(IJIRSE) International Journal of Innovative Research in Science & Engineering

ISSN (Online) 2347-3207

Synthesis, Characterization, DNA Binding-Cleavage and BSA Binding Studies of Pyridine Based Macrocyclic Binuclear Ni(II) Complexes

Vedavalli Sairaj and Muthusamy Kandaswamy*
Department of Inorganic Chemistry, University of Madras, Guindy Campus,
Chennai, 600 025, India.
E-mail: mkands@yahoo.com

Abstract- **Five new binuclear Ni (II) complexes $[Ni_2L^{1-3}].2ClO_4$ with various new unsymmetrical binucleating macrocyclic ligands were synthesized by cyclocondensation between N,N'-bis(3-formyl-5-methylsalicylidene) alkane/benzene/naphthalene-diimines (PC1-3 and 2,3-diamino -pyridine in the presence of Nickel (II) acetate and Nickel (II) perchlorate (1:1:1:1 molar ratio). Characterisation of these complexes was carried out using FT-IR, electronic and mass spectral studies and also by elemental analysis. Electrochemical properties of the complexes were studied by cyclic voltammetry. The interactions of the complexes with DNA have been measured by spectroscopic and viscosity measurements. Absorption spectroscopic investigation reveals that the Ni (II) complexes bind with DNA by intercalative binding mode, with binding constants(K_b) $1.87x10^4$, $0.94x10^4$ and $0.62x10^4$ M^{-1} for the complexes 1-3 respectively. Fluorescence spectroscopic measurement shows that the Ni (II) complexes can displace ethidium bromide and bind to DNA, with binding constants(K_{app}) of $2.5x10^6$, $3.2x10^6$and $4.3x10^6$ M^{-1} for the complexes 1-3 respectively. The interaction of the complexes with BSA has been studied by UV-Vis absorption and fluorescence spectroscopic techniques. The results indicate that the complexes have a quite strong ability to quench the fluorescence of BSA and the binding reaction is mainly a static quenching process. The complexes exhibit good binding propensity to BSA showing relatively high binding constant values. Agarose gel electrophoresis studies show that the complexes display effective DNA cleavage activity by hydrolytic mechanism.**

Keywords: **Unsymmetrical binucleating macrocyclic ligands; Ni (II) complexes; DNA and BSA binding properties; DNA cleavage studies.**

I. Introduction

The interaction of transition metal complexes with DNA has been a subject of intense research in the field of bioinorganic chemistry, ever since the discovery of cis-platin as an anticancer agent [1-2]. Although highly effective in treating a variety of cancers, the cure with cisplatin is still limited by dose-limiting side effects [3] and inherited or acquired resistance phenomena, only partially amended by employment of new platinum drugs [4,5]. These problems have stimulated an extensive search and prompted chemists to develop alternative strategies, based on different metals, with improved pharmacological properties and aimed at different targets [6]. A large number of transition metal complexes have been also explored with efficient DNA cleavage activities through either hydrolytic or oxidative pathways [7,8]. Compared with the number of studies dealing with mononuclear complexes, relatively few studies on binuclear complexes have been reported to date [9-11]. Complexes of macrocyclic polyamines with two phenol groups have good nuclease activities because of their ability to bind and cleave DNA [12]. Hence, the efficient enhancement of DNA cleavage activity for binuclear complexes stimulates us to design and synthesize new macrocyclic binuclear complexes to evaluate and understand the factors on the DNA-binding and cleavage properties [13-15].

In this paper, new binuclear Ni(II) complexes with various new unsymmetrical binucleating macrocyclic were prepared and characterized by using various spectroscopic methods. The interaction of the Ni (II) complexes with calf thymus DNA and BSA has been investigated using absorption and fluorescence spectroscopic experiments and viscosity methods. The cleavage reaction on plasmid DNA has been monitored by agarose gel electrophoresis.

II. Materials and Methods

a. General Procedures

All chemicals were purchased from commercial sources and used as received. Solvents were dried according to standard procedures and distilled prior to use [23]. HPLC grade acetonitrile and dimethyl formamide solvents were used for spectroscopic studies. CT-DNA and pBR322 DNA were purchased from Bio-Genics, Bangalore (India). The melting points were determined in an open glass capillary and were uncorrected. The elemental analysis was performed using a Carlo Erba Elemental analyzer Model 1106. The FT-IR spectra were obtained on a Perkin Elmer FTIR spectrometer (Perkin–Elmer 2000) with samples prepared as KBr pellets. Absorption spectra in the range 200–750 nm were recorded on a Perkin Elmer Lambda 35 spectrophotometer. Fluorescence spectra were recorded on a Perkin Elmer LS-45 Fluorescence spectrometer. Electrospray ionization mass spectral measurements were done using a Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer.

b. Synthesis of the Complexes.

