DNA Binding and Nuclease Activity of Structurally Characterized Triply Bridged Dinuclear Copper(II) Complex

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Abstract.

The mononuclear copper(II) complex ***[Cu(bipy)(H_2O)(NO_3)$_2$] (1)****, obtained by the reaction of 2, 2'- bipyridyl with* $Cu(NO_3)_2.3H_2O$ *in methanol solution, reacts with anionic ligand acetate to form a triply bridged dinuclear complex,* ***[Cu_2(bpy)$_2$(μ-OH)(μ-H_2O)(μ-OAc)] (NO_3)$_2$ (2).*** *The complexes are characterized based on electronic, IR and ESR spectroscopies. The dinuclear complex crystallizes in monoclinic space group P21/c P-1 with a square pyramidal Cu(II) center coordinated by the bidentate bipyridine and three bridging ligands The electrochemical properties of the complexes are investigated by cyclicvoltammetry. The interactions of these complexes with calf thymus DNA have been investigated using absorption spectrophotometry. Nuclease activities of complexes are investigated on double stranded pBR322 plasmid DNA using gel electrophoresis experiments under different conditions.*

Key words: *Dinuclear copper(II) complex, single crystal X- ray structure, DNA binding and cleavage*.

Introduction

The study of interactions of metal complexes with nucleic acids is an exciting area of research due to their potential use as drugs, tools for biochemical and biomedical applications in gene regulation. Considerable efforts are being made to design sequence-specific DNA cleaving agents that bind DNA at any desired sequence and cleave DNA efficiently at the binding site[1-2]. Several artificial DNA binding metallo-nucleases developed for the cleavage of DNA have potential applications as therapeutic agents, and as versatile replacements for nucleases as laboratory tools[3]. Investigation of transition metal complexes with catalytic activity and substrate specificity, mimicking natural enzyme, have important applications in molecular biology, and perhaps in the development of new therapeutics[4]. DNA has become an interesting target for artificial enzymes, partly because of possible therapeutic applications[5]. In past two decades, a variety of transition metal complexes have been used in developing artificial nucleases by either hydrolytic or oxidative pathway, with or without sequence specificity[6].

Currently, there is much interest to investigate catalytic activity of systems based on two metal atoms, because many enzymes that catalyze phosphate ester cleavage are activated by two or more metal ions[7] Copper complexes have been extensively investigated in this application since they possess biologically accessible reductive potentials and high affinity with nucleic acids[8]. Copper complexes of heterocyclic bases show good DNA binding propensity[9]. These ligand systems, in combination with some bridging groups may be able to hold two or more metal centers, mimicking the multinuclear metal arrays at the active sites of several metallo-enzymes[10]. In the recent past we have reported [11-14] nuclease activity of several binuclear copper(II) complexes. In continuation of our research activity[11-14] towards designing DNA cleaving agents, here in we report the synthesis, crystal structure, DNA binding and nuclease activity of novel triply bridged dinuclear copper(II) complex

Materials and Methods

Analytical grade 2, 2'- bipyridyl, $Cu(NO_3)_2$. $3H_2O$ and sodium acetate were obtained from Merck. The solvents used for synthesis of the metal complexes were distilled before use. Calf thymus DNA (CT-DNA) and plasmid pBR322 (cesium chloride purified) were purchased from Genie Bio labs, Bangalore, India. Agarose (molecular biology grade) and ethidium bromide (EB) were obtained from Sigma. Solutions of CT-DNA in 50 µM *Tris*-HCl (pH, 7.0) gave the ratio of UV absorbance at 260 and 280 nm of 1.8 indicating that the DNA was sufficiently free of protein. The DNA concentration was determined by UV absorbance at 260 nm using molar absorption coefficient 6600 M^{-1}. Stock solutions were kept at 4°C and used after not more than four days. DNA binding studies were performed in 50 mM NaCl/5mM *Tris*- base, pH, 7.0 buffer.

Physical measurements

The elemental analyses were performed using a Perkin Elmer 2400 CHNS elemental analyzer. The molar conductance of the complexes in DMF (10^{-3} M) solution was measured at 28 °C with a Systronic Model 303 direct reading conductivity bridge. The electronic spectra were recorded in DMF with a Perkin Elmer UV Lamda−50 spectrophotometer. FT–IR spectra in KBr disc were recorded in the range 4000–400 cm^{-1} with a Perkin Elmer spectrum 100 spectrometer. The cyclic voltammetry was performed with a CH instruments 660C electrochemical analyzer and a conventional three electrodes, Ag/AgCl reference electrode, glassy carbon working electrode and platinum counter electrode. Nitrogen gas was purged and measurements were made on the degassed (N_2 bubbling for 5 min) complex solution in DMF (10^{-3} M) containing 0.1 M tetrabutylammonium hexaflourophosphate (TBAHEP) as the supporting electrolyte.

