Synthesis, Characterization and Electrochemical Studies of Acyclic End-Off Copper(II) and Nickel(II) Complexes: Nuclease Activity

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A B S T R A C T

Novel acyclic binuclear copper(II) and nickel(II) complexes were synthesized and characterized by spectroscopic methods. Cyclic voltammogram of all the complexes 1-4 were recorded in the cathodic potential range 0 to −1.4 V. All the copper (II) and nickel (II) complexes exhibit two quasi-reversible steps at different potentials. In competitive binding experiments the $K_{a}$ values for complexes were $3.1 \times 10^5$ M$^{-1}$ (1), $4.2 \times 10^5$ M$^{-1}$ (2), $3.78 \times 10^5$ M$^{-1}$ (3) and $4.93 \times 10^4$ M$^{-1}$ (4) respectively. DNA cleavage mechanism is an oxidative DNA pathway in the presence of activators, in which generating active oxygen species such as hydroxyl radical, probably a copper-peroxide, degrades DNA.

1. Introduction

The war on cancer is still not won. Cancer is responsible for about 500,000 people deaths/year (20% to 50% of the total mortality) in the USA with about one million cases developing each year. The overall incidence and mortality rates in the USA between 1973 and 1990 have increased by 18.3 and 6.7% respectively. The three most common cancers in men are lung, prostate, and colorectal cancers. In women, breast, colorectal and lung cancer are the most common tumors [1]. Chemotherapy, no doubt, is the major cancer modality for patients having a tumor that has metastasized to distant sites of the body at the time of diagnosis or relapse at some time following primary surgery or radiation therapy. DNA has been used as a traditional target in chemotherapy for human cancer [2, 3]. Cisplatin is widely used as an anticancer drug that is highly effective against testicular and ovarian cancers but the major limitations are severe toxicity to fast growing normal cells in the bone marrow, hair follicles and gastrointestinal tract because of the inability of the presently available anticancer drugs to distinguish cancer cells from the normal cells [4-6]. In spite of that the high therapeutic efficiency of anticancer drugs has inspired in the development of next generation agents that are effective against cancer cells with fewer side effects. With this connection copper(II) and nickel(II) complexes tend to be strongly mutagenic, and some have shown promising chemotherapeutic activity, which correlates well with DNA-binding affinity [7-9].

In this paper, we have described the synthesis and characterization of a series of acyclic end-off copper(II) and nickel(II) complexes. Binding affinity of copper(II) and nickel(II) complexes to calf thymus (CT-DNA) was monitored using spectroscopic titration, viscosity measurements. Nuclease activities of synthesized complexes were monitored and the mechanism of cleavage also studied.

2. Experimental Methods

2.1 Materials and Methods

The precursor compound, 2, 6-diformyl-4-methyl phenol was prepared by reported procedure [10]. Tetra(n-butyl)ammonium perchlorate (TBAP) was purchased from Fluka and recrystallized from hot methanol and used as the supporting electrolyte in electrochemical measurement. (Caution! TBAP is potentially explosive and hence, care should be taken in handling the compound). All other chemicals and solvents were purified by reported procedures [11]. CT DNA and pBR322 DNA and Ethidium bromide (EtBr) were purchased from Bangalore Genie (India). Tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer was prepared using deionized water.

Elemental analysis was carried out in Carlo Erba model 1106 elemental analyzer. FT-IR spectra were recorded in (4000 − 400 cm$^{-1}$ Perkin Elmer FTIR spectrometer with samples prepared as KBr pellets. UV-visible spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200−900 nm. Emission intensity measurements were carried out using Perkin Elmer LS-45 fluorescence spectrometer. Electrochemical measurements were performed using electrochemical analyzer CHI 1008 using a three-electrode cell. Glassy carbon electrode is a working electrode with saturated Ag/AgCl electrode as the reference electrode and platinum wire as auxiliary electrode. The concentration of all the complexes was made at 10$^{-3}$ M. TBAP (10$^{-1}$ M) was used as the supporting electrolyte in all electrochemical experiments.

