N) ; 1286 (-C-O). UV-Vis λ_{max} (nm), 320, 348 (π→π*, n→π*). ¹H NMR (DMSO-d₆, δ ppm): 6.8-7.9 ppm (m, Ar-H), 8.10 ppm (s, CH=N), 9.8 ppm (Ph-OH).

Synthesis of metal complexes

An ethanolic solution (20 mL) containing L¹ (o-phenylenediamine and 2-amino 4-chlorophenol) (1 mM) and L² (1, 10-phenanthroline) (1 mM) were added to a solution of Metal(II) acetates of Co(II) and Mn(II) (1 mM) in 20 ml of ethanol. The solution was refluxed for 3 hrs and then allowed to stand at room temperature for 24 hrs. The resulting powder was filtered and dried in vacuum.

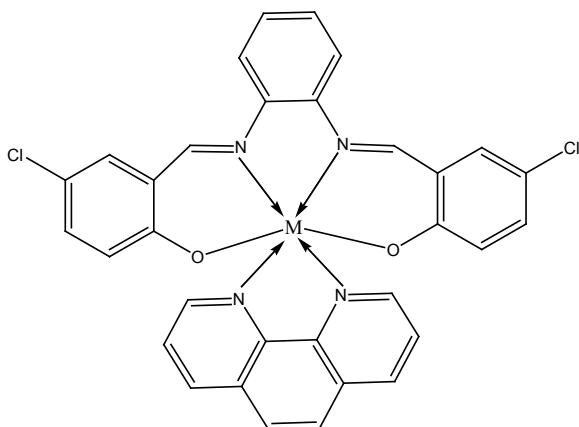
Co(C₄₂H₂₀N₄O₂Cl₂):

Yield: 73 %. M.p: 214216 °C. *Anal. Calc. For Co(C₄₂H₂₀N₄O₂Cl₂):*C, 68.02; H, 2.69; N, 7.55; Co, 7.94. Found: C, 68.01; H, 2.64; N, 7.54; Co, 7.95. (%). IR (KBr pellet, cm⁻¹): 1608 ν(-CH=N) ; 1273 ν(-C-O) ; 472 ν(-M-N) ; 532 ν(-M-O). UV-Vis λ_{max} (nm), 265, 371, 434 (π→π*, n→π*, L→MCT); 522, 551, 620 (d→d). Molar conductance x

10^{-3} (Ω^{-1} cm² mol⁻¹): 11.16; μ_{eff} (BM): 4.83.

Mn(C₄₂H₂₀N₄O₂Cl₂):

Yield: 71 %. M.p: 210213 °C. Anal. Calc. For Mn(C₄₂H₂₀N₄O₂Cl₂): C, 68.39; H, 2.71; N, 7.59; Mn, 7.40. Found: C, 68.38; H, 2.70; N, 7.57; Mn, 7.41. (%). IR (KBr pellet, cm⁻¹): 1598 ν (-CH=N); 1281 ν (-C-O); 462 ν (-M-N); 540 ν (-M-O). UV-Vis λ_{max} (nm), 262, 371, 455 ($\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, L→MCT); 519, 549, 612 (d→d). Molar conductance $\times 10^{-3}$ (Ω^{-1} cm² mol⁻¹): 8.13; μ_{eff} (BM): 5.82.



M = Co(II) & Mn(II)

Figure 1. Synthesis pathway for Schiff Base metal(II) Complexes

Methodology for DNA Cleavage Study

Agarose gel electrophoresis was used to study the DNA cleavage activity of the complexes. pBR322 DNA plasmid was cultured, isolated and used as DNA for the experiment. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μ M pBR322 DNA, 50 μ M metal complexes and 50 μ M H₂O₂ in tris-HCl buffer (pH 7.2) at 37 °C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 μ g cm⁻³ ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature (Nahid Shahabadi et al., 2010).

DNA-Binding Experiments

Electronic absorption spectroscopy has been widely employed to determine the binding characteristics of metal complexes with DNA. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M⁻¹ cm⁻¹) at 260 nm (Pradeepa et al., 2012).

RESULTS AND DISCUSSION

All the Co(II) and Mn(II) complexes were colored, stable at room temperature for extended periods, non-hygroscopic in nature and decompose on heating. These complexes are insoluble in water and many common organic solvents but are readily soluble in strong coordinating solvents like DMF and DMSO.

Molecular Modelling

The molecular modelling of Co(C₄₂H₂₀N₄O₂Cl₂) complex as a representative, is based on its distorted octahedral structure with ligand. This Molecular modeling structure also shows the stereochemistry of the complex. The details

of the bond lengths (Å) and bond angles (°) as per the 3D structure (Figure 2) respectively (Maurya et al., 2008). From the observed bond lengths and bond angles thus the proposed structure is distorted octahedral geometry and is suitable.

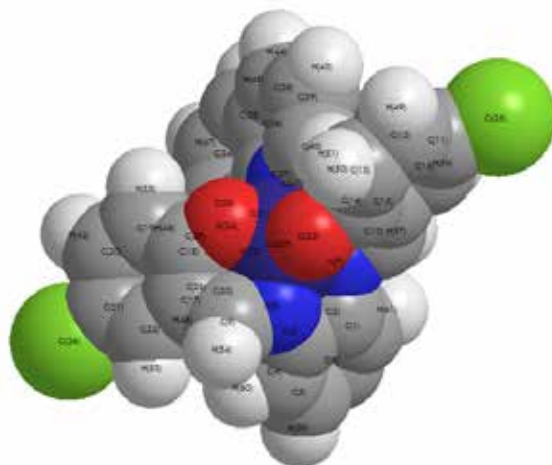
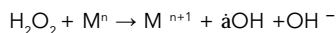