Preparation of complexes

Preparation of $[Cu(bpy)(H_2O)(NO_3)_2]$ (1): The complex was prepared by using literature procedure[15]. To a stirring solution of $Cu(NO_3)_2$. $3H_2O$ (1.21 g, 5 mmol) in MeOH (10 mL), a solution of 2, 2'- bipyridyl (0.99 g, 5 mmol) in MeOH (10 mL) was added slowly. The stirring was continued for 30 min. The blue complex was filtered off and washed with a small quantity of MeOH. Yield: 1.56 g (81%). M.P. 256 -258 ºC, F.W. 361.77. Anal. Calc. for $C_{10}H_{10}N_4O_7Cu$; C: 33.20%; H: 2.79%; N: 15.49%; Found: C, 33.0; H, 2.6; N, 15.1 %.

Preparation of $[Cu_2(bpy)_2(\mu\text{-}OH)(\mu\text{-}H_2O)(\mu\text{-}OAc)](NO_3)_2$ (2): To a stirring solution of complex (1) (0.77 g, 2 mmol) in MeOH (20 mL), excess NaOAc was added, and stirring was continued for 1 h. The dark blue solution was filtered and then evaporated slowly at room temperature. Dark blue crystals suitable for single-crystal XRD were obtained after 1 week. Yield: 1.05 g (63%) M.P. 273–275ºC, F.W. 657.56. Anal. Calc. for C_{22} H_{22} Cu_2 N_6 O_{10}. C: 40.18%; H: 3.37%; N: 12.78%; Found: C, 40.4; H, 3.4; N, 12.8%.

X-ray crystallography

Crystal data were collected using the Enraf–Nonius CAD4- MV31 single crystal X-ray diffractometer, Indian Institute of Technology-Madras, Chennai. The single crystal X-ray diffractometer is a fully automated four circle instrument controlled by a computer. It consists of an FR 590 generator, a goniometer, CAD4F interface and a microVAX3100 equipped with a printer and plotter. The detector is a scintillation counter. A single crystal is mounted on a thin glass fiber fixed on the goniometer head. The unit cell dimensions and orientation matrix are determined using 25 reflections and then the intensity data

of a given set of reflections are collected automatically by the computer. An IBM compatible PC/AT 486 is attached to micro VAX facilitating the data transfer on to a DOS floppy of 5.25″ or 3.5″. Maximum X-ray power is 40 mA × 50 kV. The data collected were reduced using SAINT program. The trial structure was obtained by direct method, using SHELXS-86, which revealed the position of all non-hydrogen atoms and refined by full-matrix least squares on F 2 (SHELXS-97), and graphic tool was DIAMOND for windows. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were treated with a mixture of independent and constrained refinements.

DNA Binding Study

The electronic spectra of metal complexes in aqueous solutions were monitored in the absence and in the presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration at 20×10^{-6} M and varying the nucleic acid concentration ($0–7.36 \times 10^{-6}$ M). The titrations were carried out by gradually increasing the concentration of CT-DNA with each addition of 10 µl DNA. The ratio of r = [complex]/[DNA] values vary from 23.41 to 2.60. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant (K_b) was calculated by using the Bebesu-Hildebrand equation, modified by Wolfe et al.[16].

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/ K_b (\varepsilon_b-\varepsilon_f) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where [DNA] is the molar concentration of DNA in base pairs, ε_a, ε_b and ε_f are apparent extinction coefficient ($A_{abs}/[M]$), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively.

Gel electrophoresis

The extent of cleavage of DNA by the copper (II) complexes was visualized using gel electrophoresis with pBR 322 DNA. After incubation for 30 min at 37 ^{o}C, the samples were added to the loading buffer containing 0.25% bromophenol blue + 0.25% xylene cyanol + 30% glycerol, and solutions were loaded on 0.8% agarose gel containing 100 µg of ethidium bromide. Electrophoresis was performed for about 1.5 hours at 75 V in TBE buffer until the bromophenol blue reached to 3/4 of the gel. Bands were visualized by UV transilluminator and photographed. The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its supercoiled (SC) form. The reactions were carried out under oxidative and/or hydrolytic conditions. Control experiments were done in the presence of hydroxyl radical scavenger DMSO (4 µl) and DTT (2 µl).

Results and Discussion

The reaction of $Cu(NO_3)_2$. $3H_2O$ with an equimolar amount of bpy in methanol results in the formation of a blue colored complex **(1).** It reacts with acetate ligand in methanol to form the stable binuclear complex **(2)**. Conductivity data (Table S1) suggest that the complex **1** is non-electrolyte while complex 2 is 1:2 electrolyte.

Magnetic and electronic spectral studies

The electronic spectra (Figure S1) of the copper(II) complexes **1** and **2** exhibit two strong bands and one weak band at 37,520, 30,320 and 14,800; 37,620, 31,345 and 15,384 cm^{-1} respectively assigned to π - π^*, CT and d-d transitions. The magnetic moment value (1.68 BM) for complex **1** is in good agreement with the value expected for mononuclear complex. For complex **2,** the observed value (0.82 BM) is subnormal to spin only value.

Infrared spectral studies

The IR spectrum of complex 1 shows strong absorptions at 1,383 and 1,294 cm^{-1}, in a region typical for ν(NO) of monocoordinated nitrates[17]. Complex **1** also exhibits a strong band at 3,459 cm^{-1} assigned to –OH stretching of a water ligand. In the IR spectra of complex **2, a** strong band is observed at 1,575 cm^{-1} assigned to bridging acetato ligand. The presence of two nitrate counter ions in complex **2** is clearly evident from conductivity data.

