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RESEARCH ARTICLE

Synthesis and DNA Binding of *cis*-Dichlorobis-benzimdazolebis-(dimethylsulfoxide)ruthenium(II)-hydrate Complexes

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Abstract: The ruthenium complex with substituted imidazole ligand of formula $[Ru(BIm)_2(DMSO)_2Cl_2].H_2O$ (BIm=Benzimidazole) was prepared. The compound has been further characterized from spectroscopic data and elemental(C, H and N) analysis. The molecular structure of this compound was determined by single crystal X-ray diffraction study. Binding of this complex with plasmid-DNA was studied by UV-Visible and Emission spectral studies. Further electrochemical studies were performed to understand the DNA binding ability of this complex. The evidences of DNA binding were found from the change of the Intensity of fluorescence band and UV-band. Binding constant (K_b) calculated from UV-Spectroscopic titration is found to be $9.09 \times 10^5 M^{-1}$.

Keywords: Benzimidazole, DMSO, X-ray diffraction, Electrophoresis, Plasmid-DNA.

Introduction

The imidazole-metal complexes are important in medicine[1] and coordination chemistry[2]. The imidazole and DMSO ligands have been extensively used in many metal complexes[3]. We have synthesized a few benzimidazole ruthenium complexes which are found to be potent anticancer agent[4]. Ligand's effect on anticancer property[5] is important and it is essential to design superior complexes in terms of effectiveness compared to that which are already available agent such as *cis*-platin. The imidazole-metal complexes are important[6-9] in medicine and coordination chemistry. The spectroscopy of these complexes in the absence and presence of plasmid DNA was examined and compared to *cis*-platin. Furthermore, benzimidazole complexes have been evaluated for antifungal activity and as antitumour agents[10,11]. Hence, the imidazole and DMSO[12] ligands have been extensively used many metal complexes[13].

Benzimidazole cyclometalated complexes were found[14] to have good anticancer activity against HT29, T47D, A2780 and A2780cisR cancer cell lines[15,16]. Representative complexes show high apoptosis, good accumulation and S-phase[17] cell arrest and strongly bind to HSA at sites I and II and also weakly bind to DNA at the minor groove[18]. Bis-benzimidazole bridged supramolecular coordination complex has potential to act as potent anticancer agents, particularly in cell lines which are resistant to Pt-based molecules[19,20]. Further studies are under progress to investigate the biological mechanism of these derivatives[21]. We have synthesized few benzimidazole ruthenium complexes which are found to be potent anticancer agent[4,22]. A single crystal X-ray diffraction study of this complex is reported.

Experimental

Analytical grade $RuCl_3.3H_2O$, Plasmid-DNA (pBR 322 DNA), Tris buffer were purchased from Sigma and Aldrich chemical companies, USA.

Characterization procedure

The IR Spectra of the compound was recorded as KBr pellets on a Perkin-Elmer FT-IR spectrophotometer. The UV-visible spectra were taken in a Shimadzu UV-2401 PC Spectrophotometer.

Preparation

Synthesis of cis-dichlorobis-(benzimdazole)bis-(dimethylsulfoxide)ruthenium(II) hydrate, $[Ru(BIm)_2D_2Cl_2].H_2O$

0.25 g $RuCl_3.3H_2O$ was refluxed in 30 mL ethanol and 5 mL, 2M HCl for 2 h, and cooled the solution. 0.1g of benzlimidazole was dissolved in 6M HCl and mixed ruthenium chloride solution. The mixture was stirred for 5 min and cooled in ice for 2 h and then at room temperature for 24 h. Yellow crystals formed, Yield = 70%. Isolated crystals were separated with 1mL DMSO in ethanol for 30 min. Light yellow crystals formed. A suitable crystal was used for XRD analysis. Molecular formula $C_{18}H_{24}Cl_2N_4O_3RuS_2$. Mol.wt = 580 g/mol. C, H, N analyzed values are C = 37.85% (37.24%), N = 9.13% (9.65%) and H = 4.01% (4.13%). The IR peaks with tentative assignments (ν_{max}/cm^{-1}) at 1647(C=N aromatic), 3116(C-H, SP^2 Carbon), 2920, 2822 (C-H methyl), 1496, 1415(C=C for side chain and aromatic), 454 (Ru-N) were observed.

