DNA Binding and Cleavage Studies of Macroyclic Complexes

Chapter – 5

DNA Binding and Cleavage studies of macrocyclic complexes of

N,N-bis (quinoline-3-yl) methyl[1,3,7,9]dithiourea

5. Introduction

The design and synthesis of macrocyclic ligands have been the subject of growing interest during the past few decades because of their importance in biomimetic studies of metal complexes, their capacity to act as catalysts for numerous chemical reactions and the possibilities for magnetic materials.[1,2] Much attention has also been paid to the construction of machine-like supramolecules[3] starting from molecular components and mechanical work can be induced at the molecular level through the controlled motion of a chosen component occurring within a molecular or a supramolecular system.

The high selectivity and strong coordination ability of macrocyclic ligands towards transition metal ions have attracted the attention of chemists all over the world due to the wide range of applications of these complexes in the areas like catalysis,[4-6] electron carriers in redox reactions,[7] dioxygen carriers,[8,9] ionophores in a number of biochemical processes,[10-12] separation and extraction of valuable and precious metals from waste materials,[13] as antitumour drugs,[14] as model compounds that mimic naturally occurring metalloproteins,[15] and metalloenzymes, as photosensitizers and in photodynamic therapy.[16]

Further, the mixed donor macrocyclic ligand metal complexes constitute a potentially important class of molecules in various fields such as medical imaging agents or supramolecular architectures [17, 18], molecular electronics, catalytic reductions and
DNA probing agents [19]. Thus, the study of complexes of macrocyclic ligands containing different donor atoms are important in fields particularly in biological chemistry.

In view of the important of macrocyclic chemistry in DNA binding and cleavage studies [20-23], herein we present the synthesis of Co(II) and Ni(II) complexes of macrocyclic ligand and studied their binding as well as oxidative and photo cleavage properties.

5.1. Experimental

5.1.1 Synthesis of ligand(qmt)

Part A: A mixture of 2-chloro-3-formyl quinoline and thiourea in the molar ratio of 2:1 was dissolved in ethanol and refluxed for 3-4 hrs. The completion of reaction was monitored by TLC. The obtained product was recrystallized using methanol.

Part B: The resulted compound of Part A and thiourea were taken in 1:1 molar ratio in the presence of K2CO3 and refluxed for 8-10 hrs. The completion of reaction was monitored by TLC. The obtained reddish coloured N,N-bis (quinoline-3-yl)methyl[1,3,7,9]dithiourea[qmt] was recrystallized using methanol. The spectral and elemental analysis data are presented in Table 5.1 and 5.2.

Figure 5.1. Structure of N,N-bis (quinoline-3-yl)methyl[1,3,7,9]dithiourea[qmt] macrocyclic ligand
5.1.2. Synthesis of (Co(II) and Ni(II) metal complexes of (qmt) ligand

N,N-bis(quinoline-3-yl)methyl[1,3,7,9]dithiourea[qmt] and the corresponding metal salts were dissolved in hot ethanolic solution in the molar ratio 1:1 and kept on the water bath (70 to 80 °C). After one hour, the contents were cooled and precipitated by the addition of hot ethanolic solution of ammonium hexafluorophosphate (NH₄PF₆). The complex was filtered and dried under vacuum before being recrystallized (acetone-ether).

\[
\begin{align*}
\text{Where } M &= \text{Co(II) or Ni(II)} \quad X = \text{H}_2\text{O}
\end{align*}
\]

Figure 5.2. Proposed structure of the metal complex

5.2. Results and Discussion

General characterization

The hexafluorophosphate salts of the complexes have been characterized by elemental analysis, UV, IR and \(^1\)H NMR and mass spectroscopic and magnetic susceptibility measurements. The data are summarized in Table 5.1 and 5.2.
Table 5.1. Analytical and physical properties of the macrocyclic ligand and its Co(II) and Ni(II) complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Found (Cal.) %</th>
<th>ΩM mols cm² mol⁻¹</th>
<th>µeff BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>C₂₄H₁₄N₆S₂</td>
<td>76</td>
<td>61.95</td>
<td>3.37</td>
<td>19.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(61.98)</td>
<td>(3.30)</td>
<td>(19.64)</td>
</tr>
<tr>
<td>C₂₄H₁₆N₆S₂</td>
<td>72</td>
<td>54.66</td>
<td>2.50</td>
<td>17.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.60)</td>
<td>(2.42)</td>
<td>(17.46)</td>
</tr>
<tr>
<td>O₂P₂F₁₂Co</td>
<td>68</td>
<td>54.69</td>
<td>2.50</td>
<td>17.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.60)</td>
<td>(2.58)</td>
<td>(17.45)</td>
</tr>
</tbody>
</table>

In the IR spectra of the ligand, the absence of band corresponding to amino groups of thiourea and carbonyl groups of 2-chloro-3-formyl-quinoline, indicates that the newly synthesized compound is macrocyclic in nature. It was supported by the observed intensive bands at 1686 cm⁻¹ due to ν(C=N) and bands at 3151 cm⁻¹ for ν(NH), respectively. Further, the formation of macrocyclic ligand was confirmed by ¹H NMR spectra. It gave multiplet at δ: 7.71-8.33 ppm for aromatic protons and singlet at δ:10.5 due to −SH.

