

Cu(II) Complex of Lincomycin: Synthesis, Spectral Characterization and Competitive DNA Binding.

Obaleye ^a, J. A. and Abosedo ^{b,*}, O. O.

^a Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

^b Department of Chemistry, Federal University Otuoke, P.M.B. 126, Yenagoa, Bayelsa State.

* Corresponding author, E-mail: funsoabosedo@gmail.com; abosedooo@fuotuoke.edu.ng

ABSTRACT

Cu(II) complex [CuLin-H(H₂O)₂](NO₃)₂ (I) of the antibiotic lincomycin has been synthesized and characterized by elemental (C,H,N) analysis, UV-Vis, FTIR, ¹H NMR and ESI mass spectra. Spectra studies revealed that monomeric 5-coordinate Cu(II) and Fe(III) complexes of lincomycin were formed. Competitive DNA binding fluorescence measurement of complex I and CT DNA-bound ethidium bromide revealed that the complex is capable of binding DNA, albeit weakly, to displace ethidium bromide from CT DNA to quench the fluorescence of ethidium bromide bound to CT DNA.

Keywords: Copper(II), Lincomycin, Complex, Antibiotic, DNA-binding

INTRODUCTION

Metals are essential for many of life's basic processes, such as catalyzing many chemical reactions or shutting electrons during energy production. Metals play essential functions as sensors and transducers of information with roles in regulation of cell function and neurotransmission in addition to their uses as drugs or diagnostic agents.¹

In addition to the roles of transition metals, metal complexes have also found applications as research probes of biological function, in probing the mechanism of non-

metal-containing therapeutics, and as potential diagnostic and therapeutic agents.² The exceptional properties of metal complexes such as hydrolytic, redox, variable valency, geometry, magnetic, spectroscopic, radiochemical properties make them suitable for the aforementioned applications.³

Copper is an example of transition metals that play vital roles in biological systems. It is found at the active sites of many enzymes and facilitates biological-oxidation reduction reactions.

Studies of the interaction of small-molecule Cu(I) complexes with dioxygen, and the subsequent oxidative processes of the resulting Cu-dioxygen complexes provide insights into the mechanism of action of Cu-containing metalloenzymes as well as aid in the development of bio-inspired Cu-oxidation catalysts.³⁻⁶ Copper enzymes exists in mononuclear and multinuclear¹ configurations in many biological systems² that are involved in carrying out essential redox processes.

New compounds (such as transition metal complexes) have added advantage of acting through different mechanisms and/or avoiding common multidrug resistance (MDR).² To this end, we have synthesized and obtained in solid state Cu(II)-lincomycin complex (**1**) and elucidated their structures by UV-Vis, FTIR and elemental analyses. DNA binding ability of complex **1** was tested by competitive DNA binding fluorescence measurement.

MATERIALS AND METHODS

All chemicals used were of analytical grade and were used as received. The chemicals and their sources were as follows: CuNO₃·3H₂O, tris (hydroxymethyl) aminomethane hydrochloride (Tris HCl) and Calf thymus DNA sodium salt (CT DNA) were obtained from SRL (India). Lincomycin hydrochloride was obtained from Drugfield Pharmaceuticals Plc, Nigeria. The solvents methanol, ethanol, dichloromethane, chloroform, acetone, diethyl ether, benzene, acetonitrile, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO) were from S. D. Fine Chemicals

Limited (India). The deuterated solvents D₂O (99.9%), CDCl₃ (99.9%) and DMSO-*d*₆ (99.5%) were obtained from Aldrich Chemical Co. (U.S.A.). All solvents used for cyclic voltammetry experiment were of HPLC grade and obtained from SRL (India)/ S. D. Fine Chemicals Limited (India).

