Reprint of Publication
Synthesis, spectroscopic characterization and comparative DNA binding studies of Schiff base complexes derived from L-leucine and glyoxal

Mohammad Shakir, Nida Shahid, Naushaba Sami, Mohammad Azam, Asad U. Khan

A Division of Inorganic Chemistry, Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India
b Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India

1. Introduction

Schiff base ligands played an integral and important role in the development of coordination chemistry since the late 19th century. Metal complexes of these ligands are ubiquitous due to their facile synthesis, wide applications and the accessibility of diverse structural modifications [1]. The chelating structures, moderate electron donation easy tunable electronic and steric effects proved Schiff bases as versatile ligands. They are capable of stabilizing different metals in various oxidation states with unusual structural features. Schiff bases are able to control the performance of metals in variety of useful catalytic transformations [2-4]. Characteristically, Schiff base provides geometrical cavity control for host–guest interaction. The modulation of its lipophilicity offers a remarkable selectivity, sensitivity and stability for specific metal ion [5]. Schiff base complexes of amino acids have gained importance because of their physiological and pharmacological activities [6,7]. The complexes of amino acid Schiff bases are considered to constitute new kinds of potential antibacterial and anticancer reagents due to the presence of azomethine functional group [8-10]. The interaction of transition metal complexes with DNA has been well documented. There has been considerable interest in DNA-binding properties of transition metal complexes with small molecules on a molecular level [11]. These metal complexes are known to bind to DNA through a series of interactions, such as π stacking interaction associated with interaction of aromatic heterocyclic groups between the base pairs, hydrogen bonding and Vander Waals interactions in the case of binding to the groove of DNA helix [12]. Chemists over a period of years reported the binding of cationic metal complexes with DNA [13,14]. The factors that determine the affinity and selectivity in the binding of small molecules to DNA would be valuable in the rational design of new diagnostic and therapeutic agents [15-17]. In continuation of our ongoing interest in the synthesis of Schiff bases and their DNA interactive studies [18-21], herein, we report synthesis of complexes, derived from the template condensation reaction between glyoxal and L-leucine, [ML(CH3OH)2] [M = Co(II), Ni(II), Cu(II) and Zn(II)] and their characterization using various physio-chemical techniques and comparative DNA binding studies.

2. Experimental

2.1. Materials and methods

All the reagents used were of A.R grade. The metal salts MCl2·nH2O [M = Co(II), Ni(II); n = 6, Cu(II); n = 3, Zn(II); n = 0] and the chemical L-leucine and Glyoxal (All E. Merck) were commercially pure samples and were used as received. Highly polymerized calf-thymus DNA sodium salt and ethidium bromide (EtBr) was purchased from Sigma Chemical Co. Calf thymus DNA was dissolved to 0.5% w/w (12.5 mM DNA/phosphate) in 0.1 M sodium phosphate buffer (pH 7.40) at 310 K for 24 h with occasional stirring to ensure formation of homogeneous solution. The purity of the DNA solution was checked from the absorbance ratio A320/A280. Since the absorption ratio lies in the range 1.8 < A320/A280 < 1.9, therefore no further deproteinization of DNA was needed. The stock solution of Schiff base complexes with 5 mg/ml concentration was also prepared.
2.2. Synthesis of complexes [ML(CH₃OH)₂]

To a solution of metal chloride (1 mmol) dissolved in 10 ml methanol, the solution of glyoxal (1 mmol) dissolved in 25 ml methanol was added and stirred at room temperature for 1 h. To this stirred solution, L-leucine (2 mmol) dissolved in 10 ml methanol containing NaOH (2 mmol) was added and stirred for 24 h at room temperature, leading to the isolation of solid product which was filtered, washed with methanol and finally dried in vacuum over anhydrous calcium chloride.

2.3. Physical measurements

The elemental analysis was made using Perkin-Elmer 2400 CHN elemental analyzer. The FT-IR spectra (4000–200 cm⁻¹) were recorded as KBr pellets on Perkin Elmer-2400 spectrometer. ¹H NMR and ¹³C NMR spectra in DMSO-d₆ at room temperature were recorded using bruker avance II 400 NMR spectrophotometer, EPR spectrum was recorded on E112 ESR spectrometer at room temperature. The electronic spectra in DMSO were recorded on Pye-unicam-8800 spectrophotometer at room temperature. Magnetic susceptibility measurements were carried out on a Faraday balance at 25 °C. The electrical conductivities of 10⁻³ solution in DMSO were obtained on a systronic type 302 conductivity bridge equilibrated at 25 °C ± 0.01. Mass spectrum was recorded on mass spectrometer (LC–MS/MS). Fluorescence measurements were performed on a spectrofluorimeter Model RF-5301PC (Shimadzu, Japan) equipped with a 150 W Xenon lamp and a slit width of 5 nm. A 1.00 cm quartz cell was used for measurements. For the determination of binding parameters, 50 μM of DNA–EtBr (1:1) complex was taken in a quartz cell and increasing amounts of tested complexes were titrated. Fluorescence spectra were recorded at temperature (310 K) in the range of 510–670 nm upon excitation at 355 (λ_em was 594 nm).

