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SYNTHESIS, CHARACTERIZATION, DNA BINDING AND CLEAVAGE STUDIES OF Fe(III) AND Zn(II) COMPLEXES CONTAINING MIXED LIGANDS

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ABSTRACT

The newly synthesized mixed complexes of the type $[\text{Fe}(\text{L}^1\text{L}^2\text{L}^3)] (\text{PF}_6)_3$ complex (1), $[\text{Zn}(\text{L}^1\text{L}^2\text{L}^3)] (\text{PF}_6)_2$ complex (2), (where $\text{L}^1 = 1, 10\text{-phenanthroline}$ and $\text{L}^2 = 1H\text{-benzimidazole-2-thiol}$ and $\text{L}^3 = 4H\text{-1,2,4-triazol-4-amine}$) were characterized by elemental analysis IR, UV and NMR spectral studies. The DNA interactions of complex (1) and complex (2) was determined by absorption spectral studies. The results of binding constant indicate that all the complexes intercalate with CT-DNA. The relative nuclease activity of these complexes were studied by gel electrophoresis using pUC 19 DNA.

KEYWORDS: Fe(III) and Zn(II) complexes; DNA binding; viscosity measurements; thermal denaturation; cleavage studies.



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INTRODUCTION

The interaction of DNA with transition metal complexes has got intensive attention in the last few years in order to develop new novel non radioactive probes of DNA structure,^{1, 2} new therapeutic agents that cleave DNA³⁻⁵ and DNA-mediated electron transfer reactions.⁶ These complexes give an opportunity to discover the effects of the central metal atom, the ligands and the coordination geometries on the binding event. As to the different ligands, it is possible to change the mode of interaction of the complex with nucleic acids that makes easy individual applications.⁷⁻⁹ The application of octahedral complexes has allowed the targeting of specific DNA sites by matching the shape, symmetry and functionality of the metal complex to that of the DNA target.¹⁰ Because of the unusual binding properties and general photo-activity, these coordination compounds act as DNA secondary structure probe, photocleavers and antitumor drugs.¹¹⁻¹⁴ Schiff bases are an important class of ligands in coordination chemistry and their complexing ability containing different donor atom is widely reported. There is a growing interest in the studies on the metal complexes of Schiff bases derived from triazoles and its derivatives which are biologically as well as industrially important ligands.¹⁵ The triazole Schiff bases constitute one of the most important classes of O, N, and S donor atoms. Several derivatives of triazole have been used as drugs and proved effective bacteriocides,^{16,17} pesticides,¹⁸ and fungicides.^{19,20} The Schiff base acts as bidentate monobasic donor ligand. In view of this, we have synthesized some new complexes of Fe(III) and Zn(II) containing ligands 1,10-phenanthroline(L¹), 1*H*-benzimidazole-2-thiol(L²) and 4*H*-1,2,4-triazol-4-amine(L³).

MATERIALS AND METHODS

All reagents and solvents were of AR grade, solvents were purified and used. Zinc chloride, ferric chloride, ammonium hexafluorophosphate (NH₄PF₆), Dimethylsulphoxide (DMSO) and *Tris*--

HCl buffer were purchased from qualigens (Mumbai, India). Calf thymus Deoxyribonucleic acid (CT-DNA) and plasmid University of California 19 Deoxyribonucleic acid (pUC19 DNA) were purchased from Bangalore Genie, Bangalore, India.

Procurement of ligands

The ligands 1,10-phenanthroline(L¹), 1*H*-benzimidazole-2-thiol(L²) and 4*H*-1,2,4-triazol-4-amine(L³) were purchased from Hi-media chemicals, Mumbai, India.

Synthesis of [Fe(L¹L²L³)] (PF₆)₃ [Complex (1)]

A solution of ferric chloride (0.27 g, 1 mmol) was prepared in ethanol and then it was added to an ethanolic solution of 1,10-phenanthroline(L¹). To this, a solution of 1*H*-benzimidazole-2-thiol(L²) in ethanol was added followed by the addition of ethanolic solution of 4*H*-1,2,4-triazol-4-amine(L³). The above mixture was refluxed on a water bath for 1h. The metal to ligands ratio maintained was 1:1:1:1 (M:L¹:L²:L³). The resulting solution was allowed to cool and precipitated by the addition of hot ethanolic solution of ammonium hexafluorophosphate to the filtrate. The complex obtained was filtered and dried under vacuum before being recrystallized using ethyl acetate. Yield of the complex 84%. Analysis: C₂₁H₁₈N₈SP₂F₁₂Fe. Calc. (%); C 33.16, H 2.37, N 14.73, Fe 7.37. Found(%): C 33.11, H 2.34, N 14.68, Fe 7.31.