ESR spectral studies

The spin Hamiltonian and orbital reduction parameters of these complexes are given in Table 1. ESR spectra of $[Cu(bipy)(OH)(H_2O)(\mu\text{-}CH_3COO^-)(NO_3)_2]$ complex at room temperature and at liquid nitrogen temperature both in solid state and DMF medium are shown in **Figure 1**. The g∥ and g⊥ were computed from the spectra using tetracyanoethylene (TCNE) as a 'g' marker. From the spectra of the complexes at 300 and 77 K in the solid state, it is clear that $g_{\parallel} > g_{\perp} > 2.00$ and the G values falling within the range 1.84- 3.86 are consistent with a d_{x2-y2} ground state in a square planar or square pyramidal geometry[18] According to Hathaway, if G > 4, the exchange interaction is negligible, whereas G < 4 indicates considerable exchange interaction between the metal centers in the solid complex. Thus, in the present case, the G values indicate considerable exchange interaction between the copper(II) atoms, which further supports the dinuclear nature of the complex **2**.

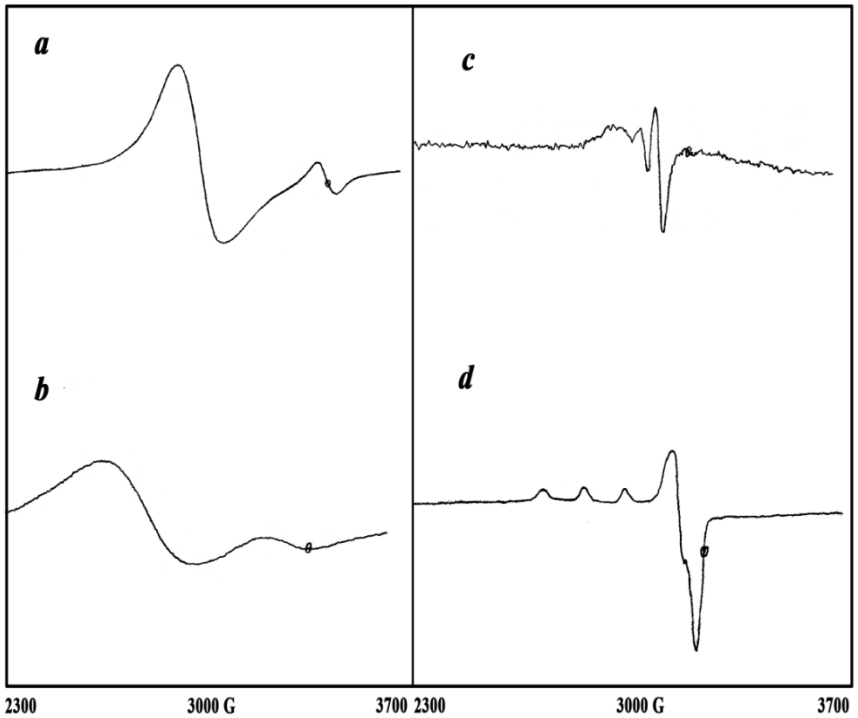

Fig 1 :(a) X-band powder ESR spectra of $[Cu(bipy)(OH)(H_2O)(\mu\text{-}CH_3COO^-)(NO_{3)2}]$ at 300K ,(b) at LNT, (c) in DMF solution at 300K and (d) at LNT in DMF solution

The ESR spectra of the complexes were also recorded in DMF at 300 K and at liquid nitrogen temperature and exhibit a set of four well-resolved peaks in the low-field region and one or two weaker signals at high field corresponding to the g∥ and g⊥ respectively.

Table 1. The Hamiltonian and orbital reduction parameters of copper(II) complexes

Complex	In solid state			In DMF solution									
	$g_{\parallel}$	$g_{\perp}$	g_{avg}	G	$g_{\parallel}$	$g_{\perp}$	g_{avg}	G	λ	$K_{\parallel}$	$K_{\perp}$	$A_{\parallel} x 10^{-5}$ cm^{-1}	$A_{\perp} x 10^{-5}$ cm^{-1}	α^2
1	2.15	2.08	2.10	1.84	2.23	2.06	2.12	3.86	444	1.93	0.51	0.0124	0.0013	0.88
2	2.16	2.05	2.08	3.36	2.20	2.07	2.11	2.09	451	1.68	0.57	0.0139	0.0046	0.30

Description of crystal structure of (2)

The X-ray crystallographic analysis reveals that complex 2 is a binuclear entity with the formula $\mathbf{C_{22}H_{22}Cu_2N_6O_{10}}$. The complex crystallizes in monoclinic, space group P21/c P-1. Crystal data and structure refinements are shown in **Table 2**. The structure of the complex is shown in **Fig. 2** together with the numbering scheme in the metal coordination sphere. Close packing diagram of the complex is shown in **Fig 3**.

Table 2 . Crystal data and structure refinement parameters for $[Cu_2(bpy)_2(\mu\text{-}OH)(\mu\text{-}H_2O)(\mu\text{-}OAc)](NO_3)_2$ (**2**).

