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Spectroscopic Analysis of DNA Binding Mode of Novel Schiff Base Vanadium Complex

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Abstract

Vanadium metal complexes based on wide range of the ligands have been explored at a greater extent for their various biological potential, in the recent times. Schiff bases are one of such fascinating ligands with donor sites varying according to the ketone or aldehyde group used. Based on these, a novel PS1V1, bis-vanadium complex was synthesized using schiff base backbone. It was characterized by using UV, FTIR, EPR and Mass spectral studies. The DNA binding ability of the characterized complex, PS1V1, was explored using UV and Fluorescence spectroscopic techniques. Furthermore, DNA binding mode of PS1V1 was also ascertained using competitive fluorescent displacement assay performed in the presence of minor groove biding agent, Hoeshcst.

Keywords: Vanadium complexes, Piperonal, Schiff base, DNA interaction, DNA binding

Introduction

Metal complexes have developed as Metallodrugs due to their multifaceted biological applications. The discovery of cisplatin, has paved a path in metallopharmaceutical field and chemotherapy as a DNA intercalator (1). The severe clinical side effects and thus limited therapeutic therapeutic efficiency of platinum based drugs have triggered a research in other metal core compounds (2).

In current times, the application of the metal complexes as anti proliferative, apoptotic and cytotoxic agents (3-6) has been reported in detail. Vanadium is a bio essential element, playing an important role in signal transduction. Currently, vanadium based metal complexes have been explred as chemotherapeutic compounds due to their wide scope of biological applications such as insulin mimetic (7,8), antibacterial (9), antioxidant (10) and anticancer (11,12) activities. Few diketone and hydrazone based vanadium complexes have also exhibited oxidative DNA cleavage and DNA binding efficacies (13,14). A number of piperonal based Schiff bases ruthenium(II) (15) complexes have been reported for cytotoxic activity.

We report, herein, the synthesis and characterization Schiff base based vanadium complex, PS1V1. Furthermore, its DNA binding mode has also been explored elaborately using spectroscopic techniques.

Experimental

Materials

All the chemicals were purchased from commercial sources and used without further purification for the experiments. 4-chlorobenzhydrazide, Piperonal (1,3-benzodioxole-5-carbaldehyde), Vanadyl acetyl acetone were purchased from Sigma Aldrich. All the solvents such as acetonitrile, methanol, ethanol and diethyl ether were purchased in AR grade and used. CT DNA, Tris HCl buffer and Hoechst 33258 were procured from Merck Genei.

Methods

Electronic spectra were recorded on a JASCO UVVIS-NIR-V-670 spectrophotometer.

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FTIR spectra were measured using KBr pellets on a Shimadzu IR affinity-1CE model with resolution IV. MS spectra were recorded by GCMS instrument. EPR was recorded on a JEOL Model JES FA200 instrument. The fluorescence spectra were recorded on a Hitachi F-7000 FL Spectrophotometer UV absorbance of commercial calf thymus DNA in a buffer gave an absorption ratio (A260/A280) of about 1.9:1, indicating that the DNA was sufficiently free from protein. The concentration of DNA was determined using molar extinction coefficient of 6600M-1cm-1 at λmax 260nm. All the experiments were carried out in a Tris buffer pH 7.2 in Mili-Q triply deionized water.

Synthesis of Ligand PS1

Methanolic solution of 4-chlorobenzhydrazide (1mmol) was added to the methanolic solution of piperonal (1mmol) and mixture was refluxed for an hour in the presence of acetic acid. The resultant pale yellow color hydrazone product was filtered and washed with petroleum ether. It was characterized using UV, FTIR, NMR and mass spectral studies.

Synthesis of Complex PS1V1

10 ml of hot methanolic solution of vanadyl acetyl acetone (1mmol) was added dropwise to the 10 ml of hot methanolic solution of ligand PS1 (3mmol). The reaction mixture was refluxed for 2h. PS1V1 = Yield: 58%. Green solid. Anal Cald. For $C_{30}H_{26}Cl_2N_4O_7V$: C: 53.75, H: 3.01, N: 8.36. Found: C: 53.73, H: 3.00, N: 8.34. UV-Vis (methanol): λ_{max} (MeOH)/nm (,dm- 3mol-1cm-1) 220(79,850), 345(61,500), 680(6550). FT-IR (KBr, vmax/cm^{-1}): 1649 (C=O), 1597(C=N), 939 (V=O). ESI-MS Calcd for $C_{30}H_{26}Cl_2N_4O_7V$: 675.19 , Found: 675.26.

DNA Binding Experiments

UV Absorption spectral titration experiments were carried out by monitoring the electronic spectrum of 20μM of the complex in the presence of varying CT-DNA concentrations (0 -120μM). The fluorescence study was carried out at the constant complex concentration of 100 μM with varying concentrations of DNA from 0 to 120μM dissolved in Tris HCl buffer.

Results and Discussion

The complex Fig.1 were synthesized and characterized by spectral, elemental and analytical techniques and found to be air stable.