Crystal structure

Fine crystals were mounted on glass capillary and data collected at room temperature. Data collection was carried out on a diffractometer (BRUKER SMART APEX2) using monochromated Mo Kα radiation. From the setting angles crystal dimension were determined. The structures (Figure 1) were solved by the Patterson method. SHELXTL routine was used for empirical absorption correction. Experimental details are given in Table 1.

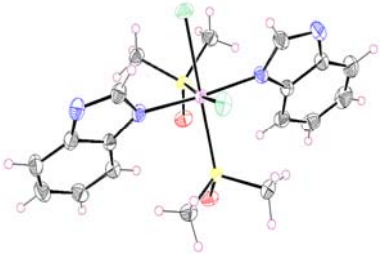

Figure 1. ORTEP diagram of $[Ru(BIm)_2D_2Cl_2].H_2O$. (*BIm=Benzimidazole, D=DMSO*)

Table 1. Crystal data and structure refinement for *cis*-dichlorobis-(benzimdazole)bis-dimethylsulfoxideruthenium(II) hydrate, $[Ru(BIm)_2D_2Cl_2].H_2O$ complexes

Identification code	$[Ru(BIm)_2D_2Cl_2].H_20$
Empirical formula	C21 H28 Cl2 N4 O3 Ru S2
Formula weight	620.56
Temperature	296(2) K
Wavelength	0.71073 A
Crystal system, space group	triclinic, P-1
Unit cell dimensions	a = 9.6901(10) A α = 73.701(4)deg. b = 11.1126(12)A β=80.993(4)deg. c = 11.7112(12)A γ=80.011(4)deg
Volume	1184.3(2) A^3
Z, Calculated density	2, 1.740 Mg/m^3
Absorption coefficient	1.097 mm^-1
F(000)	632
Crystal size	0.07mm x 0.13mm x 0.24 mm
Theta range for data collection	1.93 to 27.66 deg.
Limiting indices	-12<=h<=12, -14<=k<=14, -15<=l<=14
Reflections collected / unique	23879 / 5471 [R(int) = 0.0393]
Completeness to theta	= 27.66 99.0 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5471 / 0 / 278
Goodness-of-fit on F^2	1.119
Final R indices [I>2sigma(I)]	R1 = 0.0367, wR2 = 0.1152
R indices (all data)	R1 = 0.0384, wR2 = 0.1173
Extinction coefficient	0.0051(11)
Largest diff. peak and hole	1.020 and -1.698 e.A^-3

Results and Discussion

Spectroscopic studies on DNA binding: UV-Visible absorption titration

Interaction of the complexes with plasmid-DNA was monitored by UV-Visible absorption spectra of the complex at different concentration of DNA($2.289x10^{-6}$ M to $9.089x10^{-6}$ M). As the concentrations of the plasmid-DNA increased, red shift occurred from 351 nm to 359 nm and the absorbance decreases (Figure 2). The binding strength was estimated from the intrinsic binding constant, from the ratio of slope and intercept of the graph (Figure 3) from the following equation (1),

$$[DNA] / (\varepsilon a - \varepsilon f) = [DNA] / (\varepsilon b - \varepsilon f) + 1/(K (\varepsilon b - \varepsilon f) \quad (1)$$

where ε_a, ε_f and ε_b are the extinction coefficient of observed solution, free complex and the complex when it fully bound to plasmid-DNA respectively.

Fluorescence emission and quenching studies

Fluorescence study was perform in to phase, firstly keeping the concentration of metal complex constant and varying concentration of CT-DNA (Figure 4) and secondly concentration of CT-DNA is fixed, and varying the concentration of metal complex (Figure 5). Steady-state emission quenching experiments was performed using $[Fe(CN)_6]^{4-}$ as quencher and the Stern–Volmer quenching constant (Ksv) was calculated by using Stern–Volmer equation[12] $I_0 / I=1+Ksv [Q]$(Figure 6).