Identification code	shelxl
Formula	$C_{22}\ H_{22}\ Cu_2\ N_6\ O_{10}$
Formula weight(M)	657.54
T(K)	293(2)
Wavelength(Mo Kα) (Å)	0.71073
Crystal system,	Triclinic
space group	P-1
Lattice constants	
a(Å)	7.6860(2)
b(Å)	11.3160(3)
c (Å)	15.7910(5)
α(˚)	74.8210(10)
β(˚)	81.207(2)
γ(˚)	73.214(2)
V(A^3)	2400.02(13)
Z	4
Calculated density ρ (Mg m^{-3})	1.727 Mg/m^3
Absorption coefficient μ(mm^{-1})	1.751
F(000)	668
Crystal size (mm)	0.35 x 0.30 x 0.25 mm
Theta range for data collection (˚)	1.34 to 25.00 deg.
Limiting indices	-9<=h<=9, -13<=k<=13, -18<=l<=18
Reflections collected / unique	30433 / 4449 [R(int) = 0.0218]
Completeness to θ(%)	25.00 100.0 %
Absorption correction	Semi-empirical from equivalents

Maximum and minimum transmission	0.6697 and 0.5734
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4449 / 52 / 401
Goodness-of-fit on F^2	1.063
Final R indices [I>2σ(I)]	R1 = 0.0251[a], wR2 = 0.0656[b,c]
R indices (all data)	R1 = 0.0287[a], wR2 = 0.0681[b,c]
Largest diff. peak and hole ($eÅ^{-3}$)	0.569 and -0.320

[a] $R_1 = \sum(|F_O| - |F_C|)/\sum|F_O|$.

[b] $wR_2 = \{\sum[w(F_O^2 - F_C^2)^2]/\sum[w(F_O^2)^2]\}^{1/2}$.

[c] $w = 1/[\sigma^2(F_O^2)+(aP)^2 + bP]$ with $P = [Fo^2 + 2F_c^2]/3$, a = 0.0612 and b = 0.24.

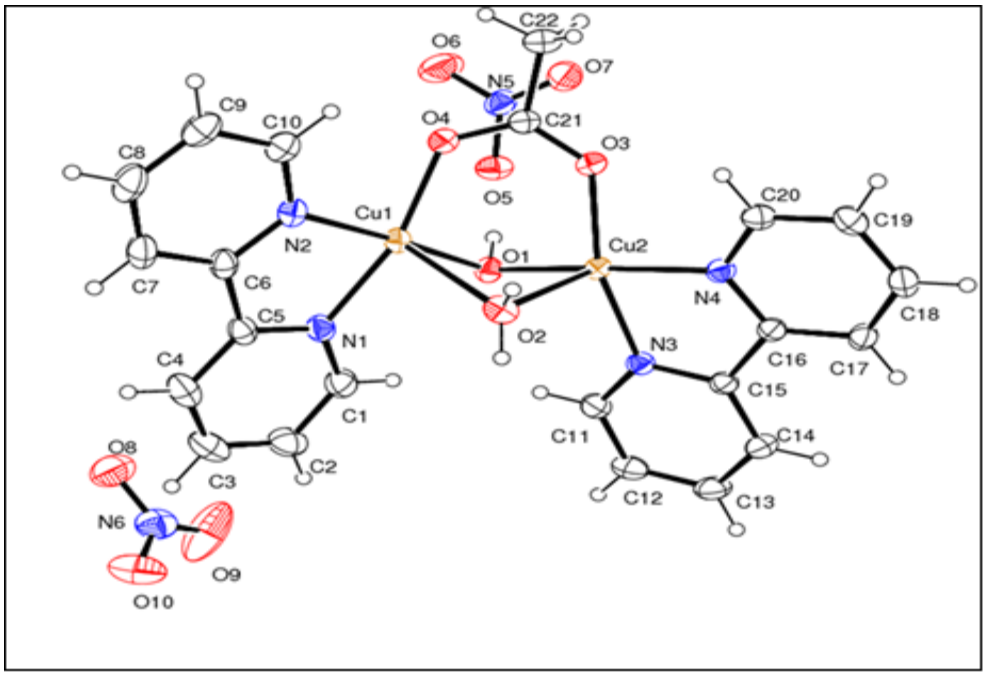

Fig . 2 ORTEP view of $[Cu_2(bipy)_2(OH)(H_2O)(\mu\text{-}CH_3COO)_2]_2.(NO_3)_2$

Dinuclear cation of the complex comprises of two [Cu(bpy)] subunits, which are interconnected through three ligand species viz. hydroxide, acetate and aquo bridges. The copper center is penta coordinate and it has square pyramidal geometry with the coordination of two nitrogen atoms of bpy and three oxygen atom from three bridging ligands. The average bond lengths of the square pyramidal coordination sphere are Cu–N(bpy) 1.99 Å, Cu–O(hydroxide) 1.91 Å, Cu–O(acetato) 1.93 Å, and Cu–O (aquo) 2.32 Å respectively. The Cu–O (aquo) bonds are longer than other two Cu-O bonds. The distance between the two copper atoms is 3.02 Å. The bridging angles of Cu-O-Cu (hydroxide) and Cu-O-Cu (aquo) are 37.99(5) and 52.40(4) respectively. Selected bond lengths and selected bond angles are presented in **Table 3 & 4**. Hydrogen bonding data are listed in **Table 5**. Thus the single crystal x-ray diffraction analysis confirms the synthesis triply bridged dinuclear copper (II) complex.