2.4. Binding property of the DNA to Schiff base complexes

Stern–Volmer equation (1) was used for data analysis to elaborate the fluorescence quenching mechanism [22]

\[
\frac{F_0}{F} = 1 + K_{SV}[Q]
\]

(1)

where \(F_0\) and \(F\) are the steady-state fluorescence intensities in the absence and presence of quencher, respectively. \(K_{SV}\) the Stern–Volmer quenching constant and \([Q]\) is the concentration of quencher (tested complexes). The \(K_{SV}\) for the tested complexes series was found to be of the order of 10⁴ and highest for Cu(II) complex (i.e., 25.7 × 10⁴ Lmol⁻¹). The \(F_0/F\) versus \([Q]\) (Stern–Volmer) plots for tested complexes (Fig. 1) depicts that the quenching may be static or dynamic, since the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. When ligand molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation [23]:

\[
\log \left( \frac{F_0 - F}{F} \right) = \log K + n \log [Q]
\]

(2)

where \(K\) and \(n\) are the binding constant and the number of binding sites, respectively. Thus, a plot of \(\log (F_0 - F)/F\) versus \(\log [Q]\) can be used to determine \(K\) as well as \(n\) (Fig. 2). The values of \(K\) and \(n\) (Table 1), suggested the high binding affinity between the complexes and the DNA, among which Cu(II) complex shows the highest binding.

3. Results and discussion

Schiff base complexes were synthesized by the condensation of L-leucine with glyoxal in the presence of metal salts in 2:1:1 molar ratio in methanol (Scheme 1). All complexes were stable at room temperature and were soluble in DMSO. The formation of the amino acid Schiff base complexes was confirmed on the basis of elemental analysis, molecular ion peaks in mass spectra, the characteristic bands in the FT-IR and resonance signals in the ¹H and ¹³C NMR spectra. The overall geometry of the complexes was inferred from the observed values of magnetic moments and the position of the bands in the electronic spectra. The molar conductance measurements of all the complexes recorded in DMSO, exhibited their non-electrolytic nature. The analytical data along with physico–chemical properties of Schiff base complexes are summarized in Table 2.
Scheme 1. Synthesis and proposed structure of Schiff base complexes $M = \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)}$ and $\text{Zn(II)}$.

3.1. IR spectra

The important IR spectral bands of amino acid Schiff base complexes are listed in Table 3. All the complexes exhibit strong band in the region 1632–1601 cm$^{-1}$ attributed to amide group [24] (Fig. 3). The complexes show strong bands in the regions 1495–1525 cm$^{-1}$ and 1281–1306 cm$^{-1}$ assigned to the $\nu_{as}(\text{COO}^-)$ and $\nu_{sy}(\text{COO}^-)$, respectively. The frequency difference $\Delta \nu$ of 214–219 cm$^{-1}$ between asymmetric and symmetric vibration suggests monodentate coordination of the carboxyl group of amino acid with metal ion [25]. This is further confirmed by the presence of the band appearing in the region 536–586 cm$^{-1}$ assigned to the $\nu(\text{M–O})$ frequency [26]. The band observed in the region 455–485 cm$^{-1}$ can be assigned to $\nu(\text{M–N})$, indicating the participation of azomethine nitrogen in the coordination to the metal ion [27]. The spectra of all the complexes show broad bands in the region 3406–3442 cm$^{-1}$ attributed to $\nu(\text{O–H})$ group indicating the presence of coordinated methanol [28]. A medium intensity band in the region 2840–2980 cm$^{-1}$ is due to the aliphatic $\nu(\text{C–H})$ stretching [29].

3.2. $^1$H and $^{13}$C NMR spectrum of Zn(II) complex

The $^1$H NMR spectrum of the Zn(II) complex shows a sharp resonance signal at 8.052 ppm assigned to $-\text{HC}\equiv\text{N}$ proton [30]. The signals appearing at 3.37 ppm and 4.0 ppm may reasonably be assigned to $-\text{CH}_3$ and $-\text{OH}$ protons, respectively indicating the presence of coordinated methanol [28]. The chemical shift values of 3.6 (t, 1H, CH), 2.07 (m, 1H, CH), 1.74 (m, 1H, CH) and 0.79 (d, 6H, CH) observed for Zn(II) complex corresponds to the aliphatic protons of amino acid Schiff base complex [29].