$[Ni_2L^1](C1O_4)_2$, l.

To a boiling solution of compound PC-1 (0.25 g, 0.07 mM) in chloroform-methanol (30: 70 v/v, 100 cm^3) was added nickel (II) acetate monohydrate (0.14 g, 0.07 mM) dissolved in methanol (15 cm^3) and the mixture was refluxed for 30 min. The precipitate formed was subsequently treated with a mixture of 2,3-diaminopyridine (0.076 g, 0.07 mM) and nickel (II) perchlorate hexahydrate (0.26 g, 0.07 mM) in methanol (10 cm^3) and refluxed for 6 h. During the course of reflux all the solid mononuclear complex dissolved and a homogeneous solution resulted. The resulting solution was filtered and the filtrate allowed to evaporate at room temperature. The dark green precipitate formed was filtered off, washed with cold methanol and vacuum dried. Yield 0.42 g (80%).
(Found: C, 55.52; H, 3.91; N, 12.95. Calc. for $C_{25}H_{29}N_5O_{14}Cl_2Ni_2$ C, 55.51; H, 3.90; N, 12.94%).

$[Ni_2L^2]$ $(C1O_4)_2$, 2.

This complex was prepared by the procedure adopted for 1 except using PC-2 (0.256 g, 0.07 mM) in place of PC-1. Dark red compound obtained. yield 0.43 g (82%). (Found: C, 56.28; H, 4.18; N, 12.62. Calc. for $C_{26}H_{31}N_5O_{14}Cl_2Ni_2$ C, 56.28; H, 4.18; N, 12.62. %).

$[Ni_2L^3](C1O_4)_2$, 3.

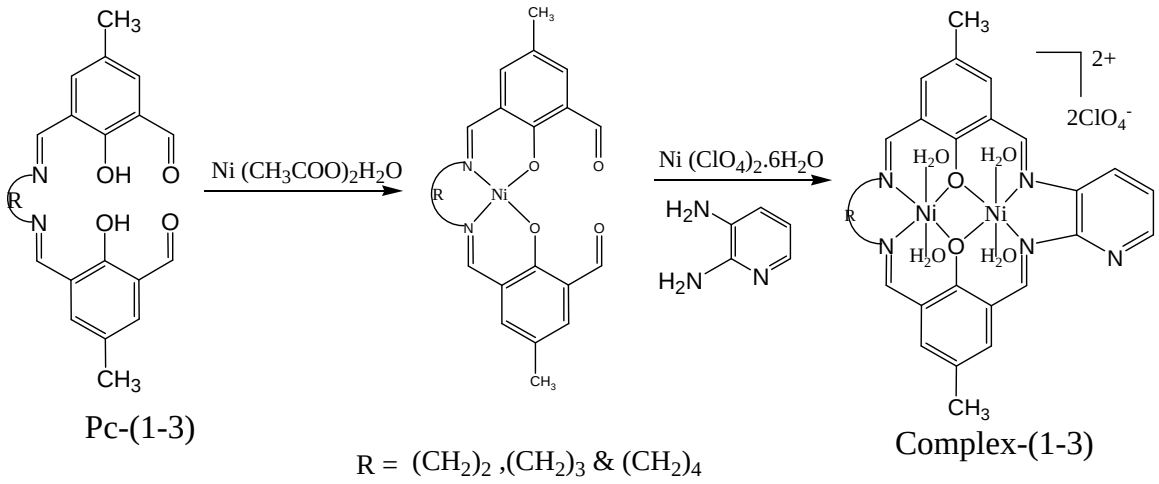
This complex was prepared by the method used for 1, using compound PC-3 (0.266 g, 0.07 mM) in place of PC-1. Red colour compound obtained. yield 0.45 g (85%). (Found: C, 57.00; H, 4.43; N, 12.31. Calc. for $C_{27}H_{33}N_5O_{14}Cl_2Ni_2$ C, 57.00; H, 4.41; N, 12.30%).