In the IR spectra of macrocyclic Co(II) and Ni(II) complexes the bands of ν(C=N) and ν(NH) shift to a lower frequency by 30 and 20 cm⁻¹. The shift of these bands in complexes suggests that the coordination of Schiff base N atoms to metal ion in tetradentate fashion [24]. The sharp band observed at 836 cm⁻¹ for ν(C=S) group in ligand indicates it has not involved in bonding with metal ions. The bonding of the metal ion to the ligand through N atoms was further supported by the presence of new low frequency bands in the region 425-490 cm⁻¹ due to ν(M-N) vibrations [25]. In addition to these bands, the complexes show broad band in the region 3102-3150 cm⁻¹ indicated that the complexes have coordinated water molecule.
Table 5.2. Some important IR stretching frequencies (cm\(^{-1}\)) and \(^1\)H NMR (\(\delta, \text{ppm}\)) of ligand and its Co(II) and Ni(II) complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (cm(^{-1}))</th>
<th>((\delta, \text{ppm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand (qmt)</td>
<td>3151, 1623, 1587, 1157, 836.</td>
<td>8.10.65 (s, 1H, SH); 8.4 (s, 1H, H-C=N); 5.9. (NH), 7.7-8.82 (m, 10H, Ar-H).</td>
</tr>
<tr>
<td>C(<em>{24})H(</em>{16})N(_6)S(_2)O(_2)P(_2)F(_2)Co</td>
<td>3102, 3150, 2920, 1654, 1601, 1445, 768, 435.</td>
<td>----</td>
</tr>
<tr>
<td>C(<em>{24})H(</em>{16})N(_6)S(_2)O(_2)P(_2)F(_2)Ni</td>
<td>3102, 3150, 2920, 1654, 1601, 1445, 768, 435.</td>
<td>----</td>
</tr>
</tbody>
</table>

Figure 5.3. \(^1\)H NMR spectrum of macrocyclic ligand [qmt]
**Figure 5.4.** IR Spectrum of the ligand N,N-bis (quinoline-3-yl)methyl[1,3,7,9]dithiourea [qmt] macrocyclic ligand

**Figure 5.5.** IR Spectrum of Co(II) complex
5.2.2. **UV-Vis spectra**

The UV-Visible spectra of the ligand and its complexes were recorded in DMF at $10^{-3}$ mol concentration. The electronic spectrum of the ligand had a strong band at $\lambda_{\text{max}} = 398$ nm and a weak band at $\lambda_{\text{max}} = 435$ nm.

The electronic spectra of the Co(II) complexes shows three absorption maxima in the regions 10970-10999, 16750-16761 and 23376-23392 cm$^{-1}$ which may be assigned to $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow ^4T_{2g}(P)$ transitions, respectively, ascertaining an octahedral geometry around the Co(II) ion [25]. The proposed octahedral geometry around the Ni(II) ion is also supported by the position of absorption bands obtained in the region 11620-11634, 16400-16434 and 27701-27708 cm$^{-1}$ attributed to $^3A_{2g}(F) \rightarrow ^3T_{2g}$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ transitions, respectively [25].
The observed magnetic moment value (Table 5.1) of the Co(II) and Ni(II) complexes corroborate the high spin octahedral geometry around the metal ion[26].

5.3. DNA binding properties

5.3.1. Absorption spectra

Absorption titration is used to monitor the interaction of cobalt(II) and nickel(II) macrocyclic complexes with CT-DNA. In general, a complex bound to DNA through intercalation usually results in hypochromism and bathochromism (red shift) of the absorption band due to strong stacking interaction between the aromatic chromophore of the complex and the base pair of the DNA. In the present study, the intense absorption band observed near 290 nm in the complexes attributed to the MLCT transition involving the macrocyclic base and the metal ion. On increasing the CT-DNA concentration the hypochromism is increased (shown in Fig. 5.8). In order to compare the binding strength of the complexes with DNA, the intrinsic binding constant $K_b$ are obtained by monitoring the changes in absorbance for both the complexes with increasing the concentration of DNA. $K_b$ using the equation 2.1.
Figure. 5.8. Absorption spectra of Co(II) complex in Tris-HCl buffer upon addition of DNA. [Co] = 0.5μM, [DNA] = 0.1μM. Arrow shows the absorbance changing upon increase of DNA concentration. Inner Plot of [DNA]/ (εrεd) vs [DNA] for the titration of DNA with Co(II) complex.