Further evidence regarding the formation of Schiff base complex comes from $^{13}$C NMR spectrum. The $^{13}$C NMR spectrum of Zn(II) complex (Fig. 4) shows a resonance signal at 162.34 ppm corresponding to azomethine carbon [31] while other signals corresponding to carboxylate carbon, carbon atom adja-

![Image](image_url)

Fig. 3. The IR spectrum of Co(II) complex.

![Image](image_url)

Fig. 4. The $^{13}$C NMR spectrum of Zn(II) complex.
cent to carboxylate carbon, coordinated methanol carbon and aliphatic carbons appears at 180.14 ppm, 70.47 ppm, 49.04 ppm and 30–41 ppm, respectively [28,32].

3.3. EPR spectrum

The EPR spectrum of Cu(II) complex has been recorded at room temperature (Fig. 5). The EPR spectrum of the polycrystalline Cu(II) complex shows one signal. The analysis of the spectrum gives \( g_z \) and \( g_\perp \) values of 2.150 and 2.080, respectively indicating the unpaired electron in the dx\(^2\) − y\(^2\) orbital. The axial spectrum with \( g_z > g_\perp > 2.04 \) is consistent with distorted octahedral geometry around Cu(II) ion [33]. The parameter \( G \) is calculated by using the expression i.e., \( G = (g_z - 2)/(g_\perp - 2) \). The calculated \( G \) value at 1.875 indicates considerable interaction in the complex [34].

### Table 3

IR spectral data (cm\(^{-1}\)) for schiff base complexes.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( v(\text{C-N}) )</th>
<th>( v(\text{COO}^-) )</th>
<th>( v_{\text{as}}(\text{COO}^-) )</th>
<th>( v(\text{M-N}) )</th>
<th>( v(\text{M-O}) )</th>
<th>( v(0-H) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CuH}_2\text{N}_2\text{O}_2\text{Co} \left( \text{CH}_3\text{OH} \right)_2 )</td>
<td>1632</td>
<td>1495</td>
<td>1281</td>
<td>568</td>
<td>487</td>
<td>3445</td>
</tr>
<tr>
<td>( \text{CuH}_2\text{N}_2\text{O}_2\text{Ni} \left( \text{CH}_3\text{OH} \right)_2 )</td>
<td>1600</td>
<td>1495</td>
<td>1300</td>
<td>602</td>
<td>482</td>
<td>3357</td>
</tr>
<tr>
<td>( \text{CuH}_2\text{N}_2\text{O}_2\text{Cu} \left( \text{CH}_3\text{OH} \right)_2 )</td>
<td>1627</td>
<td>1490</td>
<td>1289</td>
<td>571</td>
<td>480</td>
<td>3406</td>
</tr>
<tr>
<td>( \text{CuH}_2\text{N}_2\text{O}_2\text{Zn} \left( \text{CH}_3\text{OH} \right)_2 )</td>
<td>1600</td>
<td>1525</td>
<td>1306</td>
<td>536</td>
<td>490</td>
<td>3462</td>
</tr>
</tbody>
</table>

![Fig. 5. The EPR spectrum of Cu(II) complex at room temperature.](image)

### 3.4. Mass spectroscopy

The observed molecular ion peak(s) at m/z 436.2, 436.7, 442.9 and 442.5 for Co(II), Ni(II), Cu(II) and Zn(II) complexes, respectively, are consistent with the proposed molecular formulae of the complexes (Table 2).

### 3.5. Electronic spectra and magnetic moments

The electronic spectra, log \( \varepsilon \) value and magnetic moment data of the complexes are summarized in Table 4. The electronic spectrum of Co(II) complex shows bands appearing at 1176 nm (8500 cm\(^{-1}\)) with log \( \varepsilon = 5.84 \), 655 nm (15,245 cm\(^{-1}\)) with log \( \varepsilon = 5.69 \), and 476 nm (20,990 cm\(^{-1}\)) with log \( \varepsilon = 5.30 \) assigned to \( 4T_{2g}(F) \rightarrow 4T_{2g}(F), 4T_{1g}(F) \rightarrow 4A_{2g}(F) \) and \( 4T_{1g}(F) \rightarrow 4T_{1g}(P) \), respectively consistent with octahedral geometry around the cobalt(II) ion which is further supported by a magnetic moment value of 4.7 B.M. [35].