Ultraviolet spectroscopy

The UV-visible spectra of complex PS1V1 is shown in Fig. 2 The vanadium complex, PS1V1, has shown the intraligand π-π* transition at 220 nm and strong metal ligand charge transfer at 345 nm and the complex has shown d-d transitions at 680 nm.

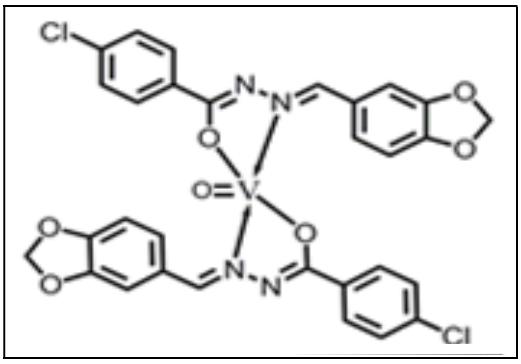

Fig 1. Structure of the Complex PS1V1

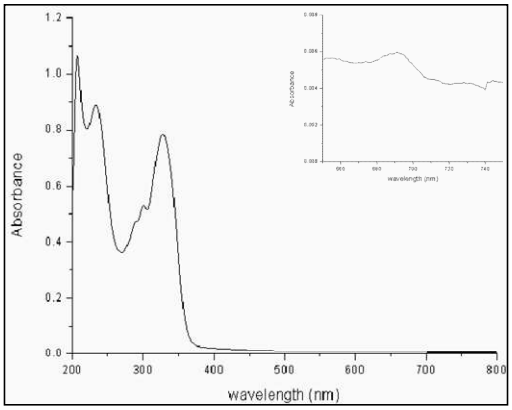

Fig 2. UV-Visible Spectrum of the Complex PS1V1

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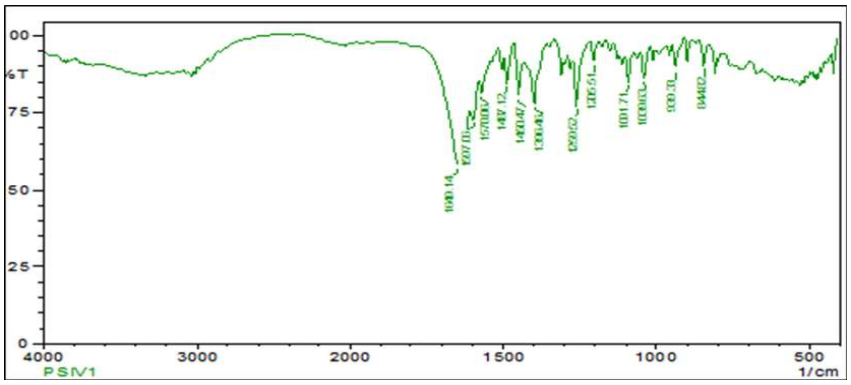

Fig 3. FTIR Spectrum of the Complex PS1V1

IR Spectral Analysis

The FTIR spectrum of complex PS1V1 is shown in Fig. 3. FTIR stretching band of carbonyl carbon was found to be significantly reduced at 1597cm^{-1} compared to the ligand PS1. The typical V=O stretching frequency was observed at 939cm^{-1}.

EPR Spectra

The EPR spectra Fig. 4 for the complex in polycrystalline form at RT displayed g value of about 1.925.

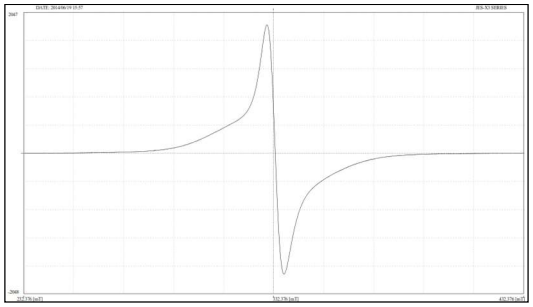

Fig 4. EPR Spectrum of the Complex PS1V1

Mass Spectral Analysis

The Mass spectrum of the complex is shown in Fig. 5. The mass spectrum of the complex was recorded in the methanol and M^+ peak was observed at 675.25 m/z

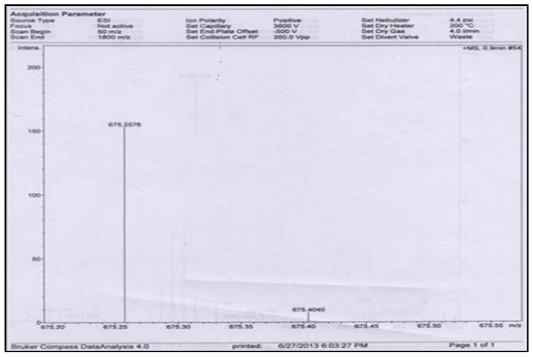

Fig 5. ESI MS Spectrum of the Complex PS1V1

DNA Binding Studies

UV Absorption Titration

The binding mode of complex PS1V1 was assessed by UV absorption titration with calf thymus (CT) DNA. The UV spectra of 20μM solutions of in the absence and with successive increment of CT DNA are shown in Fig.6. The typical hyperchromic effect with negligible shifts in absorption maxima was observed in the bands of the complex (Fig. 6) indicating the groove binding nature. The intrinsic binding constant K_b with the help of the absorbance spectra of the same. K_b was calculated using the formula (16),