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Scheme 1 synthesis of new copper(II) and nickel(II) complexes (1-4)

2.2 Synthesis of Binuclear Copper(II) Complexes

To ethanolic solution of Salicylaldehyde (0.11 mL, 1 mmol) was added diethylamine (0.11 mL, 1 mmol) to prepare complex 1 and/or for complex 3 dipropalene triamine in the manner of dropwise under constant stirring (Scheme 1). To further ethanolic solution of (M(CIO)3, 6H2O (1 mmol) was added and the solution was stirred well and refluxed for 1 h. The resulting hot solution was evaporated under room temperature for about one fourth of the solution, and then precipitated color solid was separated out and washed well with cold ethanol. For complex 1: Yield: 0.24 g (60 %); Brown color solid; Anal.Calc for C14H13CuNO5.3C; 55.84; H. 5.29; N, 12.6; Found: C55.77; H, 5.27; N, 12.62 and for complex 3: Yield: 0.211 g (54 %); Green color solid; Anal.Cal for C16H15CuN6O6.5C; 58.16; H; 6.0; N, 11.63; Found: C; 58.11; H; 5.94; N, 11.66. The binuclear Cu (II) complex was prepared from a vigorously stirred suspension of 2,6-diformyl-4-methyl phenol (0.328 g, 2 mmol) in 50 mL ethanol/chloroform (9:1 mL) then ethanolic solution of complex 1 and complex 3 (4 mmol) was added slowly and the mixture was stirred for 15 min to obtain a clear solution. The reaction mixture was refluxed for 3 h, the hot solution was filtered and evaporated at room temperature, the dark red solid was separated out and dried well recrystallized in ethanol.

For complex 2: Red color solid; Yield: 0.449 g (61 %); Anal.Cal for C16H14CuN6O6.5C; 56.67; H; 5.37; N, 12.79; Found: C; 56.61; H; 5.31; N, 12.82 and for complex 4: Green color solid; Yield: 0.450 g (57 %); Anal.Cal for C16H15CuN6O6.5C; 58.95; H; 6.04; N, 11.78; Found: C; 58.89; H; 5.98; N, 11.82.

2.3 DNA Binding Experiments

Fluorescence quenching experiment were carried out by addition of complexes to sample solution containing EtBr-DNA. The spectra were recorded at excitation wavelength 520 nm and emission wavelength between 610 nm. Using the fluorescence quenching spectra, the reduction in emission intensity measures the binding propensity of complex to CT DNA. Stern-Volmer quenching constant (Ksv) and apparent binding constant (Ksv) were calculated using I0/I = 1 + Ksvr and Ksv = [EB][complex]/[I] where I0 and I corresponds fluorescence intensities of ETBr-DNA in absence and presence of complex, r is the ratio of the total concentration of complex to that of DNA, and Ksv = 1 × 103 [EB] [in µM] [complex] is the concentration of the complex at 50% reduction of emission intensity of ETBr respectively [12].

2.4 DNA Cleavage Studies

The DNA cleavage experiments were performed by agarose gel electrophoresis, by incubation at 37 °C as follows: pBR322 DNA (0.1 µg µl) in 50 µl of 0.1 M HCl buffer (pH 7.2) was incubated with 1 µl of complexes containing 1% DMF. The samples were incubated for 3 h, and then loading buffer (1 µl) was added. The sample was electrophoresed for 3 h at 50 V on 0.8% agarose gel using Tris–Acetic acid-EDTA buffer. After electrophoresis, bands were visualized by UV light and photographed. To identify the reactive oxygen species (ROS) involved in the cleavage reaction to introduce the scavengers like Na2S2O4, L-histidine (singlet oxygen), SOD (superoxide), and DMSO (hydroxyl). The extent of cleavage of the super coiled DNA (SC DNA) was determined by measuring the intensities of the bands using the UVITEC Gel Documentation System [13].

3. Results and Discussion

3.1 FTIR Spectral Characterization

All the complexes are stable in air and good solubility in DMF, DMSO, methanol, ethanol and 1% DMF/50 mM Tris-HCl buffer solution. The complexes are characterized by various spectroscopic and analytical methods. ESI-MS spectra of the copper(II) complexes (1-4) showed an essentially molecular ion peaks and their isotopic peak [M+1]. From IR spectra of acyclic copper(II) complexes shows the three important peaks (Table 1).