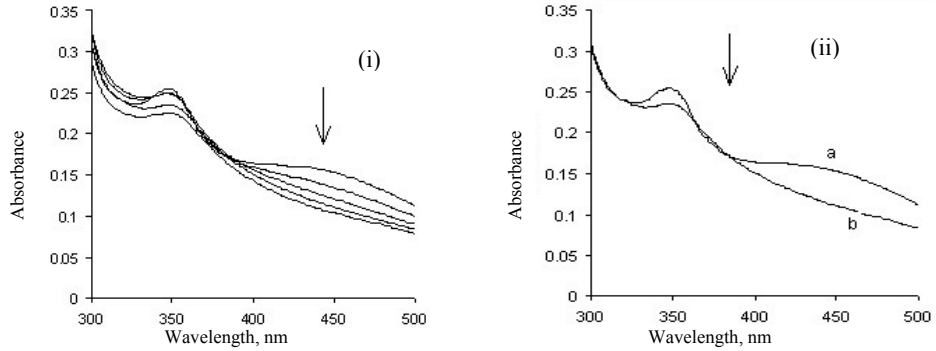

Figure 2. (i) UV-Visible spectra of $[Ru(BIm)_2D_2Cl_2]$ in tris-buffer(pH=7.4) with increasing concentrations of plasmid-DNA and (ii) with maximum concentration of plasmid-DNA indicating red shift from 351 nm (a) for the complex without DNA to 359 nm (b) with maximum concentration of DNA)

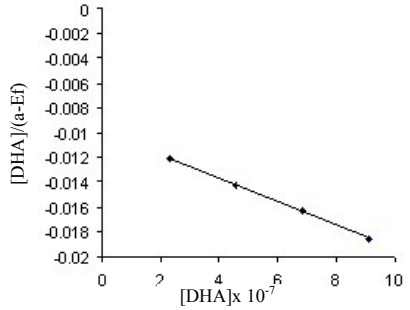

Figure 3. Plote of [DNA]/(εa-εf) *vs.* [DNA]. Binding constant = 9.09 x 10^5 M^{-1}

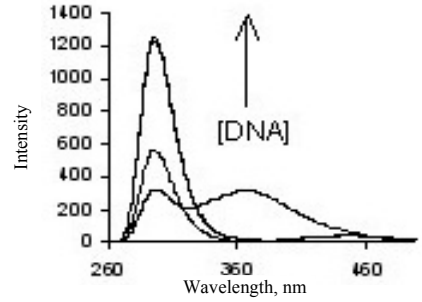

Figure 4. Fluorescence spectra of $[Ru(BIm)_2D_2Cl_2]$, 10^{-3} M with different concentration of DNA (0 to 9.089 μ M). Exciting wavelength = 220 nm. Intensity at 300nm increases but at 370 nm intensity completely diminished

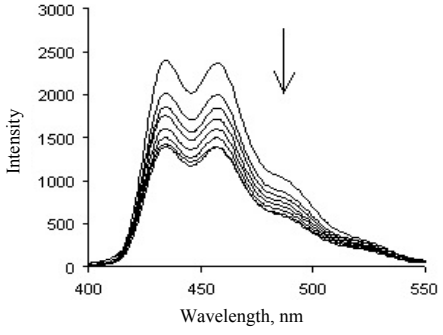

Figure 5. Fluorescence spectra of plasmid-DNA with different concentration of $[Ru(BIm)_2D_2Cl_2]$ (0 to 7.6x10^{-4} M). Exciting wavelength = 220 nm. Intensity decreases with increasing concentration of drug

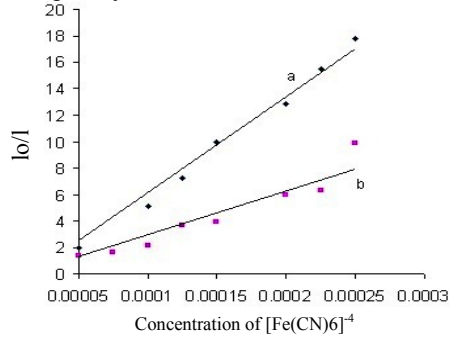

Figure 6. Stern-Volmer plot a) without plasmid DNA and b) with 30 μL of 0.1 mM plasmid DNA. Slope of line a is 72079 and that of line b is 33131

Electrochemistry

Cyclic voltammetric study for $[Ru(BIm)_2D_2Cl_2]$ complexes were carried out in water/ethanol solution containing 0.1M $NaNO_3$ as supporting electrolyte using Ag/Ag^+ as reference electrode, and a glassy carbon was used as working electrode[1]. The inert environment was maintained by passing N_2 gas through the solution to remove oxygen. The voltamogram shows distinct oxidation and reduction peaks of a reversible electron transfer reaction. The shifts of redox potentials due to binding with CT-DNA are shown in Figure 7, 8 and 9. Interacting with DNA, by intercalative mode increases the hydrophobicity of the complex. As a result there is a positive shifting of potential.

