

Synthesis, characterization, DNA- Binding Interaction studies and antimicrobial screening mixed-ligand Cu (II) and Mn (II) complexes

Omar H. Al-Obaidi

Chemistry Department, Education College for Women, Al-Anbar University

Abstract: The interaction of ligands and their transition metal complexes with plasmid (pUC19 DNA) and calf Thymus DNA were investigated by UV spectroscopic studies and gel electrophoresis measurements. The interaction between ligand/metal complexes and DNA was carried out by increasing the concentration of DNA from 0 to 10 μ l in UV spectroscopic study, while the concentration of DNA in gel electrophoresis remained constant of 10 μ l. These studies supported the fact that, the complex binds to DNA by intercalation via ligand into the base pairs of DNA. The relative binding efficacy of the complexes to DNA was much higher than the binding of ligands, especially the complex of [Cu (L1) (L2) Cl₂] had the highest binding ability to DNA. The mobility of the bands decreased as the concentration of the complex was increased, indicating that there was increase in the interaction between the metal ion and DNA. Complexes of L1 were excellent for DNA binding as compared to L2.

The complexes show enhanced antimicrobial activities complexes with the free ligand.

A theoretical treatment of the formation of complexes in the gas phase was studied, this was done using the HYPERCHEM-6 program for the Molecular mechanics and Semi-empirical calculations.

I. Introduction

DNA is often referred as the molecule of heredity, as it is responsible for the genetic propagation of all traits^(1,2). Over the past decades, there has been a considerable interest in DNA binding properties towards different types of metal complexes. Further, many transition metal complexes have been used as tools for understanding DNA structure, as agents for mediation of DNA cleavage or as chemotherapeutic agents. DNA is a significant cellular receptor, many chemicals bring to bear their antitumor effects by binding to DNA and by this means changes the replication of DNA and inhibits the growth of the tumor cells. Which is the basis of designing new and more efficient antitumor drugs. Moreover, their effectiveness depends on the mode and affinity of their binding ability to the DNA strands⁽³⁾. A number of metal chelates, as agent for mediation of strand scission of duplex DNA and as chemotherapeutic agents, have been used as probes of DNA structure in solution^(4,5).

In medicine particularly, a new class of drugs have been reported as potent HIV-1 inhibitors^(6,7), protein tyrosine kinase inhibitors⁽⁸⁾, protozoal-retroviral co-infections⁽⁹⁾, anti HIV-1 agent⁽¹⁰⁾ and therapeutic drugs for the inflammatory diseases⁽¹¹⁾.

Looking to the above important of DNA binding study of ligands and metal complexes^(12,13), the present paper describes mixed ligand synthesis and DNA binding interaction assay of L1 and L2.

II. Experimental:

1- INSTRUMENTATION:

A Fisher-100 infrared spectrophotometer was used to recorded the ir spectra as KBr and CsI disc, UV/VIS spectra were measured by a BIOTECH 80D spectrophotometer, Elemental Analysis (C.H.N) founded on (Carlo Erloa microanalyzer type 1106),determination of all metals percentage by atomic absorption spectrophotometry on AA-680G (Shimadzu). Electrical conductance was measured on conductivity CDC304 (Jenway4070). Melting points determined by an electric heated block apparatus (Gallen Kamp), and were uncorrected. Room temperature magnetic susceptibility measurements were carried out on a B.M 6 BRUKER type magnets, balance; Diamagnetic correction was done using pascal constants⁽¹⁴⁾.

2-MATERIALS:

Plasmid (pUC19 DNA) and calf Thymus (CT) DNA were purchased from sigma-Aldrich chemicals. All solvents and reagents were purchased commercially from Merk, and were used without further purification. A solution of DNA in the buffer (phosphate, pH 7.0) gave a ratio of UV absorbance at 260 and 280 nm of about 1.90 indicatly that the DNA was sufficiently free of protein⁽¹⁵⁾. The CT-DNA concentrations in terms of base pair L⁻¹ and in terms of nucleotide L⁻¹ were determined spectrophotometrically by employing an extinction of

$13200 \text{ M}^{-1} \text{ cm}^{-1}$ (bas pair) $^{-1}$ and $6600 \text{ M}^{-1} \text{ cm}^{-1}$ (nucleotide) $^{-1}$ at 260 nm, respectively⁽¹⁶⁾, Deionized, triply distilled water used for preparing various buffers.