Synthesis of complex 1

[CuLin(H₂O)₂](NO₃)₂ (**1**)

A 0.461 g (1mmol) of lincomycin hydrochloride was dissolved in 10 ml acetone and 0.4 ml triethyl amine was added. The mixture was stirred for 30 minutes at 50 °C followed by addition of 0.242 g Cu(NO₃)₂·3H₂O (1 mmol). The mixture was further stirred for 2 hours and the green precipitate formed was filtered, washed with acetone and dried in vacuum desiccator. Recrystallized from methanol. UV-Vis (MeOH, nm): 343, 638, 726, 757. ESI-MS: [CuLin(H₂O)₂] at 504.1; [CuLin(H₂O)₂+NO₃] at 567; [CuLin(H₂O)₂](NO₃)₂ at 567 and [2Lin+Na⁺] at 835.4 FT-IR (KBR, v/cm⁻¹): 3331, 3234, 3057, 2958, 2918, 2872, 2683, 1676 (amide I C=O), 1608 (amide II C=O), 1381, 1369, 1311, 1240 (C-O-C), 1149 (C-O-C), 1076 (S-CH₃ stretching), 1061, 995, 900, 869, 800, 696, 611, 590.

Fluorescence measurements

Competitive binding fluorescence measurements of complex **1** was determined by a fluorescence spectral technique using ethidium bromide (EtBr)-bound CT DNA solution in Tris-HCl buffer (pH, 7.2). The changes in fluorescence intensities at 600 nm (510 nm excitation) of EtBr bound to DNA were recorded with an increasing amount of the complex concentration.

Concentrations of DNA and EtBr were 20 μM .

RESULTS AND DISCUSSION

Characterization of complex 1

Homoleptic copper(II) complex of lincomycin have been synthesized and isolated in solid state. The isolated complexes were characterized and the coordination mode of lincomycin assigned based on the only possible coordination mode for lincomycin as previously reported.⁷⁻¹¹ Complex is soluble in water but insoluble in organic solvents except in hydroxylic solvents such as methanol. The NMR spectra of complexes **1** is not well resolved because it is a paramagnetic complex. However, broad peaks observed in the aliphatic region are due to lincomycin

and the spectra of the complex are available upon request.

The ESI-MS spectra of complex **1** was taken in methanol and gave peaks at 504.1 corresponding to $[\text{CuLin}(\text{H}_2\text{O})_2]$ while the peak at **835.4** is suspected to be due to dimerization of lincomycin with the sodium ion introduced by the spectrometer $[2\text{Lin}+\text{Na}^+]=\mathbf{835.4}$. Peaks corresponding to $[\text{CuLin}(\text{H}_2\text{O})_2+\text{NO}_3]$ at 567 are also observed in the mass spectrum. The peak at 393.2 is due to the dissociation of methyl group from the pyrrolidene group of lincomycin. The assigned coordination of the complex is in agreement with literature report for the complexation of Cu^{II} -lincomycin in water solution except for coordination of an extra molecule of water. The proposed structure for the complex is confirmed by both ESI-MS (**Figure 1**) and elemental analysis of complex **22**.