Synthesis of [Zn(L¹L²L³)] (PF₆)₂ [Complex (2)]

Zinc chloride (0.136 g, 1 mmol) was dissolved in ethanol and it was then added to an ethanolic solution of 1,10-phenanthroline(L¹). This was followed by the addition of ethanolic solution of 1*H*-benzimidazole-2-thiol(L²) and solution of 4*H*-1,2,4-triazol-4-amine(L³) in ethanol. The resulting solution was refluxed on a water bath for 1h. The metal ion-ligands ratio maintained was 1:1:1:1 (M:L¹:L²:L³). The contents were cooled and precipitated by the addition of hot ethanolic solution of ammonium hexafluorophosphate to the filtrate. The compound was filtered and dried

under vacuum before being recrystallized using ethyl acetate. Yield of the complex 72%. Analysis: $C_{21}H_{18}N_8SP_2F_{12}Zn$: Calc. (%); C

32.73, H 2.34, N 14.54, Fe 8.48. Found(%): C 32.68, H 2.31, N 14.48, Zn 8.41. The proposed structure of the complexes is given in Fig. 1.

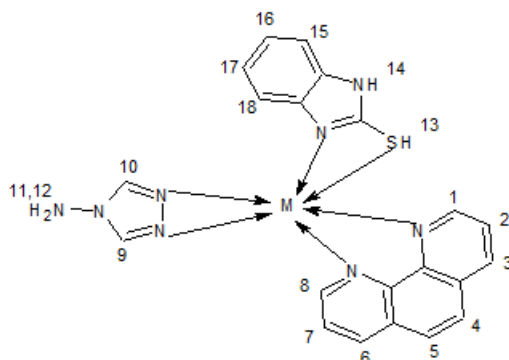


Figure 1

The proposed structure of the complexes where $M = Fe^{3+}$ or Zn^{2+}

RESULTS AND DISCUSSION

Characterization of complexes

The elemental analysis data are agreed with the theoretical values within the limit of experimental error. These complexes are soluble in DMF, DMSO and in buffer (pH 7.2) solution. The observed conductivity measurement of $10^{-3}M$ solution values in DMF fall in the region $149-188\Omega^{-1}cm^2mole^{-1}$ indicate that complex (1) showed uni-trivalent and complex (2) showed uni-bivalent nature.

IR spectra

The IR spectra of complexes were recorded in the range of $4000-400cm^{-1}$ on KBr pellets. The spectra of the ligand 1H-benzimidazole-2-thiol (L^2) showed bands at $1622cm^{-1}$ assigned to $\nu C=N$ aromatic hydrocarbon, $3100cm^{-1}$ assigned to $\nu C-H$ group and $3200cm^{-1}$ assigned to $\nu O-H$ group. The spectra of both the complexes showed a peak in the range $1625cm^{-1}$ to $1690cm^{-1}$ for $\nu C=N$ group are shifted slightly indicating that the coordination taken place through nitrogen atom. The band due to $\nu S-H$ appeared around $2940cm^{-1}$. The position of S-H band of uncoordinated ligand found to be shifted by $10-20cm^{-1}$ after the formation of complexes. Besides, the complexes showed new bands at $400-430cm^{-1}$

are assigned to $\nu(M-N)$ bands.²¹

¹H NMR spectra

The ¹H NMR spectra of the uncoordinated ligand and the complex (2) were recorded in DMSO- d_6 . The complex (1) was found to be paramagnetic in nature. The NH and SH proton signals in the free ligand appeared at 12.5ppm and the position of the peak at 7.1ppm is due to the benzimidazole ring protons. In the complex (2), a sharp peak at 9.0ppm is assigned to eight protons i.e., $H_1, H_8, H_9, H_{10}, H_{11}, H_{12}, H_{15}$ and H_{18} , another sharp signal appeared at 8.3ppm is due to four protons H_2, H_7, H_{16} and H_{17} , the singlet's observed at 8.4ppm and 6.3ppm are due to $-NH$ and $-SH$ group protons respectively.

Absorption Spectral Studies

Absorption titration is used to monitor the interaction of the complexes (1) and (2) with CT-DNA. The absorption spectra of the complexes (1) and (2) in the absence and presence of CT-DNA are given in Fig. 2 and 3 respectively. The Fig. 2 depicts well resolved band at 270nm for complex (1) and the Fig. 3 depicts well resolved band at 272 nm for complex (2), with increasing the DNA concentration (0-200 μM). The result

showed that the absorbance (hypochromism) decreased by the successive addition of CT-DNA to the complex solution. The hypochromism observed for the bands of complex (1) and (2) are accompanied by small bathochromic shift of 4 and 3 nm in Fig.2 and 3 respectively. The hypochromism and bathochromic shift are observed for the complexes suggest that binding is intercalative mode. In order to compare quantitatively, the DNA binding strengths of these complexes, the intrinsic DNA binding

constants K_b are determined from the decay of the absorbance at 270 nm for complex (1) and 272 nm for complex (2) with increasing concentrations of DNA. The observed K_b values for complex (1) and (2) are equal to the classical intercalators bound to CT-DNA. The K_b values were determined as per the procedure reported.²² The K_b values for complex (1) and (2) are $2.5 \times 10^5 \text{ M}^{-1}$ and $4.1 \times 10^5 \text{ M}^{-1}$, respectively. So, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