Table 1 FT-IR spectral data of all the complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>ν(H-N) (cm⁻¹)</th>
<th>ν(C-N) (cm⁻¹)</th>
<th>ν(C=O) (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3176, 3264</td>
<td>1623</td>
<td>1558</td>
</tr>
<tr>
<td>2.</td>
<td>3162, 3265</td>
<td>1623</td>
<td>1568</td>
</tr>
<tr>
<td>3.</td>
<td>3205, 3293</td>
<td>1640</td>
<td>1582</td>
</tr>
<tr>
<td>4.</td>
<td>3142, 3230</td>
<td>1640</td>
<td>1538</td>
</tr>
</tbody>
</table>

One is sharp peak observed in the region of 1000-1100 cm⁻¹ and sharp band in the region of 626 cm⁻¹ which indicates that the perchlorate anion of antisymmetric stretching and antisymmetric bending respectively. Third a strong absorption band around 1600-1635 cm⁻¹ is due to the azomethine (C=N) bond as a place of 1680 cm⁻¹ as a result of this, the formation of Schiff base product is confirmed. Absorption for the aromatic rings occurs around in the 1560 cm⁻¹ region. Complexes (1-4) shows weak band around 3220 cm⁻¹ and 3100 cm⁻¹ indicates for the N-H stretching vibration of coordinated secondary amine groups. The non-ligand peaks at around 500-600 cm⁻¹ were assigned to (Cu-N) and (Cu-O) stretching vibration respectively.

3.2 Electronic Spectroscopy

The electronic spectra were recorded in DMF solvent in the region 200-900 nm. The electronic spectra of the binuclear acyclic copper(II) complexes observed three main transitions (Table 2). One has less intense band in the range of 500-600 nm is due to d-d transition of the metal ion, a moderately intense band in range of 300-400 nm is due to ligand to metal charge transfer transition and a band observed at below 300 nm is assigned to an intraligand (π-π*) charge transfer transition. The electronic spectra of the copper(II) complexes 2 and 4 have higher wavelength compared to complexes 1 and 3. As a result of red shift, the distortions of geometry increase with increase the aliphatic group of the complexes. The d-d band below 600 nm corresponds to [B3 – B2g] [A2g – A1g] to d-d transition which is is consistent with that of a square pyramidal geometry around the copper(II) complexes. Therefore, it appears that there is a distorted square-pyramidal geometry of the Cu(II) centers in the copper(II) complexes and Ni(II) centers in the nickel(II) complexes.

Table 2 UV-Vis spectral data of the complexes (1-4)

<table>
<thead>
<tr>
<th>Complexes</th>
<th>λmax (nm)</th>
<th>ε (m²·cm⁻¹·mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>560(ε=125)</td>
<td>362(ε=96,000)</td>
</tr>
<tr>
<td>2.</td>
<td>630(ε=580)</td>
<td>358(ε=45,000)</td>
</tr>
<tr>
<td>3.</td>
<td>576(ε=300)</td>
<td>357(ε=70,000)</td>
</tr>
<tr>
<td>4.</td>
<td>620(ε=320)</td>
<td>346(ε=58,000)</td>
</tr>
</tbody>
</table>

3.3 Electrochemical Studies

Redox behavior of all the complexes reported in the present work were studied by suing cyclic voltamogramme in the potential ranges 0 to 21.6 V and 0 to 1.6 V in dimethylformamide containing 0.1 M tetraprobutylammonium perchlorate (Caution! TBA is potentially an explosive compound, hence care should be taken in handling the compound) as a supporting electrolyte. All the nickel(II) complexes undergo both reduction and oxidation in cathodic and anodic potentials, respectively. Systematic electrochemical studies on various metal complexes can distinguish three types of electrochemical behaviors, reversible, quasi-reversible and irreversible electron transfer processes.

(i) Reversible Systems

- The cathodic and anodic potential are independent of scan rate.
- ΔE value is 59 mV at 25 °C.
- The anodic to cathodic current ratio is unity.
- The wave shape doesn’t change with change in scan rate.

(ii) Quasi-Reversible Systems

- The anodic and cathodic peak potential varies with respect to scan rates.
- ΔE value is higher than 59 mV and increases with this scan rate.
- The anodic to cathodic current ratio is greater than unity.
- The nature of the wave shape broadens as this scan rate increases.

(iii) Irreversible Systems

- Ep value shift cathodically by 30 mV per tenfold increase in the scan rate.
- There is no current on the reverse scan.
- The wave shape in determined by the charge transfer coefficient and it is independent of the scan rate.

Two different reduction and oxidation waves of all the copper(II) complexes maybe due to copper metal ions present in two different compartments during redox processes.