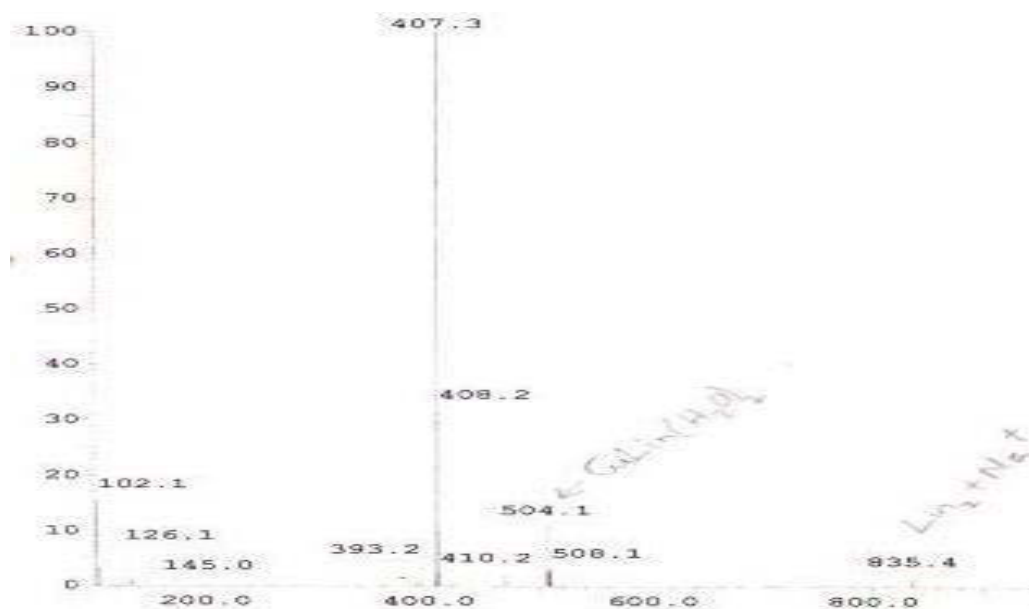


Figure 1: ESI-MS (positive ionization mode) spectrum of **1**

The FTIR spectra of lincomycin and the copper complex (**1**) were taken and compared to establish the point of coordination of copper to lincomycin. The amide N-H stretching of lincomycin at 3400-3500 cm^{-1} were absent in the copper-lincomycin complex showing that the amide N-H of lincomycin was deprotonated and coordinated to copper. The amide I and amide II C=O absorption frequencies at 1656 and 1566 cm^{-1} were still present in the spectrum of complex **1** though shifted to 1676 and 1608 cm^{-1} respectively.¹² This shows that the amide C=O group is not involved in coordination and the shift in frequency was due to the participation of amide N in coordination to copper. The S-CH₃ stretching at 1076 in lincomycin remained the same in the copper-lincomycin complex (**1**) showing that it is not involved in coordination. The other major difference in the spectra of lincomycin and its copper complex is in the region for C-O, C-C and C-O-C ring vibrational stretching between 1105 and 1261 cm^{-1} .¹³⁻¹⁴ This corroborates the participation of the OH group at C-2 in coordination to copper. Comparison of FTIR spectra of lincomycin and its copper complex is summarized in table 1.

Table 1: Summary of diagnostic bands in the FT-IR Spectra of lincomycin and its copper complex (1), (wavenumber in cm^{-1})

| Lincomycin | 1 | Assignment |
|------------------|----------|---------------------------|
| 3533, 3452, 3487 | - | Amide N-H stretch |
| 1656 | 1676 | Amide I C=O stretch |
| 1566 | 1608 | Amide II C=O stretch |
| 1261 | 1240 | C-O-C ring stretching |
| 1105 | 1149 | C-O-C ring stretching |
| 1076 | 1076 | S-CH ₃ stretch |

Competitive binding fluorescence measurements of 1

Fluorescence is a frequently used technique for investigating the binding mode of small molecules to DNA. For compounds that do not display luminescence either alone or in aqueous buffer, ethidium bromide is commonly used. Ethidium bromide is an intercalating molecule emitting intense fluorescence when bound to DNA.¹⁵ Decrease in emission intensity results when a second DNA binding molecule either replaces EthBr¹⁶ or accepts the excited state electron from EtBr.¹⁷ Ethidium bromide is non-emissive in aqueous buffer due to fluorescence quenching of the free ethidium bromide by the solvent molecules. In the presence of DNA, ethidium bromide shows enhanced emission intensity due to its intercalative binding to DNA. If competitive binding of a compound to DNA resulted in the displacement of the DNA-bound ethidium bromide and considerable decrease in ethidium bromide emission intensity is observed, then the mode of binding of the complex to DNA is by intercalation.

When complexes **1** was added to CT DNA pretreated with EtBr ([DNA]/[EtBr]=1:1), the DNA-induced emission intensity of EtBr decreased slightly (**Figure 2**) indicating the weak binding of these complexes to DNA. The quenching of EtBr emission is by acceptance of excited state electron from EtBr by the complexes since lincomycin is known not to interact with DNA.¹⁷

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