A- Preparation of the ligand:

The ligand L1(phenyl-3-methyl-5-pyrazolone Semicarbazone) was prepared by the reaction of (benzaldehyde) with (Semicarbazide), according to the literature⁽¹⁷⁾. The physical properties of these compounds (L₁) are yellow color, yield: 85%, M.P: 174-176 °C, Elemental analysis: (%cal.) %found C: (52.98) 53.42, H: (5.37) 5.30, N: (28.24) 28.32. The characters ir bands and uv/vis spectrum in DMSO as shown in table (1).

B- Synthesis of complexes [M (L1) (L2) Cl₂]; M=Cu, Mn:

Transition metal complexes of L1 and L2 as down in fig. 1. The physical properties of prepared complexes are listed in table (1).

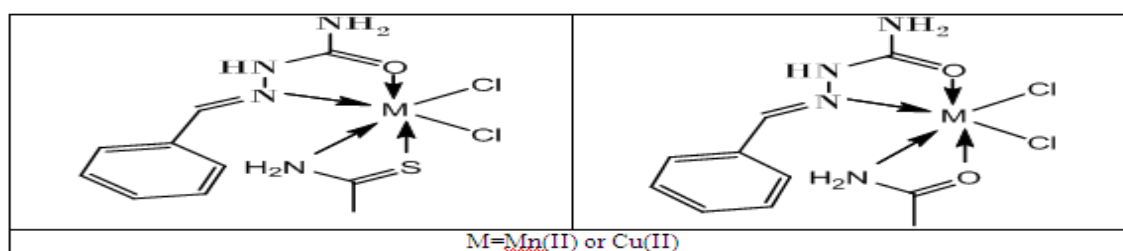


Fig. 1. Structure of metal complexes [M (L1) (L2) Cl₂] and [M (L1) (L3) Cl₂]

C- Methods and Measurements:

The DNA – binding experiments were performed^(18,19) at room temperature for ligands and its transition metal complexes. The solutions were prepared in DMSO at 10 μM concentration.

UV-Visible experiment :

UV-Vis. Spectra were recorded on BIOTECH UV-VIS. Spectrophotometer 80D(UK). UV absorption spectra were studied in the absence and in the presence of DNA (pUC19 and calf Thymus) at a constant of the ligand and complexes (10 μM) in phosphate buffer (pH 7.0). For that, the appropriate volume of the ligand/complex (10 μl) and solution of 0-12 μl DNA (pUC19 and calf Thymus) were added in the phosphate buffer by maintaining the pH of the solutions at 7.0.

D- pUC19 DNA cleavage study:

For the gel electrophoresis experiments, DNA (pUC19 and calf Thymus) was treated with ligand / metal complexes in phosphate buffer at pH 7.0. A garose gel (0.8% w/v) was prepared in TBE buffer (45 mM Tris, 45 boric acid and 1 mM EDTA, pH 7.3). Then 10 μl each of the incubated ligand/metal complexes and DNA mixture was incubated at room temperature for 2hr. then it was loaded on the gel tracking dye (0.25% bromo phenol blue, 40% sucrose, 0.25% xylene cyanole, and 200 mM EDTA) and electrophoresis was carried out TBE buffer system at 50 V for run. At the end of electrophoresis i.e. the end of DNA migration, the electric current was turned off. Then, the gel was stained by immersing it in water containing ethidium bromide (EB)(0.5 μg/ml) for 30-45 min at room temperature and later visualized under UV light using a trans illuminator. The illuminated gel images were captured with an attached camera. photo quantization of the gel after electrophoresis was estimated using AlphDigiDoc.RT. Version v.4.0.0 Pc-image software,(USA).

E- Antibacterial activity studies :

The in vitro antibacterial activity of the ligand and its complexes were tested against the bacteria Staphylococcus aureus and Escherichia coli by the paper disc method using nutrient agar as the medium. The extract loaded discs inoculated with microorganisms were incubated at 30°C for 24 hr. for the bacteria. During the incubation period, the test solution diffused and the growth of the inoculated microorganisms was affected.