III. Results and discussion

a. Synthesis and characterization of the complexes

The binuclear nickel (II) complexes were prepared in good yields from the one molar ratio of the 2,3-diaminopyridine and the corresponding PC(1-3) and one molar ratio of nickel (II) acetate and nickel (II) perchlorate in acetonitrile with reflux (scheme-1).

The elemental analysis data of the complexes are consistent with the calculated results from the empirical formula of each compound**.** The positive ion ESI mass spectra for the nickel (II) complexes 1-3 showed a major peak at m/z = 639, 653 and 667 respectively corresponding to the $[Ni_2L^{1-3}.ClO_4]^+$ ion. Also mass spectra of the complexes show peaks at 270, 277 and 284 confirm the presence of a dipositive binuclear core $[Ni_2L^{1-3}]^{2+}$ ions respectively. The analytical and ESI mass spectral data are consistent with the proposed formula of the dinickel (II) complexes. The characteristic IR bands for the binuclear complexes are different from those of the related PC1-3 compounds. The effective Schiff base condensation in the complexes is indicated by the formation of a peak around 1620 cm^{-1} corresponding to imine group ν(C=N) and the disappearance of the formaldehyde ν(HC=O) peak around 1670 cm^{-1} observed for the precursors. The binuclear nickel (II) complexes 1–3 show two peaks near 1100 cm^{-1} due to the antisymmetric stretch and one peak near 630 cm^{-1} antisymmetric bend of perchlorate ions, respectively. Among the two peaks near 1100 cm^{-1}, one peak shows splittings, which is due to the presence of coordinated perchlorate ion. A broad band around 3400 cm^{-1} suggests the presence of lattice water.



R = $(CH_2)_2$,$(CH_2)_3$ & $(CH_2)_4$

Scheme 1

b. Interaction of Nickel (II) Complexes with DNA.

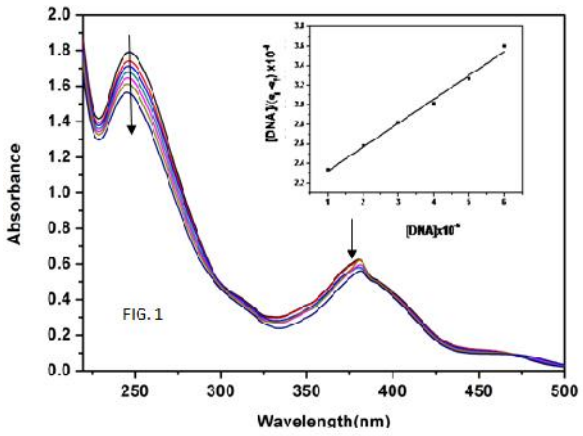
Absorption Spectral Titration.

Electronic spectra of the complexes show two ligand-centered transitions (LC) around 350–365 nm and 260–270 nm regions, corresponding to π- π * and n- π * transitions, respectively. The nickel(II) complexes were dissolved in DMF at a concentration of 5 X 10^{-5} M. The UV absorption titrations were performed by keeping the concentration of the complex fixed while varying the DNA concentration. Complex-DNA solutions were allowed to incubate for 30 min at room temperature before measurements were made. Absorption spectra were recorded using cuvettes of 1 cm path length at room temperature. The absorption spectra of the complexes 1 in the presence of increasing amounts of DNA are shown in Fig 1. Upon addition of DNA to the solution of the complexes, the peak at 255 nm and a peak at 326 nm decreased. Hypochromism in the band reaches as high as 40% after adding DNA, indicating that the interaction mode between CT-DNA and the complex is intercalative [16]. In order to quantitatively investigate the binding strength of the complex with CT-DNA, the intrinsic binding constant Kb was obtained by monitoring the changes in absorbance at 255 nm for the complex as increasing concentration of CT-DNA. The intrinsic DNA binding constants, Kb, of the complexes have been obtained by monitoring the changes in absorbance at 255 nm for complexes 1−3 with increasing concentrations of DNA. The DNA binding constants for complexes 1−3 have been found to be $1.87x10^4$, $0.94x10^4$ and $0.62x10^4$ M^{-1} respectively.