3.3.1 Reduction Process

The electrochemical property of all the complexes 1-4 were recorded by cyclic voltammetry. The electrochemical data of cathodic peak potential(Ec), anodic peak potential(Epa), redox peak E1/2, and peak

separation ($\Delta E_p$) are given in Table 3. In the cathodic potential range 0 to -1.4 V all the copper (II) and nickel(II) complexes exhibit two quasi-reversible steps at different potentials. In all the four complexes well-defined peaks, observed in the potential range from -0.81 to -0.93 V were due to the reduction of Cu$^{2+}$-Cu$^{4+}$ to Cu$^{2+}$-Cu$^{4+}$ and Ni$^{2+}$-Ni$^{4+}$ to Ni$^{2+}$-Ni$^{4+}$. The second reduction waves observed the potential range from 1.04 to -1.17 V are due to reduction of Cu$^{2+}$-Cu$^{4+}$ to Cu$^{2+}$-Cu$^{4+}$ and Ni$^{2+}$-Ni$^{4+}$ to Ni$^{2+}$-Ni$^{4+}$. (Fig 1) Based on the observations, it is reasonable to suggest that the reduction process may involve the following steps [14].

\[ \text{Cu}^{2+} \rightarrow \text{Cu}^{0} \rightarrow \text{Cu}^{2+} \rightarrow \text{Cu}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{0} \rightarrow \text{Ni}^{2+} \rightarrow \text{Ni}^{4+} \]

\[ \text{Cu}^{2+} \rightarrow \text{Cu}^{0} \rightarrow \text{Cu}^{2+} \rightarrow \text{Cu}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{0} \rightarrow \text{Ni}^{2+} \rightarrow \text{Ni}^{4+} \]

### 3.3.2 Oxidation Process

Fig. 2 shows the cyclic voltammogram of the binuclear Ni(II) complexes, which exhibit two quasi-reversible oxidation waves. The first oxidation potential ranges from 0.64 to 0.66 V and the second oxidation potential falls in the range from 1.08 to 1.10 V. The oxidation process can be described as below. The values are shown in Table 4.

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{3+} \rightarrow \text{Ni}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{3+} \rightarrow \text{Ni}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{3+} \rightarrow \text{Ni}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{3+} \rightarrow \text{Ni}^{4+} \]

\[ \text{Ni}^{2+} \rightarrow \text{Ni}^{3+} \rightarrow \text{Ni}^{4+} \]

### Table 3 Electrochemical reduction data of copper(II) and nickel(II) complexes

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$E_{pa}(V)$</th>
<th>$E_{pc}(V)$</th>
<th>$I_{pa}(A)$</th>
<th>$I_{pc}(A)$</th>
<th>$\Delta E_{pa}(mV)$</th>
<th>$\Delta E_{pc}(mV)$</th>
<th>$\Delta E_{re}(mV)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-0.88</td>
<td>-0.74</td>
<td>-0.81</td>
<td>140</td>
<td>-1.17</td>
<td>-1.10</td>
<td>-1.14</td>
</tr>
<tr>
<td>2.</td>
<td>-0.78</td>
<td>-0.65</td>
<td>-0.72</td>
<td>120</td>
<td>-1.15</td>
<td>-1.06</td>
<td>-1.11</td>
</tr>
<tr>
<td>3.</td>
<td>-0.93</td>
<td>0.73</td>
<td>-0.93</td>
<td>200</td>
<td>-1.2</td>
<td>-1.1</td>
<td>-1.15</td>
</tr>
<tr>
<td>4.</td>
<td>-0.81</td>
<td>-0.56</td>
<td>-0.69</td>
<td>250</td>
<td>-1.04</td>
<td>-0.90</td>
<td>-0.96</td>
</tr>
</tbody>
</table>

### Table 4 Electrochemical oxidation data of nickel(II) complexes

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$E_{pa}(V)$</th>
<th>$E_{pc}(V)$</th>
<th>$I_{pa}(A)$</th>
<th>$I_{pc}(A)$</th>
<th>$\Delta E_{pa}(mV)$</th>
<th>$\Delta E_{pc}(mV)$</th>
<th>$\Delta E_{re}(mV)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.64</td>
<td>0.46</td>
<td>0.55</td>
<td>180</td>
<td>1.08</td>
<td>0.88</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>0.66</td>
<td>0.48</td>
<td>0.72</td>
<td>180</td>
<td>1.10</td>
<td>0.82</td>
<td>0.96</td>
</tr>
</tbody>
</table>