III. RESULT AND DISCUSSION:

3.1 Synthesis and characterization:

The IR, UV-Vis. And magnetic moment data of the section and data are provided in table (1). Semi-empirical methods are done on hyperchem program version (6) running on a windows XP work station with a Pentium IV PC. Geometry optimization has been carried out by using a conjugate gradient method⁽²⁰⁾.

3.2 Molar Conductance Measurements:

The molar conductance values of the complexes as shown in table (1) carried out in DMSO solvent in 10^{-3} M at 25 °C indicate that the complexes are electrolytic nature⁽²¹⁾. The values suggest that an ions present outside the coordination spheres.

3.3 IR Spectra

The spectra of the ligands show a band within the range $(1650) \text{ cm}^{-1}$ which is assigned to $\nu^{(\text{C}=\text{N})}$ of azomethine group^(22,23), table (2). The observed low frequency shift of $\nu^{(\text{C}=\text{N})}$ for all complexes relative to that of the corresponding free ligand is due to the participation of azomethine group in coordination.

The spectra are also characterized by a new band in range $(440-460) \text{ cm}^{-1}$ assigned to $\nu^{(\text{M}-\text{N})}$ ^(24,25), which indicating the involving of nitrogen atoms in coordination with metallic ion.

The band at 825 cm^{-1} in the spectrum of L1 due to C=S stretching vibrations. In the metal complexes this band is weakened and lowered⁽²⁶⁾ (table 2), the observations indicate the coordination of the ligand through sulphur atom.

The coordination through oxygen atom of carbonyl group was also confirmed by decreases to $(1640-1660) \text{ cm}^{-1}$ compared to the free ligand at $(1690) \text{ cm}^{-1}$ and the appearance of a medium new band at $500-550 \text{ cm}^{-1}$ which is assigned to $\nu^{(\text{M}-\text{O})}$ ^(24,27).

3.4 Electronic Absorption Spectra and Magnetic Moment :

The electronic absorption spectra of the ligands and its Mn(II) and Cu(II) complexes are recorded at room temperature using DMSO as the solvent.

The electronic absorption spectra of the Schiff bases complexes as shown in table (2), from their values the following can be pointed out.

Manganese Complexes

The spectra of Mn (II) complexes display a strong band at approximately $15050-13450 \text{ cm}^{-1}$ assigned for the ${}^2\text{T}_{2g} \rightarrow {}^2\text{T}_{1g}(\text{G})$ transition. this may indicate an octahedral geometry for Cu (II) complexes. these bands gather with the μ_{eff} value (5.93- 5.88 B.M) indicate an octahedral geometry^(28,29).

Copper Complexes

Abroad band at 16150 cm^{-1} due to ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$ transition in Cu(II) complexes and effective magnetic moment of 1.70-1.75 B.M. are suggestive of an octahedral geometry around Cu(II) ion^(30,31).

3.6 Theoretical Study:

The ball and cylinders and some of selected structural parameters (bond length and angles) of the optimized geometries are shown in fig.(2) table (3).

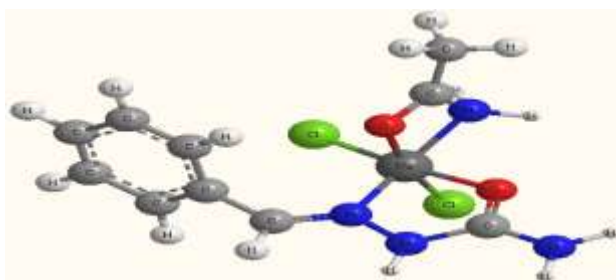


Fig.(2): The optimized structural geometry of Cu(II) complexes

As shown in this figure, there is no obvious trend for the variation of these parameters. The values of the bond length and angles of the optimized geometries are quite similar to the experimental results of the corresponding compounds.