The binding constant obtained for cobalt and nickel complexes are 1.4 x 10⁵ M⁻¹ and 1.7 x 10⁶ M⁻¹, respectively. The Kₘ values obtained for these complexes are lower than those observed for typical classical intercalators [EthBr, Kₘ, 1.8x10⁶ M⁻¹ in 25 mM Tris-HCl/40 mM NaCl buffer, pH 7.9) and partial intercalating metal complexes [Ru(phen)₂(dppz)]²⁺, dppz = dipyrido-[3,2-d: 2',3'-f]-phenazine, Kₘ>10⁶ M⁻¹] bound to CT-DNA(23). This is indicative of binding the complexes with DNA with an affinity less than the classical intercalators.

5.4. Viscosity measurements

Further more the interactions between the complex and DNA were investigated by viscosity measurements. Optical photophysical probes provided necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements that were sensitive to length change (i.e., viscosity and sedimentation) were regarded as the least
ambiguous and the most critical tests of binding mode in solution in the absence of
crystallographic structural data (27, 28). A classical interaction model usually resulted in
lengthening the DNA helix, as base pairs were separated to accommodate the binding
ligand leading to the increase of DNA viscosity. The viscosity plots for Co(II) and Ni(II)
macroyclic complexes are shown in Fig. 5.9. In both the cases the viscosity increased
with increase in the complex concentration, which indicates that the complexes bound to
DNA. This is parallel to the above spectroscopic results, such as hypochromism and
bathochromism shift of complexes in the presence of DNA.

![Graph showing viscosity changes with complex concentration](image)

**Figure. 5.9.** Effect of increasing amounts of the complex Co(II) [----•----] and Ni(II) [----▲----] on
the relative viscosities of CT-DNA at 25 (±0.1)°C.

### 5.5. Thermal denaturation studies

The interaction of classical intercalators with DNA such as ethidium can facilitate
the stability of the DNA double helix and increase the $T_m$ value of DNA(29). Fig.5.10.
shows the behaviours of thermal denaturation of CT-DNA+Co(II) complex and CT-
DNA+Ni(II) complex systems. The melting point of free CT-DNA was 61.0 °C where as
the \( T_m \) value of CT-DNA was increased by 5 and 4 °C in the presence of Co(II) and Ni(II) complexes, respectively, may be due to the complexes stabilized the double helix DNA. These results consistent with the interaction of the two metal complexes to the DNA helix.

![Graph showing melting curves of CT-DNA in the absence and presence of complexes](image)

**Figure. 5.10.** Melting curves of CT-DNA in the absence and presence of complexes

### 5.6. Oxidative DNA Cleavage Studies

In order to determine the ability of Co(II) and Ni(II) complexes for DNA scission, the complexes were incubated at different concentrations with supercoiled pUC19 DNA for 1 h in 50 mM tris-Cl/50 mM NaCl buffer (pH 7.2) using hydrogen peroxide H\(_2\)O\(_2\) activation. Control experiments using H\(_2\)O\(_2\) do not show any apparent cleavage of DNA (Fig. 5.11, lane 1). At the concentration of 40 μM, the Co(II) complex is able to convert only 22% of the initial SC (Form I) to NC (Form II), while as the Ni(II) complex is able to convert 35% of the initial SC (Form I) to NC (Form II) (lane 2 and 4; Fig. 5.11.). While at higher concentration of 60 μM the Co(II) complex exhibit 64% conversion, (lane 3)
and in the same concentration the Ni(II) complex exhibit 75% conversion (lane 5; Fig. 5.11). In conclusion, at higher concentration of 60 µM, both the complexes show more cleavage activity. From these results, we infer that the Ni(II) complex act as a potent nuclease agent in the presence of H₂O₂ agent.

Lane 1 2 3 4 5

Form II
Form I

Figure. 5.11. Cleavage of supercoiled pUC19 DNA (0.5 µg) by the Co(II) and Ni(II) complexes in a buffer containing 50 mM Tris-HCl and 50 mM NaCl at 37 °C. Lane 1, DNA alone; Lane 2, DNA+40 µM of Co(II) complex+H₂O₂; Lane 3, DNA+60 µM of Co(II) complex+H₂O₂; Lane 4, DNA+40 µM of Ni(II) complex+H₂O₂; Lane 5, DNA+60 µM of Ni(II) complex+H₂O₂; Forms I and II are supercoiled and nicked circular forms of DNA, respectively.