$$(DNA) / (\varepsilon_a - \varepsilon_f) = (DNA) / (\varepsilon_a - \varepsilon_f) + 1/ K_b (\varepsilon_b - \varepsilon_f)$$

where, ε_a, ε_f and ε_b correspond to A_{obsd}/(PS1V1), the extinction coefficients for free vanadium complex and that of vanadium complex in fully bound form, respectively. Intrinsic binding constant for complex PS1V1 was found to (2.05 ± 0.09) × 10^{3}M^{-1}.

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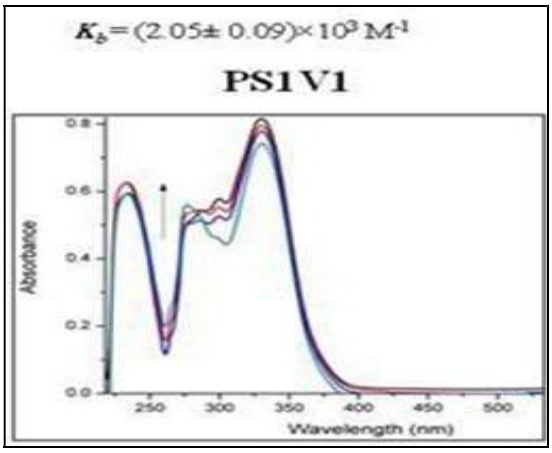

Fig 6. UV Absorption Spectrum of the Complex PS1V1 with CT DNA

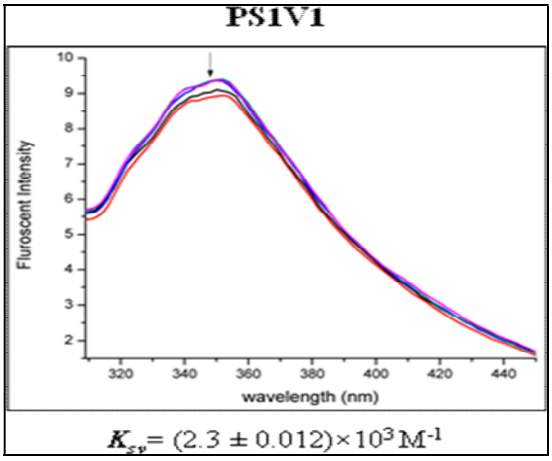

Fig 7. Fluorescence Emission Spectrum of the Complex PS1V1 with CT DNA

Fluorescence Spectral Studies

Emission spectral titrations were carried out to ascertain the binding mode of the complex PS1V1 with CT DNA. Complex PS1V1 was excited at 274nm. The emission maximum of the complex was observed in the presence of DNA. The successive addition of DNA resulted in the quenching of the emission intensities of both the complexes thus suggesting a groove binding mode rather than intercalation (Fig 7)

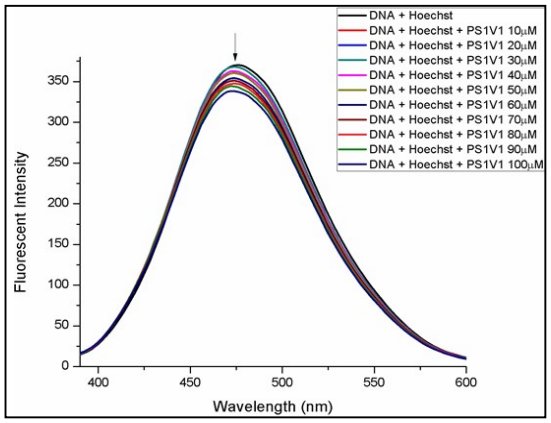

Fig 8. Fluorescence Emission Studies of the Complex PS1V1 in the Presence of Hoechst-DNA

supporting the results of absorption spectral studies. K_{sv} value for PS1V1 was found to be (2.3 ± 0.012) × 10^3 M^{-1}.

Competitive Fluorescence Displacement Assay

The Hoechst–CT DNA complex containing was excited at 355nm and fluorescence emission spectra were recorded at 480nm by titrating with increasing concentrations of complex. The quenching in the emission intensity of the Hoechst bound DNA was observed (Fig. 8), supporting a groove binding nature. K_{sv} value for PS1V1 was found to be 0.94×10^3 M^{-1}.

Conclusion

PS1V1, vanadium complex was synthesized based on Schiff base ligand and was characterized using UV, FTIR, EPR and Mass spectral studies. UV absorption titration of the complex PS1V1 with CT DNA suggested the groove binding mode. The fluorescence spectral titration with DNA also supported the groove binding mode of the complex. Later, Hoechst fluorescence displacement assay ascertained the minor groove binding mode of the complex, PS1V1, as the emission intensity of the hoechst-DNA complex was quenched at a greater extent.

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