Fluorescence spectroscopic studies

Ethidium bromide (EB) emits intense fluoresence light in the presence of DNA, due to its strong intercalation between the adjacent DNA base pairs. It was previously reported that the enhanced fluoresence can be quenched by the addition of a second molecule. The quenching of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA. Fluorescence quenching experiments were performed by adding a solution of the complex at different concentrations to EB-bound CT-DNA solution (1.5 mL). All experiments were carried out using cuvettes of 1 cm path length at room temperature. Samples were excited at 510 nm, and emission was recorded at 550–700 nm. In our experiments, when the Ni(II) complex was added to the EB-DNA system, the emission intensity was reduced. The emission spectra of EB bound to DNA in the absence and presence of the complex are given in fig.2. The significant reduction in fluorescence intensity of the EB-DNA solution on addition of the complex suggests that the Ni (II) complex can displace the EB and therefore bind to the DNA. Such a characteristic change is often observed in the intercalative DNA interaction [17]. The binding of the complex to DNA can be determined according to the classical Stern–Volmer equation. The quenching constant K obtained for the complexes is given by the slope of the plot in Fig. 2, as K_{app} $2.5x10^6$, $3.2x10^6$and $4.3x10^6M^{-1}$ for the complexes 1-3 respectively. These constants are similar to those of DNA-intercalative complexes, so we deduce that the Ni(II) complex binds to DNA with a moderate intercalative mode.

ISSN (Online) 2347-3207



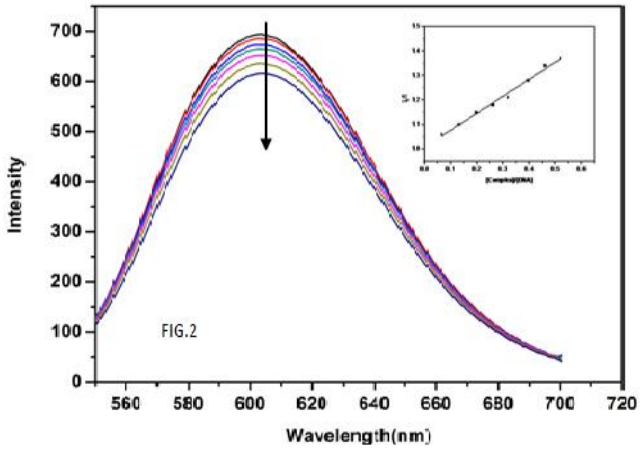


Fig. 1. UV-visible spectra of the Ni(II) complex in the presence of increasing amounts of CT-DNA in 50 mM Tris–NaCl aqueous buffer solution (pH = 7.2). Insert:Plot of [DNA]/(ε_a - ε_f) versus [DNA] for absorption titration of CT-DNA with the Complex

Fig. 2. Emission spectra of EB bound to DNA in the absence and presence of the complex 1. .Insert : Stern–Volmer quenching plot of EB bound to DNA by complex.

Viscosity experiments

To further confirm the interaction mode of the Ni (II) complex with DNA, a viscosity study was carried out. Hydrodynamic method, such as determination of viscosity, which is exquisitely sensitive to the change of length of DNA, may be the most effective means studying the binding mode of complexes to DNA in the absence of X-ray crystallographic or NMR structural data. Since the relative specific viscosity (η/η_0) of DNA (η and η_0 are the specific viscosities of DNA in the presence and absence of the complexes, respectively) gives a measure of the increase in contour length associated with separation of DNA base pairs caused by intercalation, a classical DNA intercalator like EB shows a significant increase in the viscosity of the DNA solutions[18]. In contrast, the molecules that bind to DNA either in the grooves or on the external surface give rise to irregularity or no changes in the viscosity of CT DNA[19]. The specific viscosity of the DNA sample increases obviously with the addition of the complexes and the relative viscosities of DNA was increased with the increase of the concentration of the complexes. The plots of relative viscosity $(\eta/\eta_0)^{1/3}$ vs [complex]/[DNA] ratio for the complexes are given in the figure 5. The viscosity increase of DNA is ascribed to the intercalative binding mode of the complex because this could cause the effective length of the DNA to increase.

c. Protein binding studies

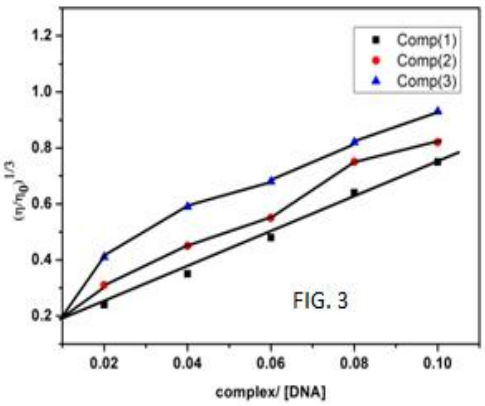
The investigation of compounds with respect to their binding to albumins becomes important because of the pharmacokinetic and pharmacodynamics role of such binding.