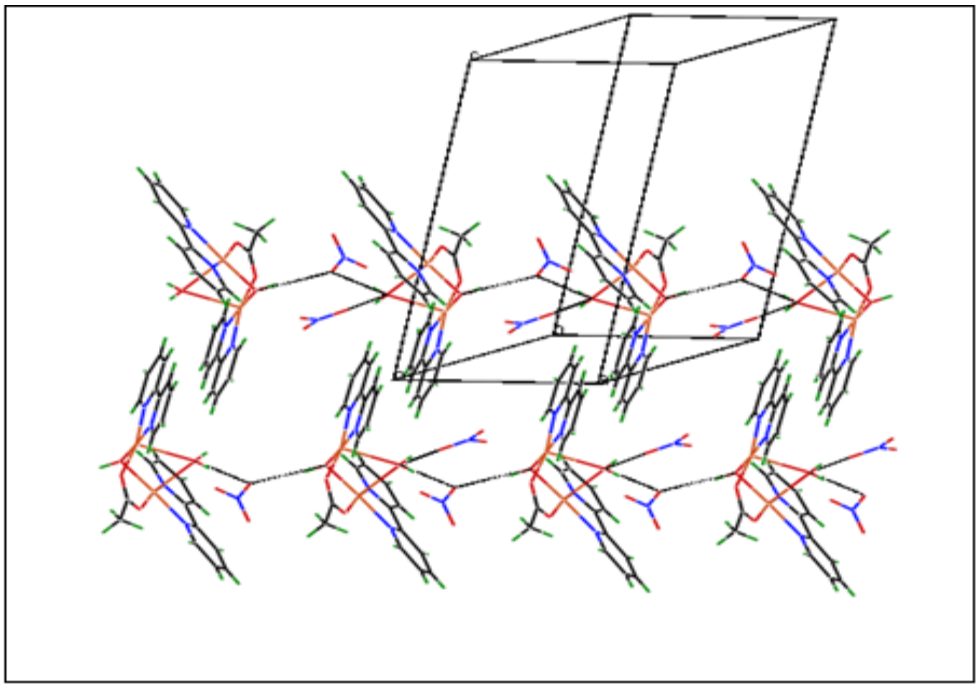

Fig . 3 Packing of the dinuclear complex in the unit cell viewed along 'a' axis

Table 3. Selected Bond lengths in $[Cu_2(bpy)_2(\mu\text{-}OH)(\mu\text{-}H_2O)(\mu\text{-}OAc)](NO_3)_2$ (2).

O(1)-Cu(1)	1.9155(15)	
	O(1)-Cu(2)	1.9206(15)
	O(1)-H(1A)	0.848(18)
	O(2)-Cu(2)	2.3262(16)
	O(2)-H(2A)	0.860(17)
	O(2)-H(2B)	0.855(17)
	O(3)-Cu(2)	1.9425(16)
	O(4)-Cu(1)	1.9311(16)
	Cu(1)-Cu(2)	3.0210(4)
N(1)-Cu(1)	1.9928(19)	
	N(2)-Cu(1)	1.9953(19)
	N(3)-Cu(2)	1.9979(17)
	N(4)-Cu(2)	1.9981(18)

Table 4. Selected bond angles in $[Cu_2(bpy)_2(\mu\text{-}OH)(\mu\text{-}H_2O)(\mu\text{-}OAc)](NO_3)_2$ (2).

Cu(1)-O(1)-Cu(2)	103.91(7)
Cu(1)-O(1)-H(1A)	108(2)
Cu(2)-O(1)-H(1A)	107(2)
Cu(2)-O(2)-H(2A)	113.4(19)
Cu(2)-O(2)-H(2B)	109.7(19)

H(2A)-O(2)-H(2B)	112(2)
C(21)-O(3)-Cu(2)	130.73(16)
C(21)-O(4)-Cu(1)	126.95(15)
O(1)-Cu(1)-O(4)	93.92(7)
O(1)-Cu(1)-N(1)	95.70(7)
O(4)-Cu(1)-N(1)	164.85(8)
O(1)-Cu(1)-N(2)	176.18(7)
O(4)-Cu(1)-N(2)	89.10(8)
N(1)-Cu(1)-N(2)	80.87(8)
O(1)-Cu(1)-Cu(2)	38.10(5)
O(4)-Cu(1)-Cu(2)	79.34(5)
N(1)-Cu(1)-Cu(2)	115.24(6)
N(2)-Cu(1)-Cu(2)	145.19(6)
O(1)-Cu(2)-O(3)	95.41(7)
O(1)-Cu(2)-N(3)	95.05(7)
O(3)-Cu(2)-N(3)	162.20(7)
O(1)-Cu(2)-N(4)	174.72(7)
O(3)-Cu(2)-N(4)	87.81(7)
N(3)-Cu(2)-N(4)	80.80(7)
O(1)-Cu(2)-O(2)	81.75(6)
O(3)-Cu(2)-O(2)	95.78(7)
N(3)-Cu(2)-O(2)	99.95(7)
N(4)-Cu(2)-O(2)	102.12(6)
O(1)-Cu(2)-Cu(1)	37.99(5)
O(3)-Cu(2)-Cu(1)	76.45(5)
N(3)-Cu(2)-Cu(1)	120.02(5)
N(4)-Cu(2)-Cu(1)	147.25(5)
O(2)-Cu(2)-Cu(1)	52.40(4)