Figure. 5.12. Quantification of gel electrophoresis bands originating from SC and NC DNA in our cleavage experiments. The sum of intensities of both bands is standardized to 100% for each individual lane. Metal complexes and concentrations are annotated (dd H₂O: doubly distilled water as background). See text and experimental section for details.
5.7. DNA Photo cleavage studies

Irradiation of the plasmid pUC19 DNA in presence of complex and reactive species in tris-borate buffer (pH = 7.2) at 365 nm results in cleavage of the supercoiled Form I of the plasmid pUC19 DNA to the nicked Form II. In order to establish the reactive species responsible for the photoactivated cleavage of the plasmid pUC19 DNA, the following experiments were carried out (Fig. 5.13). Selective DNA photo cleavage data are given in Table 5.3. Both the complexes with the absence of reactive species shows apparent photo cleavage activity [lane 5 and 10 for Co(II) and Ni(II) complex Fig. 5.13.]. The activities were studied in the presence of D$_2$O to assess the possibility that photoactivated cleavage involves the formation of singlet oxygen, which is known to react with guanine residues at neutral pH [30]. Singlet oxygen would be expected to induce more strand scission in D$_2$O than in H$_2$O, due to its longer lifetime in the former solvent. As seen [lane 4 and 9 for Co(II) and Ni(II) complexes, Fig.5.13] the enhancement in photocleavage activity is observed for the reaction carried out in D$_2$O. This indicates that singlet oxygen is likely to be the cleaving agents. Studies with singlet oxygen quencher, sodium azide were then carried out. The cleavage is strongly inhibited [lane 2 and 7 for Co(II) and Ni(II) complexes, Fig. 5.13], which further confirmed that the singlet oxygen may be the reactive species. The same results were observed in the presence of the hydroxyl radical (OH$^*$) scavenger DMSO [lane 3 and 8 for Co(II) complex and Ni(II) complex, Fig. 5.13].
Figure. 5.13. Gel electrophoresis diagram of the control experiments using SC DNA (0.5 μg), Co(II) and Ni(II) (60 μM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; lane 2, DNA + NaN₃ (38 μM) + Co(II) complex; lane 3, DNA + DMSO (4 μL) + Co(II) complex; lane 4, DNA + D₂O (14 μL) + Co(II) complex; lane 5, DNA + Co(II) complex; lane 6, DNA Control; lane 7, DNA + NaN₃ (38 μM) + Ni(II) complex; lane 8, DNA + DMSO (4 μL) + Ni(II) complex; lane 9, DNA + D₂O (14 μL) + Ni(II) complex; lane 10, DNA + Ni(II) complex.

Fig. 5.13. Quantification of gel electrophoresis bands originating from SC and NC DNA in our cleavage experiments. The sum of intensities of both bands is standardized to 100% for each individual lane. Metal complexes and concentrations are annotated (dd H₂O: doubly distilled water as background). See text and experimental section for details.
Table 5.3. Selected DNA (SC pUC19 DNA, 0.5 μg) cleavage data of Co(II) and Ni(II) complexes in tris-buffer (pH 7.2)

<table>
<thead>
<tr>
<th>Reaction condition</th>
<th>λ (nm)</th>
<th>t (h)</th>
<th>[Complex] (μM)</th>
<th>Form-I (%)</th>
<th>Form-II (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA control</td>
<td>365</td>
<td>1.5</td>
<td></td>
<td>100</td>
<td>00</td>
</tr>
<tr>
<td>DNA + (1)</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>DNA control</td>
<td>365</td>
<td>1.5</td>
<td></td>
<td>94</td>
<td>06</td>
</tr>
<tr>
<td>DNA + (2)</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td>DNA + D₂O (14μl) + Co(II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>DNA + D₂O (14μl) + Ni(II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>DNA + DMSO (4μl) + Co(II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>DNA + DMSO (4μl) + Ni (II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>DNA + NaN₃ (38μM) + Co(II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>79</td>
<td>21</td>
</tr>
<tr>
<td>DNA + NaN₃ (38μM) + Ni (II) complex</td>
<td>365</td>
<td>1.5</td>
<td>60</td>
<td>83</td>
<td>17</td>
</tr>
</tbody>
</table>

λ, excitation wavelength; t, exposure time; Form-I and Form-II are SC and NC forms of DNA respectively.

5.8. Conclusion

The cobalt(II) and nickel(II) complexes of the macrocyclic ligand were synthesized and characterized. Spectroscopic studies, together with viscosity experiments and thermal denaturation studies, supports the fact that both the complexes binds to DNA by partial intercalative interaction in to the base pairs of DNA. Obviously, in the oxidative cleavage studies, the complexes have been found to cleave plasmid pUC19 DNA from the supercoiled Form I to the nicked circular Form II upon H₂O₂ activation. Where as in photocleavage studies these complexes in the absence of various ‘inhibitors’ shows more cleavage properties than in the presence of these ‘inhibitors’, which may taken as these complexes are potential DNA cleaving agents.
References


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