UV-visible spectral absorption spectral analysis

The absorption band obtained for BSA at 280 nm in the absence of metal complex showed an increase in the intensity of absorption after the addition of complex.

Fluorescence quenching of BSA.

Fluorescence spectroscopy is an effective method to examine the interactions between metal complexes and BSA. Here we investigated the binding affinity of the dinickel(II) complexes 1–3 with BSA under physiological conditions. The effect of Ni(II) complex 2 on the BSA fluorescence intensity is shown in Fig. 4, from which we can see clearly that BSA had a strong fluorescence emission band around 360 nm by fixing the excitation wavelength at 280 nm. The addition of Ni(II) complexes to the solution of BSA resulted in the quenching of its fluorescence emission without any shift, possibly due to changes in the secondary/tertiary structure of BSA in the phosphate buffer, affecting the orientation of the tryptophan residues of BSA. The K_{BSA} values of the complexes 1-3 are 2.75 x 10^5 M^{-1}, 2.98 x 10^5 M^{-1} and 3.5 x 10^5 M^{-1} respectively.



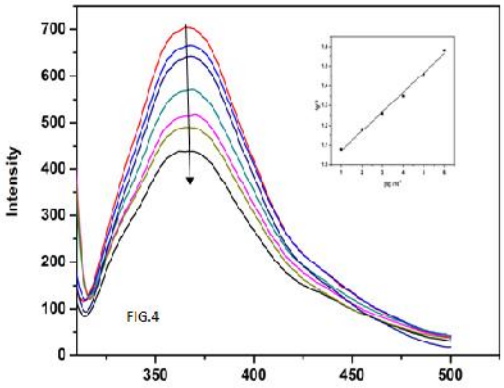


Fig. 3. Effects of increasing amounts of the nickel complex on the relative viscosities of CT-DNA at 23.0 (±0.1) °C; [DNA] = 100 µM, r = [M]/[DNA].

Fig. 4. Fluorence spectra of BSA in presence of various concentration of complex-2 Vs [BSA], [BSA]=1× 10^{-6} M^{-1}, [complex]=(0 - 10 × 10^{-6} M^{-1}). Insert : Plot of [Q] versus (I_0/I) for emission titration of BSA with complex.

d. Chemical nuclease activity

To assess the DNA cleavage ability of the complexes 1–3, supercoiled (SC) pBR322 DNA (100 ng μL^{-1}) was incubated with different concentrations of the complexes in 5% DMF in Tris–HCl/NaCl (5:50 mM) buffer (pH 7.2) for 30 min using H_2O_2 as an activator (Fig. 5). When plasmid pBR322DNA was incubated with the complexes in the presence of H_2O_2, the supercoiled DNA (SC, form I) was transited to nicked circular DNA(NC, form II) and linearized DNA(LC, form III). The conversions of form I to form II and to form III were observed with increases in the concentration of the complexes.

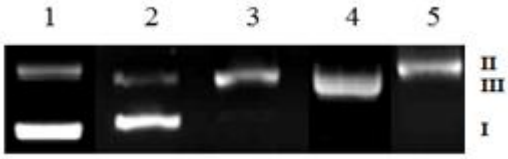


Fig.5. Cleavage of SC pBR322 DNA (0.1 lg) by Ni(II) complexes (15µg) in 50 mM Tris– HCl/NaCl buffer (pH 7.2). Lane 1: DNA control, lane 2: DNA +H_2O_2, lane 3: DNA + H_2O_2 + complex 1, lane 4: DNA + H_2O_2 +complex 2, lane 5: DNA + H_2O_2 +complex 3.

IV. Conclusion

Three new binuclear Ni(II) complexes [Ni_2L^{1-3}].2ClO_4 with various new unsymmetrical binucleating macrocyclic ligands were synthesized. The interactions of the complexes with DNA and BSA have been measured by spectroscopic and viscosity measurements. The complexes exhibit good binding propensity to DNA and BSA showing relatively high binding constant values. Agarose gel electrophoresis studies show that the complexes display effective DNA cleavage activity by hydrolytic mechanism.

Acknowledgement

The author Vedavalli Sairaj is thankful for the financial support provided by the MHRD special grants, New Delhi, INDIA under NCNSNT Program - research grants.

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