Table 5. Hydrogen bonding data in complex **2**

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(1)-H(1)...O(1)	0.93	2.56	3.069(3)	115.0
C(1)-H(1)...O(9)#1	0.93	2.61	3.369(5)	138.9
C(2)-H(2)...O(10')	0.93	2.56	3.200(9)	126.7
C(7)-H(7)...O(5)#2	0.93	2.65	3.376(3)	136.0
C(10)-H(10)...O(4)	0.93	2.38	2.884(3)	114.1
C(11)-H(11)...O(1)	0.93	2.56	3.070(3)	114.7
C(11)-H(11)...O(9')#1	0.93	2.59	3.299(11)	133.7
C(17)-H(17)...O(5)#3	0.93	2.57	3.216(3)	127.3
C(17)-H(17)...O(7)#3	0.93	2.51	3.426(3)	167.3
C(19)-H(19)...O(8')#4	0.93	2.40	3.116(12)	133.8
C(20)-H(20)...O(3)	0.93	2.38	2.864(3)	112.4
O(2)-H(2A)...N(5)#5	0.860(17)	2.549(17)	3.386(3)	165(2)

O(2)-H(2A)...O(5)#5	0.860(17)	1.876(19)	2.716(2)	165(3)
O(2)-H(2B)...O(10)#6	0.855(17)	1.961(19)	2.777(5)	159(3)
O(2)-H(2B)...O(10')#6	0.855(17)	1.85(2)	2.691(8)	166(3)
O(1)-H(1A)...O(5)	0.848(18)	2.076(19)	2.920(2)	173(3)

Symmetry transformations used to generate equivalent atoms:
#1 -x+3,-y,-z #2 -x+2,-y+1,-z #3 -x+2,-y,-z+1
#4 x-1,y,z+1 #5 x-1,y,z #6 -x+2,-y,-z

Electrochemical studies

Redox behaviour of complexes has been investigated by cyclic voltammetry in DMF using 0.1M tetrabutylammonium hexafluorophosphate as supporting electrolyte. Table 6 gives the electrochemical data obtained at the glassy carbon electrode in DMF. **Fig. 4** shows the profile of complex 2 at 25 – 75 mVs^{-1} scan rates. The cathodic peak current function values were found to be independent of the scan rate. The non-equivalent current intensity of the cathodic and anodic peaks [ic/ia = 0.945 (for complex **1**) and 0.779 (for complex **2**)] indicates quasi-reversible behavior[19], in reduction. The complexes have large separation (153–359 mV) between the anodic and cathodic peaks. The difference ΔEp = Epc - Epa for complexes exceeds the Nernstian requirement 59/n mV (n = number of electrons involved in the redox process) which suggests quasi-reversible character[20]

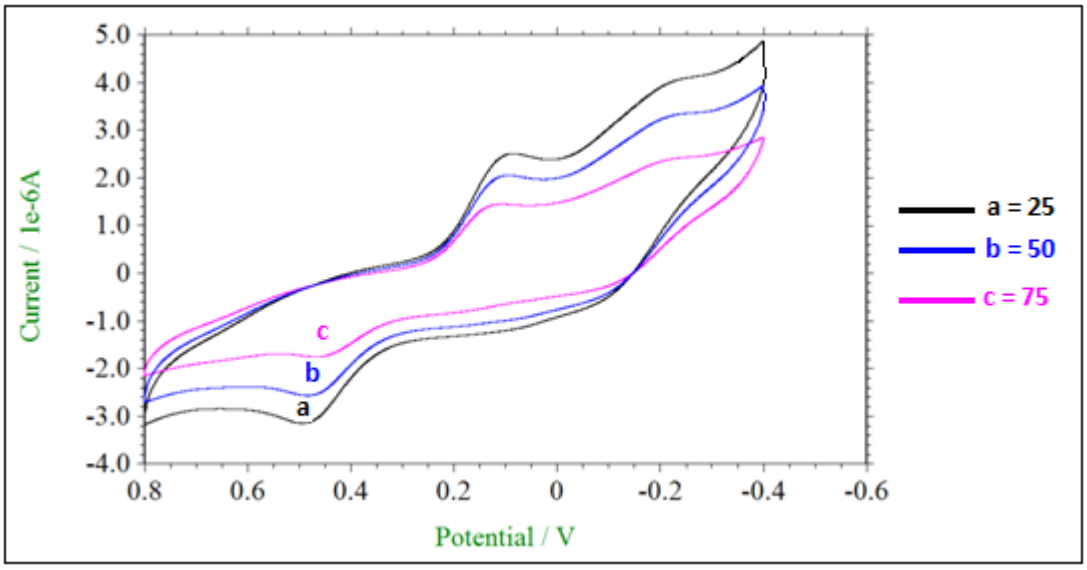

Fig 4: Cyclic voltammetric profile of $[Cu_2(bipy)_2(OH)(H_2O)(\mu\text{-}CH_3COO)_2]_2.(NO_3)_2$ complex