UV absorption spectroscopic titration:

Absorption titration was carried out to monitor the interaction of a compound with DNA. The interaction of the ligand/transition metal complexes with DNA (pUC19 and calf Thymus) was investigated using the UV absorption spectra of complex in the absence and presence of increasing concentration of DNA and at a constant concentration of the complexes (10 μl). The observable hypochromism and red shift are usually characterized by the non-covalently intercalative binding of compound to DNA helix, due to the strong stacking interaction between the aromatic chromophore of the compound and base pairs of DNA^(32,33). Results of absorption titration for pUC19 DNA as well as calf-Thymus DNA showed that no major difference was observed in the value of λ_{max} . Fig. 2 represent the absorption spectra of complex in the absence and presence of increasing amounts of DNA. In the UV region,

the intense absorption bands were observed due to the metal complex, which is believed to be the band of interligand transition of the coordinated groups. An addition of increasing amounts of the DNA resulted in hypochromism and bathochromic shift in the UV spectra. The hypochromism in the intraligand band reaches as high as at around 280 nm with red shift at the ratio of [DNA]/[Cu]. These spectral characteristics suggest that the complexes had been bound to the base pairs DNA by intercalation. This bathochromism result might be due to the decrease in the energy of $\pi-\pi^*$ transition, when the π -orbital of the base pairs of DNA coupled with the $\pi-\pi^*$ orbital of the intercalated ligand.

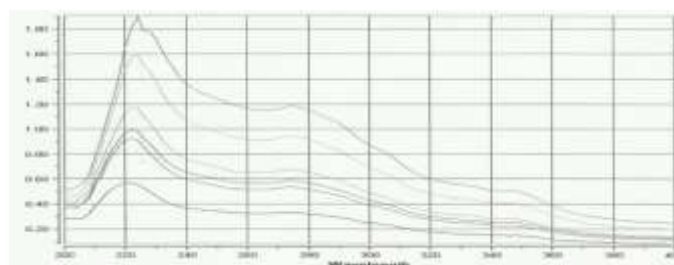


Fig. (2): UV/Vis. Spectra of $[Cu(L1)(L2)Cl_2]$ in the absence (top curve) and presence (subsequent curve) of increasing concentrations of CT DNA(0-300 μM).

Gel electrophoresis:

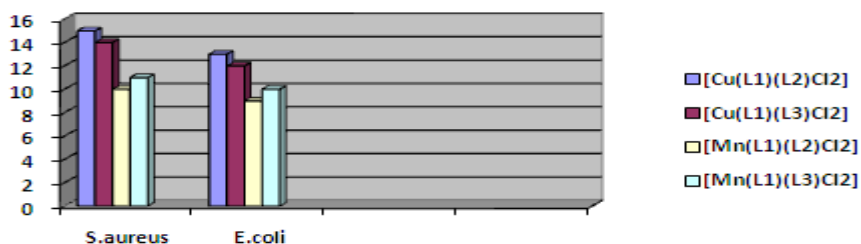
The interaction of ligands /transition metal complexes with DNA was studied by electrophoresis and the results were represented in (fig. 4). In this study , DNA may allowed to interact with the ligands/ metal complexes in presence of TAE buffer at pH 7.3 in air when DNA was subjected to the electrophoresis after interaction and upon illumination of gel (fig. 4), the fastest migration was observed for super coiled (SC) form I, were as the slowest migration was observed for open circular (OC) form II . The intensity of unsaturated DNA band appeared as such even after electrophoresis. Furthermore , the DNA cleavage data showed that the complexes $[Cu(L1)(L2)Cl_2]$, $[Cu(L1)(L3)Cl_2]$, $[Mn(L1)(L2)Cl_2]$ and $[Mn(L1)(L2)Cl_2]$ showed maximum cleavage ability compared to all compounds. The variation in DNA- cleavage efficiency of ligands / transition metal complexes was due to their difference in binding ability of ligands / complexes to DNA . The intensity of L2-complexes lane were higher than others in L3-complexes (fig. 4), which indicated that complex of Cu(II) and Mn(II) showed more strong binding ability with DNA. However in case of L2-complexes (fig. 4), all metal complexes showed moderate binding with DNA. The relative binding efficacy of the complexes to DNA was much higher than ligands, and among the complexes, the binding efficacy for complexes of L2 were better than that of L3-complexes. Among, the tested compounds $[Cu(L1)(L2)Cl_2]$ was found to have excellent binding ability for DNA.