The electronic spectrum of the Ni(II) complex shows three bands at 1023 nm (9769 cm\(^{-1}\)) with log \( \varepsilon = 5.47 \), 595 nm (16,800 cm\(^{-1}\)) with log \( \varepsilon = 5.69 \) and 497 nm (20,100 cm\(^{-1}\)) with log \( \varepsilon = 5.95 \) assigned to \( 3A_{2g}(F) \rightarrow 3T_{2g}(F), 3A_{2g}(F) \rightarrow 3T_{1g}(F) \) and \( 3A_{2g}(F) \rightarrow 3T_{1g}(P) \), respectively, corresponding to an octahedral environment around Ni(II) ion [36]. The magnetic moment value of 3.0 B.M. further supports the electronic spectral findings.

The electronic spectrum of the Cu(II) complex show a broad band centres at 661 nm (15,120 cm\(^{-1}\)) with log \( \varepsilon = 5.77 \) corre-

### Table 4

Magnetic moment, log \( \varepsilon \) values, electronic spectral data with their assignments.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( \mu_{\text{eff}} ) (B.M.)</th>
<th>Bands positions, ( \lambda_{\text{max}} ) (nm)</th>
<th>Assignments</th>
<th>log ( \varepsilon )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CoL(CH}_3\text{OH)}_2 )</td>
<td>4.7</td>
<td>1176</td>
<td>( 4T_{2g}(F) \rightarrow 4T_{2g}(F) )</td>
<td>5.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>655</td>
<td>( 4T_{2g}(F) \rightarrow 4A_{2g}(F) )</td>
<td>5.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>476</td>
<td>( 4T_{1g}(F) \rightarrow 4T_{1g}(P) )</td>
<td>5.30</td>
</tr>
<tr>
<td>( \text{NiL(CH}_3\text{OH)}_2 )</td>
<td>3.0</td>
<td>1023</td>
<td>( 3A_{2g}(F) \rightarrow 3T_{2g}(F) )</td>
<td>5.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>595</td>
<td>( 3A_{2g}(F) \rightarrow 3T_{1g}(F) )</td>
<td>5.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>497</td>
<td>( 3A_{2g}(F) \rightarrow 3T_{1g}(P) )</td>
<td>5.95</td>
</tr>
<tr>
<td>( \text{CuL(CH}_3\text{OH)}_2 )</td>
<td>2.0</td>
<td>661</td>
<td>( 2E_g \rightarrow 2T_{2g} )</td>
<td>5.77</td>
</tr>
</tbody>
</table>
3.6.2. Interaction

The fluorescence spectroscopy provides an in depth information regarding the changes taken place in the microenvironment of DNA–ligand interaction. The interaction of Schiff base complexes with calf thymus DNA were studied by monitoring the changes in the extrinsic fluorescence of EtBr at varying concentration of the tested complexes (Fig. 6) shows the representative fluorescence emission spectra of the DNA–EtBr complex. The addition of complex caused a gradual decrease in the fluorescence emission intensity. The quenching of the compound fluorescence spectra clearly indicated that the binding of the compound to DNA has changed the microenvironment of fluorophore residue. The reduction in the fluorescence of DNA–EtBr upon interaction with the compound could be due to masking or burial of fluorophore upon interaction between the stacked bases with in the helix and/or surface binding at the reactive nucleophile sites on the heterocyclic nitrogenous bases of DNA molecule.

3.6.2. Absorption spectroscopy

UV–vis absorption studies were performed to further ascertain the predicted binding trend of Schiff base complexes with DNA. The UV absorbance showed an increase with the increase in drug concentration (Fig. 7). Since the Schiff base complexes does not show any peak in this region (Fig. 7), hence the rise in the DNA absorbance is indicative of the complex formation between DNA and the tested molecules. Cu(II) complex at 260 nm exhibited highest hyperchromism of 30% at 1:1 molar ratio. So we primarily speculate that complex interacting with the secondary structure of the calf thymus DNA resulting in its breakage and perturbation. After interaction with the base pairs of DNA, the π−π* orbital of the bound ligand can couple with the π orbital of the base pairs, due to the decrease π−π* transition energy, which results in bathochromic shift [37]. The prominent shift in the spectra also suggests the tight complexation of synthesized molecule with DNA, which resulted in the change in the absorption maxima of the DNA. Hence the overall results suggest that Cu(II) complex have higher binding affinity toward DNA than Co(II), Ni(II), Zn(II) complexes.

References