Electronic absorption titrations

The binding interactions of the complexes with CT-DNA were monitored by comparing their absorption spectra with and without CT-DNA. With increasing DNA amounts, the hypochromism of π - π* absorption band increased upto +20.25% per complexes, together with red shift of 2 nm indicating the binding of the complexes to DNA. Figure 5 shows absorption spectra of complex **2** in the presence of increasing amounts of DNA. The binding of an intercalative molecule to DNA is generally characterized by large hypochromism and significant red shift due to strong stacking interactions between the aromatic

chromophore of the ligand and DNA base pairs, with the extent of hypochromism and red shift commonly consistent with the strength of intercalative interaction[21, 22].

Table 6 . Cyclic voltammetric data of copper(II) complexes

S.no	Complex	Redox couple	C_V cathodic E_{pc}	C_v anodic E_{pa}	Δ_{EP} (mv)	$E_{1/2}$	i_c/i_a	Log K_{C_b}	$-\Delta G^0$
1.	$[Cu(bipy)(H_2O)(NO_3)_2]$	II/I	0.281	0.128	153	0.268	0.945	0.0823	473
2.	$[Cu(bipy)_2(OH)(H_2O)(\mu\text{-}CH_3COO)_2]_2.[NO_3]_2$	II/I	0.480	0.121	359	0.301	0.779	0.0933	535

However, in the present case, the magnitude of hypochromism and red shift observed for the copper complexes are lower than those observed for typical classical intercalators or partially intercalating complexes. To enable quantitative comparison of DNA binding affinities, the intrinsic binding constants Kb of the complexes for binding were obtained using Eq. 1. Electronic absorption spectral data upon addition of CT-DNA and binding constants of these complexes are given in the **Table 7**. The K_b value for the complex **2** is high, probably due to the strong electrostatic attraction between the cationic complex (**2**) and the negatively charged phosphodiester backbone of DNA[17]. Since the complexes are bulky and lack planarity, groove binding[23], of the complexes with DNA is suggested (rather than base pair intercalation). We predict groove binding of complex to DNA via hydrophobic interactions involving the phenyl groups of the complex and/or electrostatic interactions between the DNA and complex.

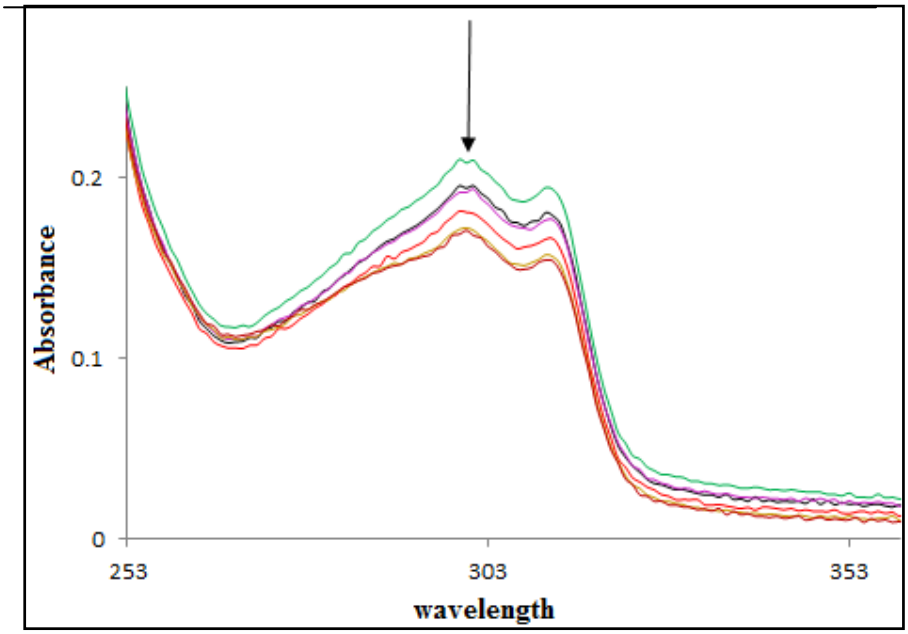

Fig 5: Absorption spectra of **$Cu_2(bpy)_2(\mu\text{-}OH)(\mu\text{-}H_2O)(\mu\text{-}OAc)](NO_3)_2$ (Complex 2)** in the absence and in the presence of increasing amounts of CT-DNA.