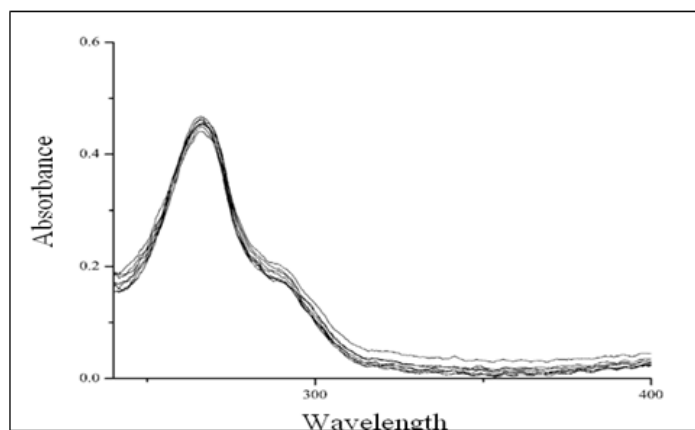


Figure 2

Absorption spectra of complex (1) in Tris-HCl buffer upon addition of DNA. $[\text{Fe}] = 0.5 \mu\text{M}$, $[\text{DNA}] = 0-200 \mu\text{M}$. The arrow shows the absorbance changing upon the increase of DNA concentration.

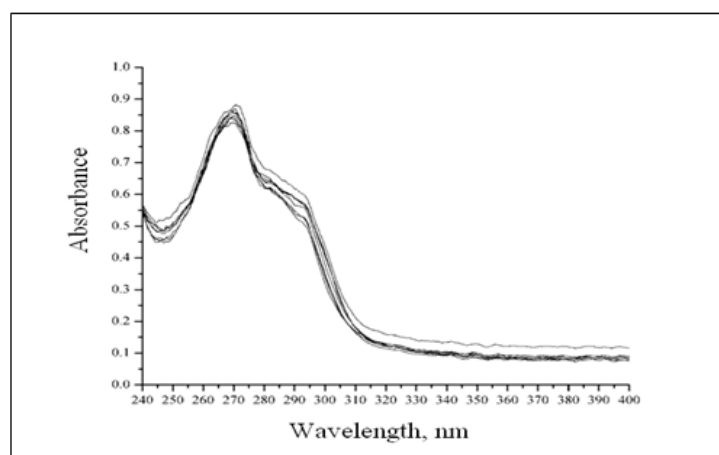


Figure 3

Absorption spectra of complex (2) in Tris-HCl buffer upon addition of DNA. $[\text{Zn}] = 0.5 \mu\text{M}$, $[\text{DNA}] = 0-200 \mu\text{M}$. The arrow shows the absorbance changing upon the increase of DNA concentration.

Viscosity measurements

The DNA binding modes of complexes were further investigated by viscosity measurement, which is sensitive to the increase in length of DNA and is regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data.²³ To understand the nature of DNA binding of mixed ligand Fe(III) and Zn(II) complexes, the viscosity measurements were carried out on CT-DNA by varying the concentration of the added complex. Representative plots of relative viscosity (η/η_0) vs. [Complex]/DNA are shown in Fig. 3. As can be seen, there is positive change in viscosity with increasing addition of the concentration of the complexes to DNA. These results suggested that, both the complexes intercalated between two adjacent base pairs of DNA through a classical intercalation mode.

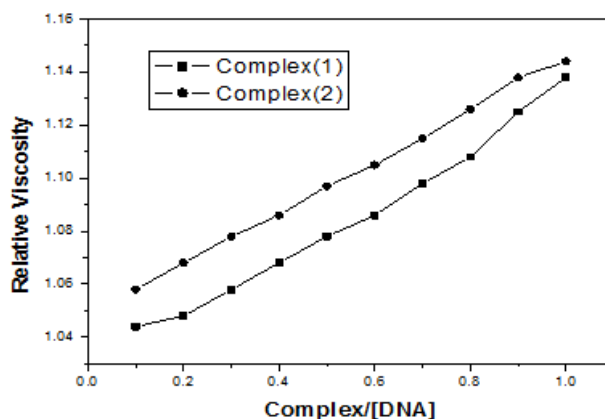


Figure 4

Plot of relative viscosity Vs [complex]/[DNA]. Effect of complex(1) and complex(2) on the viscosity of CT-DNA at 25 (± 0.1) °C, [Complex] = 0-100 μ M, DNA = 50 μ M.