Figure 2. 3D Structure of Compound of Co(C₄₂H₂₀N₄O₂Cl₂)

DNA Cleavage Studies

Agarose gel electrophoresis assay is an effective method to investigate various binding modes of synthesized complexes to supercoiled DNA (Neelakantan et al., 2008). Natural-derived plasmid DNA mainly has a closed-circle supercoiled form (Form I), as well as nicked form (Form II) and linear form (Form III) as small fractions. Intercalation of synthesized complexes to plasmid DNA can loosen or cleave the supercoiled form DNA, which decreases its mobility rate and can be separately visualized by agarose gel electrophoresis method. To assess the DNA cleavage ability of complexes, supercoiled (SC) pBR322 DNA was incubated in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2 for 2 hrs. The relatively fast migration is the intact supercoil form (Form I) and the slower moving migration is the open circular form (Form II), which was generated from supercoiled. The mononuclear Schiff base metal complexes is able to perform cleavage of pBR322 DNA (lane 3 and 4; Figure 3). The supercoiled SC (Form I) gradually converted to nicked form NC (Form II). The production of a hydroxyl radical due to the reaction between the metal complex and oxidant may be explained as shown below



The OH· free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate backbone. The increase in hydroxyl radical leads to the pronounced nuclease activity in the presence of oxidant H₂O₂. Control experiments using DNA alone do not show any significant cleavage of pBR322 DNA even on longer exposure time. From the observed results, it is concluded that all the complexes effectively cleave the DNA as compared to control DNA.

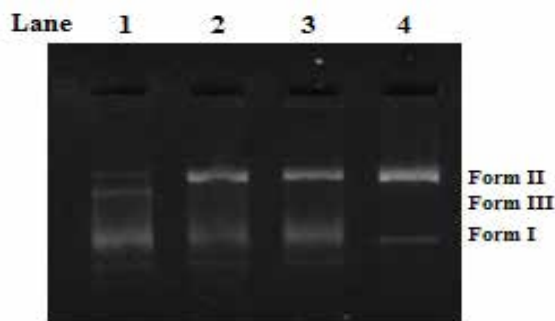


Figure 3. Changes in the agarose gel electrophoretic pattern of pBR322 DNA induced by H_2O_2 and metal complexes. From left to right: Lane 1, pBR322 DNA - Control; Lane 2, DNA + H_2O_2 (1mM); Lane 3, DNA + H_2O_2 (1mM) + $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ (50 μM); Lane 4, DNA + H_2O_2 (1mM) + $\text{Mn}(\text{C}_{42}\text{H}_{34}\text{N}_4\text{O}_6)$ (50 μM) + H_2O_2 .

Electronic Absorption Spectroscopy

Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. The interaction of $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ complex with CT-DNA were investigated by UV absorption titrations by following the intensity changes of the intraligand $\pi-\pi^*$ transition band 260 and 320 nm. Absorption titration experiments of $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ complexes in buffer were performed by using a fixed complex concentration to which increments of the DNA stock solution were added. The binding of $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ complex to duplex DNA led to a decrease in the absorption intensities (Figure 4) with a small amount of red shift in the UV-Vis absorption spectra. With increasing concentration of DNA, the absorption bands of the complex were affected to a considerable extent. For $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ complex, the absorption spectra show clearly that the addition of DNA to the complexes yields hypochromic and a red shift with 2 nm to the ratio of $[\text{DNA}]/[\text{Co}]$. Obviously, these spectral characteristics suggest that the Co(II) complex interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA (Kollur Shiva Prasad et al., 2011).

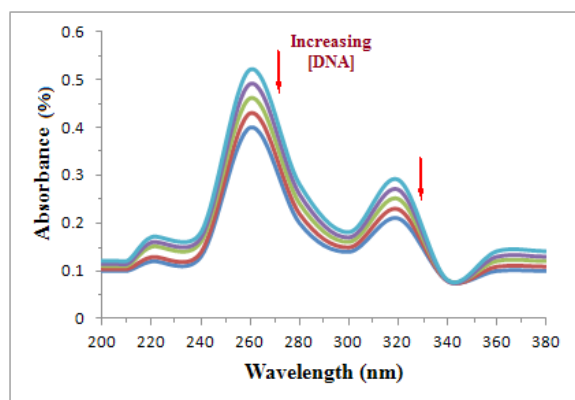


Figure 4. Electronic spectra of complexes $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ in DMF in the absence and presence of plasmid DNA. Arrow shows the absorbance changes upon increasing DNA concentrations.

From the absorption data, the intrinsic binding constant

(K_b) was determined from the plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ versus $[\text{DNA}]$ using the following equation:

$$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + [K_b(\epsilon_b - \epsilon_f)]^{-1}$$

where $[\text{DNA}]$ is the concentration of CT DNA in base pairs. The apparent absorption coefficients ϵ_a , ϵ_f and ϵ_b correspond to $A_{\text{obs}}/[\text{M}]$, the extinction coefficient for the free metal(II) complex and extinction coefficient for the metal(II) complex in the fully bound form, respectively. K_b is given by the ratio of slope to the intercept. The magnitudes of intrinsic binding constants (K_b) were calculated to be $2.3 (\pm 0.12) \times 10^4 \text{ M}^{-1}$ for $\text{Co}(\text{C}_{42}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2)$ complex respectively.

CONCLUSION

In conclusion, the synthesis, structural elucidation and DNA interaction properties of new mononuclear Schiff base metal complexes have been reported in this paper. The DNA-binding results revealed that the complexes can bind CT-DNA moderately in an intercalation mode. The synthesized complexes show DNA cleavage activity via hydroxyl radical pathway.

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