Table 7. Electronic absorption data upon addition of CT-DNA to the complex

S.No.	Complex	λ max. (nm)		Δλ/nm	H%	K_b (M^{-1})
		Free	Bound			
1.	[Cu (bipy)(H_2O)(NO_3)$_2$]	312	313	1	+1.96	3.92×10^6
2.	[Cu bipy (OH)(H_2O)(μ-CH_3COO)]$_2$. [NO_3]$_2$	297	298	1	+20.25	7.73×10^5

Nuclease activity

Nuclease activity of complexes **1** and **2** has been studied by agarose gel electrophoresis using pBR322 plasmid DNA in *Tris*-HCl/ NaCl (50mM/ 5mM) buffer (pH, 7) in the presence and absence of H_2O_2 after 30 minutes incubation period at 37°C [26, 28]. Nuclease activity of complexes was also investigated in presence of free radical scavenger (DMSO) and reducing agent DTT. DNA cleavage activity of complexes was studied at different concentrations. It was found that there is a nominal effect of concentration. Even at lower concentrations the complexes show much nuclease activity. The percentage of the three forms of DNA is presented in the **Table S2 & S3.** The decrease in percentage of supercoiled form of DNA may be considered to estimate the cleavage activity of complex**.** In the absence of H_2O_2 the complexes cleaved supercoiled DNA (Form 1) into nicked DNA (Form II) only. From **Figures 6 & 7 and** data from **Table S2 & S3**, it is evident that copper complexes cleave DNA more effectively in the presence of oxidant indicating that the Cu(II) complex may be reduced by the peroxide to produce hydroperoxo species. Lane 5 of Fig 6 and lanes 5 & 7 of Fig 7 are almost invisible. It indicates that the DNA is completely degraded by the complex in presence of the oxidant. The hydroxyl free radical formed in the second step leads to DNA damage. This is consistent with the production of hydroxyl radicals by cuprous ions similar to the well known Fenton reaction[24]

$$Cu(II) + H_2O_2 \rightleftharpoons Cu\,(I) + \dot{O}OH + H^+$$

$$Cu\,(I) + H_2O_2 \rightleftharpoons Cu(II) + \dot{O}H + {}^{-}OH$$

These hydroxyl radicals participate in the oxidation of the deoxyribose (sugar) moiety. In presence of free radical scavenger (DMSO) nuclease activity of copper complexes is diminished whereas the reducing agent DTT enhances the cleavage activity of copper complexes. This may be due to formation of copper(I) complex by catalytic reduction which causes the production of more hydroxyl radicals which may support the oxidative cleavage.

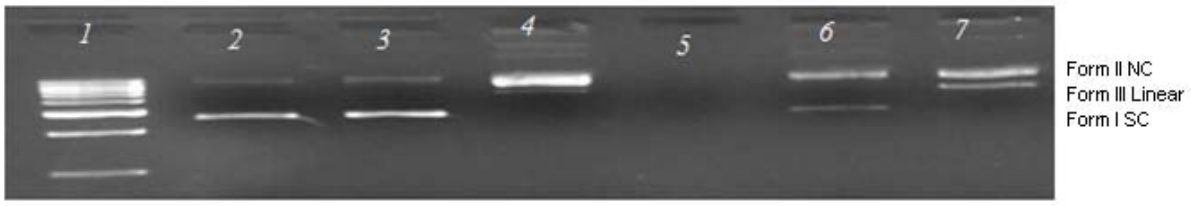

Fig 6: DNA Cleavage of Complex 1:
Lane 1: DNA ladder; Lane 2: DNA Control; Lane 3: DNA + H_2O_2; Lane 4: DNA+ Complex (150μM) ; Lane 5: DNA + Complex + H_2O_2 ; Lane 6: DNA + Complex + DMSO; Lane 7: DNA + Complex + DTT

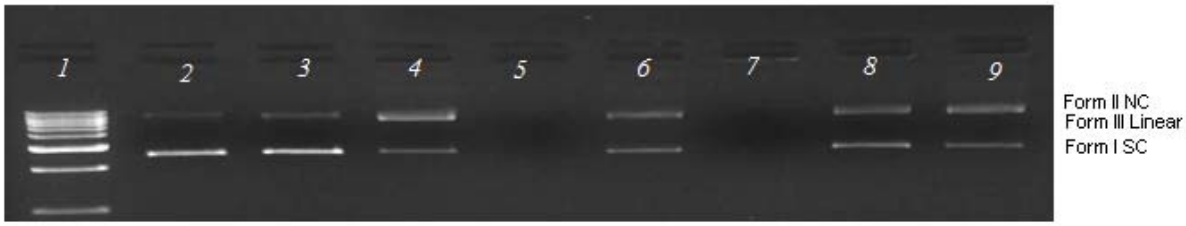

Fig 7: DNA Cleavage of Complex 2:

Lane 1: 1kb DNA ladder ; Lane2: DNA Control ; Lane 3: DNA + H_2O_2 ; Lane 4: DNA+ Complex(100μM) ; Lane 5: DNA + Complex + H_2O_2 ; Lane 6: DNA + Complex(150 μM); Lane 7: DNA + Complex + H_2O_2 ; Lane 8: DNA + Complex + DMSO ; Lane 9: DNA + Complex + DTT.

Conclusions

In the present studies a new triply bridged dinuclear complex is structurally characterized by using single crystal X-Ray diffraction studies for the first time. The dinuclear complex has positive charge and DNA is polyanion. We predict that the dinuclear complex binds DNA via strong electrostatic attraction between the cationic complex (**2**) and negatively charged phosphodiester backbone of DNA. The complexes exhibit DNA cleavage activity even in the absence of oxidant, but this activity becomes more pronounced in the presence of an oxidant.

Supplementary material

CCDC 1029633 contains the supplementary crystallographic data for Cu complex. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary information is available at www.ias.ac.in/chemsci.

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