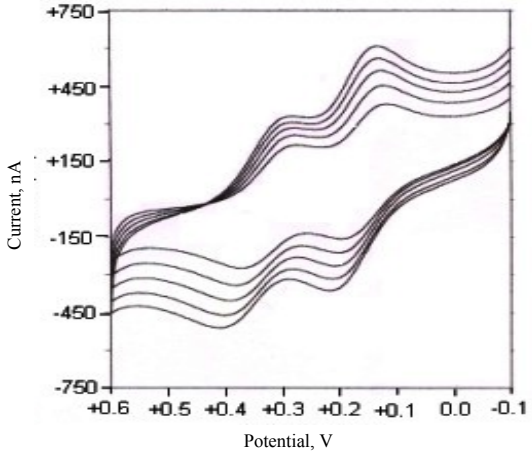

Figure 7. Cyclic voltammogram of $[Ru(BIm)_2D_2Cl_2]$ at different scan rate(50 mV/s to 350 mV/s) in water/0.1M $NaNO_3$ solution

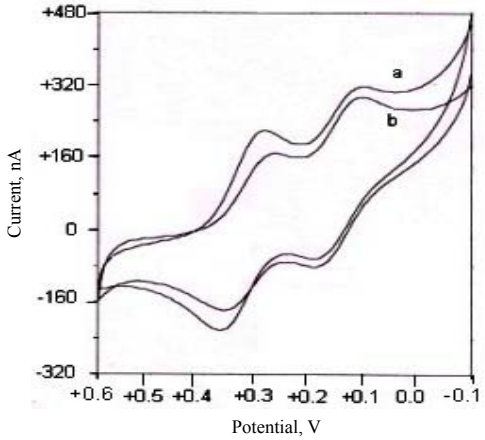

Figure 8. Cyclic voltammogram of $[Ru(BIm)_2D_2\ Cl_2]$ (a) without Plasmid-DNA (Segment I : E_1= +174 mV, current = -8.296 x 10^{-8}A and E_2 = +361 mV; current = -1.069 x 10^{-7}A; Segment II: E_1 = +103 mV, current = +1.324 x 10^{-7}A and E_2 = +268 mV, current = +1.626 x 10^{-7}A) and (b) with 60 µL 0.5 mM plasmid-DNA. (Segment I: E_1= +169 mV, current = -6.366 x 10^{-8}A and E_2 = +369 mV; current = -1.696 x 10^{-7}A; Segment II: E_1= +105 mV, current =+1.270 x 10^{-7}A and E_2 = +292 mV; current = +1.984 x 10^{-7}A)

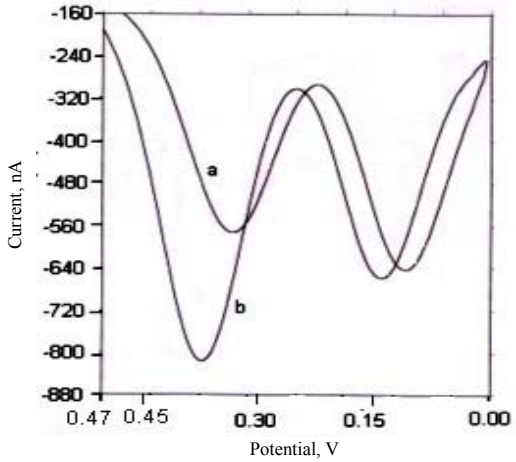

Figure 9. OSWV of (a) $[Ru(BIm)_2D_2Cl_2]$, $(E_{1/2})1$ = +135 mV; current = -6.505 x10^{-7}A and $(E_{1/2})2$ = 321 mV, current = -5.121 x 10^{-7}A; (b) $[Ru(BIm)_2D_2Cl_2]$ with 30 µL 0.5 mM plasmid-DNA. $(E_{1/2})1$ = 142 mV; current = -3.905 x 10^{-7}A and $(E_{1/2})2$ = 331 mV; current = -5.697 x10^{-7}A. Scan rate = 50 mV/s.

Conclusion

The structural analysis was performed by single crystal XRD study, which shows six coordinated octahedral geometry. The results characterized by various techniques give evidences binding of the complex with plasmid-DNA. On the basis of spectroscopic shift, the intrinsic binding constant (K_b) is 9.09x$10^5$$M^{-1}$. Emission quenching with $[Fe(CN)_6]^{4-}$ in presence and absence of plasmid-DNA shows the Stern-volmer constant 33131 and 72079 respectively . Based on the observed distinct electrochemical $E_{1/2}$ and electrophoresis band shifts, the complex is expected to bind with plasmid-DNA.

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