Fig. 4 :DNA binding results of $[Cu(L1)_2(L2)Cl_2]$ based on Gel electrophoresis . Lanes 1 : Plasmid (pUC 19) DNA Lanes 2,3 and 4: (pUC 19) DNA + 10, 20, 30 μM of $[Cu(L1)_2(L2)Cl_2]$ respectively ; Lanes 5,6 and 7 : (pUC 19) DNA + 10, 20, 30 μM of $[Cu(L1)_2(L3)Cl_2]$ respectively; Lanes 8,9 and 10 : (pUC 19) DNA + 10, 20, 30 μM of $[Mn(L1)_2(L2)Cl_2]$ respectively; Lanes 11,12 and 13 : (pUC 19) DNA + 10, 20, 30 μM of $[Mn(L1)_2(L3)Cl_2]$ respectively

Antimicrobial screening :

The synthesized ligand and its complexes were tested for their in vitro antimicrobial activity. They were tested against the bacterial *Staphylococcus aureus* and *Escherichia coli* . A comparison of the value of L with these of the complexes indicates that the metal complexes exhibited higher antibacterial activity than ligands. Such increased activity of the complexes can be explained based on Overtones concept⁽³⁴⁾ and the tweedy chelation theory⁽³⁵⁾. Moreover, the copper complexes were more active than the zinc complexes against the tested microorganisms as shown in fig. 5.



Fig(5): The effect of ligands and their metal complexes toward bacteria

Table(1): Physical characterization ,analytical and Molar Conductance data of the compounds

Compound	Yield %	Colour	$\Delta M/S \text{ cm}^2 \text{ mol}^{-1}$ In DMSO	μ_{eff} (BM)	Elemental analysis (% found) % cal	
					Cl	M
[Cu(L1) ₂ (L2)Cl ₂]	85	Pale green	9	1.70	(18.98) 19.02	(17.00) 17.05
[Cu(L1) ₂ (L3)Cl ₂]	80	Pale green	8	1.75	(19.84) 19.88	(17.77) 17.82
[Mn(L1) ₂ (L2)Cl ₂]	75	Pale yellow	6	5.93	(19.38) 19.43	(15.05) 15.09
[Mn(L1) ₂ (L3)Cl ₂]	70	Pale yellow	7	5.88	(20.31) 20.37	(15.32) 15.37

Table(3): Infrared and UV-Visible Spectra data of the prepared compounds

Compound	IR Spectra (Cm ⁻¹)					
	$\nu^{(C=N)}$	$\nu^{(C=S)}$	$\nu^{(C=O)}$	$\nu^{(M-O)}$	$\nu^{(M-N)}$	$\nu^{(M-S)}$
[Cu(L1) ₂ (L2)Cl ₂]	1625	1215	1660	530 m	420 m	375 w
[Cu(L1) ₂ (L3)Cl ₂]	1590	1210	1640	500 w	430 m	350 w
[Mn(L1) ₂ (L2)Cl ₂]	1600	1225	1655	550 m	440 m	355 w
[Mn(L1) ₂ (L3)Cl ₂]	1615	1220	1650	525 m	460 m	370 w

Table (4) : structural parameters, bond length (°A) and angles(°) of the [Ni₂(L₂)₂Cl₄] complex.