### 3.4 DNA Binding and Cleavages Studies

#### 3.4.1 Ethidium Bromide Displacement Assay

The competitive DNA binding of complexes has been studied by monitoring changes in emission intensity of ethidium bromide (EtBr) bound to CT-DNAs as a function of added complex concentration. Though the emission intensity of EtBr in buffer medium (50 mM Tris-HCl) is quenched by the solvent molecules, it is enhanced by its stacking interaction between adjacent DNA base pairs. When complexes were added to DNA preincubated with EtBr ([DNA]/[EtBr]=1:1), the DNA-induced emission intensity of EtBr was decreased (Fig 3) [15]. Addition of a second DNA binding molecule would quench the EtBr emission by either replacing the DNA-bound EtBr (if it binds to DNA more strongly than EtBr) or accepting an excited state electron from EtBr. Because the complexes have planar ligands, they efficiently compete with strong intercalators like EtBr for intercalative binding sites on DNA by replacing EtBr, which is reflected in quenching of emission intensity of DNA-bound EtBr. The apparent binding constant ($K_{app}$) has been calculated from the following equation

$K_{app}$ = $K_{b}$ [complex]

where $K_{b}$ is 1 x $10^{7}$ M$^{-1}$ and the concentration of EtBr is 20 µM [complex] is the concentration of the complex causing 50% reduction in the emission intensity of EtBr. The $K_{app}$ values for complexes were 3.1 x $10^{8}$ M$^{-1}$ (1), 4.2 x $10^{7}$ M$^{-1}$ (2), 3.7 x $10^{7}$ M$^{-1}$ (3) and 4.9 x $10^{7}$ M$^{-1}$ (4) respectively. The higher values of $K_{app}$ indicate that these complexes bind to DNA by intercalation. The Stern-Volmer quenching constant $Ksv$ of the complex 1 to DNA was calculated from the equation $I_0/I = 1 + Ksv \cdot [Q]$ where $I_0$ and $I$ are fluorescence intensity of EB-DNA in the absence and presence of complex respectively. $Ksv$ is the Stern-Volmer quenching constant, $r$ is the ratio of [Complex]/[DNA]. The calculated value of $Ksv$ is 0.18 (1), 0.41 (2), 0.39 (3) and 0.55 (4).

#### 3.4.2 DNA Cleavage of pBR322

In order to assess the chemical nuclease activities of the copper(II) complexes for DNA strand scission, pBR322 DNA was incubated with the copper(II) complexes under the reaction conditions. The cleavage reaction can be monitored by gel electrophoresis. When pBR322 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoiled form will relax to generate a slower-moving nicked form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated [16].

Firstly, the chemical nuclease activities of complex 2 has been studied using supercoiled pBR322 plasmid DNA as a substrate in the medium of 50 mM Tris-HCl/NaCl buffer (pH = 7.2) in the presence of hydrogen peroxide under physiological conditions. Complex 2 cleaves the supercoiled DNA(SC) to nicked circular DNA(NC) and linearised forms. In order to obtain information about the active oxygen species which was responsible for the DNA damage, we investigated the DNA cleavage in the presence of hydroxyl radical scavengers (DMSO), singlet oxygen quenchers (NaN$_3$, L-Histidine), superoxide scavenger (SOD) and chelating agent (EDTA) under our experimental conditions. From Fig. 4, we can see that no obvious inhibitions are observed for the complex 2 in the presence of SOD (lane 4), L-Histidine (lane 6) and NaN$_3$, as the results rule out the possibility of DNA cleavage by superoxide and singlet oxygen. The addition of EDTA (Lane 3) partly diminishes the nuclease activity of the complex which is indicative of the involvement of metal ion like entity in.

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the cleavage process. The inhibitory activity of DMSO (Lane 4) is more comparable to other inhibitors, so strongly suggests that the hydroxyl radical was involved in the cleavage mechanistic pathway. The copper(II) complex examined here may be capable of promoting DNA cleavage through an oxidative DNA damage pathway in the presence of activators, in which giving active oxygen species such as hydroxyl radical, probably a copper-peroxide, that cleaves DNA. As follows the same concentration of complex and scavenger used for the complexes 1, 3 and 4 which have the same results collected for the gel electrophoresis picture (Fig. 5).

4. Conclusion

The competitive DNA binding of complexes has been studied by monitored the changes in emission intensity of ethidium bromide (EtBr) bound to CT-DNAs as a function of added complex concentration. The K_{\text{app}} values were calculated as 3.1x10^{3} \ M^{-1} \ L \ (2), 3.78x10^{3} \ M^{-1} \ L^{2}) and 4.93x10^{3} \ M^{-1} \ L^{2} \ (3) respectively. The higher values of Kapp indicate that synthesized complexes bind with DNA by intercalation mode. In cleavage studies, synthesized complexes cleave the DNA in presence of H_{2}O_{2} as an activator and the reactive oxygen species were responsible for the DNA damage. In mechanistic investigation, the best inhibition activity as found in presence of DMSO, as result hydroxyl radical induces the DNA cleavage process.

References