Thermal denaturation studies

Thermal behaviors of DNA in the presence of complexes can give insight into their conformational changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hypochromic effect on the absorption spectra of DNA bases ($\lambda_{\max} = 260$ nm). In order to identify this transition process, the melting temperature T_m is usually introduced, which is defined as the temperature where half of the total base pairs is bounded. According to the literature, the intercalation of natural or synthesized organic and metallointercalators generally results in a considerable increase in melting

temperature (T_m). As shown in Fig.5, the T_m was found to be $55 \pm 1^\circ\text{C}$ under experimental conditions. Under the same set of conditions, addition of complex (1) and (2) increased $T_m (\pm 1^\circ\text{C})$ by 5°C and 3°C respectively, which indicates that these compounds stabilize the double helix of DNA. The increase in T_m of the latter is comparable to that of classical intercalators.²⁴ So from the above data it is concluded that the new Fe(III) and Zn(II) mixed ligand complexes act as a new class of DNA intercalators. The observations made during the absorption titration, viscosity measurements and thermal denaturation experiments are reminiscent of those reported earlier for various metallointercalators, thus suggesting that the complexes (1) and (2) bound to DNA by intercalations.²⁵⁻³⁸

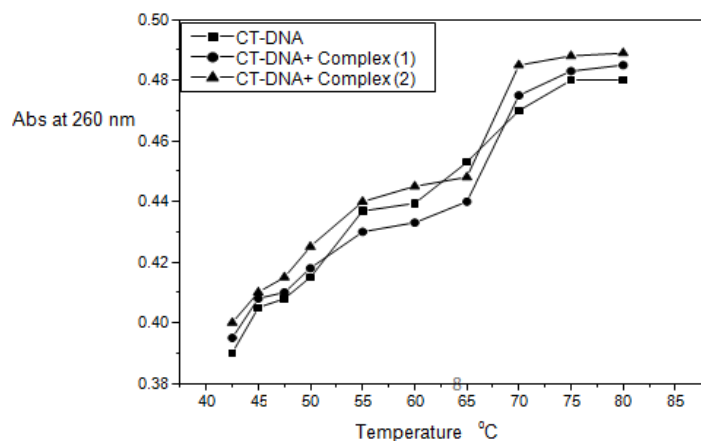


Figure 5
Melting curves of CT-DNA in the absence and presence of complexes.

DNA cleavage studies

In order to determine the ability of complexes (1) and (2) for DNA scission, the complexes were incubated at different concentrations with supercoiled pUC19 DNA for 1 hour in 50 mM Tris-HCl/50 mM NaCl buffer (pH 7.2) using hydrogen peroxide (H_2O_2) activation. Control experiments using H_2O_2 do not show any

apparent cleavage of DNA (Fig. 6, lane 1). At the concentration of 40 μM and 80 μM in Fig. 6, complex (1) is able to convert 85 % and 90 % of the initial SC (Form I) to NC (Form II) (lane 2 and 3) and the complex (2) is able to convert 90 % (40 μM) and 90% (80 μM) of the initial SC (Form I) to NC (Form II) (lane 4 and 5).

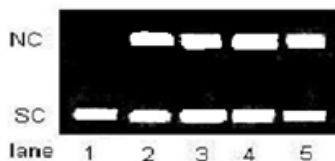


Figure 6

Agarose gel electrophoresis of supercoiled pUC 19 DNA (0.5 μg) by the Complexes (1) and (2) in a buffer containing 50 mM Tris-HCl and 50 mM NaCl at 37°C. Lane 1, DNA alone; Lane 2, DNA+40 μM of complex (1); Lane 3, DNA+80 μM of complex (1); Lane 4, DNA+40 μM of complex (2); Lane 5, DNA+80 μM of complex (2). Forms I and II are supercoiled and nicked circular forms of DNA respectively.

CONCLUSION

The experimental results obtained by absorption spectra, viscosity and thermal denaturation studies indicate that the new metal complexes viz, $[Fe(L^1L^2L^3)](PF_6)_3$ [Complex (1)] and $[Zn(L^1L^2L^3)](PF_6)_2$ [Complex (2)], bound to the double stranded DNA with binding constant $K_b = 2.5 \times 10^5 M^{-1}$ for complex (1) and $K_b = 4.1 \times 10^4 M^{-1}$ for complex (2) respectively. The viscosity of solution of the DNA bound to the complexes

increased with increase in concentration of the complexes suggesting the intercalation of both the complexes with DNA. Thermal denaturation experiments also revealed the intercalation of both the complexes with DNA. Further the complexes have been investigated for their cleavage activity. The results showed that both the complexes were exhibiting fairly good nuclease activity.

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