Parameter		
Bond lengths (°A)	Bond angles(°)	
O(18)-Cu(19) 1.8100	H(31)-C(16)-H(30) 109.5198	O(12)-Du(19)-N(8) 103.9999
N(17)-H(32) 1.0120	H(31)-C(16)-H(29) 109.4618	H(28)-N(11)-C(10) 125.7933
N(17)-Cu(19) 1.8460	H(31)-C(16)-C(15) 109.4616	H(28)-N(11)-N(8) 125.7920
C(16)-H(31) 1.1130	H(30)-C(16)-H(29) 109.4419	C(10)-N(11)-N(8) 108.4147
C(16)-H(30) 1.1130	H(30)-C(16)-C(15) 109.4421	Du(19)-N(8)-N(11) 103.9998
C(16)-H(29) 1.1130	H(29)-C(16)-C(15) 109.5001	Du(19)-N(8)-C(2) 127.9998
C(15)-O(18) 1.2080	O(18)-C(15)-N(17) 97.7967	N(11)-N(8)-C(2) 128.0003
C(15)-N(17) 2.6140	O(18)-C(15)-C(16) 131.1015	H(24)-C(6)-C(7) 120.0002
C(15)-C(16) 1.5090	N(17)-C(15)-C(16) 131.1018	H(24)-C(6)-C(5) 120.0000
Cl(14)-Cu(19) 2.1600	Du(19)-O(18)-C(15) 89.9996	C(7)-C(6)-C(5) 119.9999
Cl(13)-Cu(19) 2.1600	H(32)-N(17)-Du(19) 151.0654	H(23)-C(5)-C(6) 120.0001
O(12)-Cu(19) 1.8100	H(32)-N(17)-C(15) 151.0651	H(23)-C(5)-C(4) 119.9996
N(11)-H(28) 1.0120	Du(19)-N(17)-C(15) 57.8695	C(6)-C(5)-C(4) 120.0003
C(10)-O(12) 1.2080	H(27)-N(9)-H(26) 120.0000	H(22)-C(4)-C(5) 120.0003
C(10)-N(11) 1.4233	H(27)-N(9)-C(10) 119.9997	H(22)-C(4)-C(3) 120.0000
N(9)-H(27) 1.0120	H(26)-N(9)-C(10) 120.0003	C(5)-C(4)-C(3) 119.9997
N(9)-H(26) 1.0120	Du(19)-O(12)-C(10) 103.9999	H(25)-C(7)-C(6) 120.0000
N(9)-C(10) 1.3690	O(12)-C(10)-N(11) 119.5857	H(25)-C(7)-C(1) 119.9997
N(8)-Cu(19) 1.3030	O(12)-C(10)-N(9) 120.2070	C(6)-C(7)-C(1) 120.0003
N(8)-N(11) 1.3520	N(11)-C(10)-N(9) 120.2074	H(21)-C(3)-C(4) 120.0000
C(7)-H(25) 1.1000	O(18)-Du(19)-N(17) 114.3341	H(21)-C(3)-C(1) 119.9999
C(6)-H(24) 1.1000	O(18)-Du(19)-Cl(14) 25.9681	C(4)-C(3)-C(1) 120.0001
C(6)-C(7) 1.3370	O(18)-Du(19)-Cl(13) 147.1672	H(20)-C(2)-N(8) 120.0000

C(5)-H(23) 1.1000	O(18)-Du(19)-O(12) 109.4999	H(20)-C(2)-C(1) 120.0001
C(5)-C(6) 1.3370	N(17)-Du(19)-Cl(14) 140.2841	N(8)-C(2)-C(1) 119.9999
C(4)-H(22) 1.1000	N(17)-Du(19)-Cl(13) 32.8331	C(7)-C(1)-C(3) 119.9998
C(4)-C(5) 1.3370	N(17)-Du(19)-O(12) 109.4999	C(7)-C(1)-C(2) 120.0003
C(3)-H(21) 1.1000	N(17)-Du(19)-N(8) 109.5001	C(3)-C(1)-C(2) 119.9999
C(3)-C(4) 1.3370	Cl(14)-Du(19)-Cl(13) 173.0779	Cl(14)-Du(19)-N(8) 94.8580
C(2)-H(20) 1.1000	Cl(14)-Du(19)-O(12) 93.595	Cl(13)-Du(19)-O(12) 90.0000
C(2)-N(8) 1.2600	O(18)-Du(19)-N(8) 109.500	Cl(13)-Du(19)-N(8) 89.9999
C(1)-C(7) 1.3370		
C(1)-C(3) 1.3370		
C(1)-C(2) 1.3370		

IV. Conclusion:

The results of UV spectroscopy and electrophoresis measurement showed that the metal complex binds with DNA via interaction as well as intercalation with the base pairs of DNA (pUC19 and calf Thymus). It also suggests that the covalent binding of the metal complex caused a change in the conformation of DNA such as more of intercalated and thus an increase in intensity of the band was generally observed. Moreover, the results described in this study showed that changing the ligand environment can modulate the binding property of the complex with DNA. The relative binding efficacy of the complexes to DNA was much higher than binding efficacy of ligands, especially the Cu-L1 complex has the highest binding ability to DNA, and complexes of L1 were better for DNA binding.

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