DNA binding and cleavage activity of a structurally characterized oxobridged diiron(III) complex

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Received 1 January 2013; revised and accepted 12 November 2013

The synthesis, crystal structure and variable temperature magnetic measurement of an oxobridged dinuclear Fe(III) complex \([\text{Fe}_2\text{O}(\text{phen})_2\text{Cl}_2\text{]}(\text{NO}_3)_2\) (I) \([\text{phen}=1,10\text{-phenanthroline}]\) is reported. From X-ray diffractometry, it is shown that each Fe(III) in the compound is in distorted octahedral environment. Variable temperature magnetic study reveals strong antiferromagnetic behaviour of (I) with \(\chi = -104\times10^{-6}\ \text{cm}^3\text{mol}^{-1}\) at \(T = 298\text{K}\). The binding property of the complex with calf thymus DNA has been investigated using absorption and emission studies, circular dichroism spectral investigations, thermal melting and viscosity experiments. The binding constant and the linear Stern-Volmer quenching constant of the complex have been determined to be \(1.49\times10^3\ \text{M}^{-1}\) and \(2.02\times10^3\ \text{M}^{-1}\), respectively. Spectroscopic and hydrodynamic investigations reveal a mixed mode involving groove binding along with partial intercalative interaction of (I) with CT-DNA. (I) is found to induce oxidative cleavage of the DNA from its supercoiled to both nicked circular and linear forms in a concentration-dependent manner.

**Keywords:** Coordination chemistry, Iron, Dinuclear compounds, Oxobridged diiron(III) complex, Crystal structure, DNA binding, DNA cleavage

Interaction between small molecules and DNA has emerged as an area of research being widely investigated\textsuperscript{1-7}. These interactions play a central role to define the local structure of nucleic acids as well as to design novel drug, chemotherapeutic agents, etc. Another aspect which makes the polynuclear iron complexes enticing is their relevance to a large number of non-heme iron proteins, such as ribonucleotide reductase, which converts ribonucleotides into deoxyribonucleotides in the first step in the biosynthesis of DNA\textsuperscript{8,9}. Metal complexes can bind to DNA through a series of interactions like coordination by the DNA bases, intercalation and non-covalent interaction including hydrogen bonding between the coordinated ligands and phosphate oxygen atoms of the sugar-phosphate group\textsuperscript{10,11}. Binding may induce cleavage of nucleic acids which may be considered as an enzymatic reaction comprising various biological processes as well as biotechnological manipulation of genetic materials. The application of artificial DNA cleaving agents is many fold: biotechnological applications, structural studies of nucleic acids, development of new drugs, etc\textsuperscript{1,3,12-14}. Among the various DNA binding small molecules, iron complexes with 1,10-phenanthroline (phen) as ligand are of particular importance because these complexes can bind to DNA in different modes\textsuperscript{15-19}. It is found that dinuclear iron-phen compounds interact more effectively with DNA than the mononuclear ones\textsuperscript{20}. Several studies addressing the synthesis of mononuclear iron-phen complexes and their interaction with DNA have been reported\textsuperscript{16-20}. Compared to the mononuclear complexes, reports on dinuclear complexes of iron-phen and their interaction with DNA has been paid less attention\textsuperscript{21}. In continuation to our recent\textsuperscript{22} work with mononuclear iron(II) complex, herein we report the synthesis, X-ray structural data and magnetochemical characterizations of an oxobridged dinuclear Fe(III) complex \([\text{Fe}_2\text{O}(\text{phen})_2\text{Cl}_2\text{]}(\text{NO}_3)_2\) (I) \([\text{phen}=1,10\text{-phenanthroline}]\). We also present the
DNA binding characteristics of this metal complex from different physicochemical studies along with its DNA cleavage activity.

Materials and Methods

High purity 1,10-phenanthroline (E. Merck, India), anhydrous iron(III) chloride and all other solvents were purchased and used as received. The calf thymus (CT) DNA and ethidium bromide (EB) were obtained from Sigma. All other chemicals and solvents were of analytical grade and were used as received without further purification. Agarose and ethidium bromide were purchased from Sigma Chemicals. pUC 18 was further purified. Agarose and ethidium bromide of analytical grade and were used as received without

...was collected on polycrystalline samples with a Quantum Design MPMS-XL SQUID magnetometer equipped with a 7 T magnet. The diamagnetic corrections were evaluated from Pascal’s constants. The fit was performed minimizing the function $R$ (agreement factor defined as $\Sigma [(\chi_M T)_{\text{exptl}} - (\chi_M T)_{\text{calcd}}]^2 / \Sigma (\chi_M T)_{\text{exptl}}^2$). Ground state absorption was measured with a Jasco V-530 UV-vis spectrophotometer. The fluorescence spectra were recorded on Hitachi-2000 fluorimeter. Absorbance versus temperature profiles (optical melting curves) of DNA and alkaloid-DNA complexes were measured on a Shimadzu Pharmaspec 1700 unit (Shimadzu, Kyoto, Japan) equipped with the peltier controlled accessory (model TMSPC-8) in an eight chambered quartz cuvette of 1 cm path length. The temperature was ramped from 40–100 °C at a scan rate of 0.5 °C/min, monitoring the absorbance changes at 260 nm. The viscosity of the DNA-metal complex was determined by measuring the time needed to flow through a Cannon-Manning semimicro size 75 capillary viscometer (Cannon Instruments Company, USA) that was submerged in a thermostated water bath (20±1 °C). Flow times were measured in triplicate to an accuracy of ±0.01 s with a Casio electronic stopwatch (Model HS-30W) (Casio Computer Co. Ltd., Tokyo, Japan).

The circular dichroism (CD) spectra were recorded on a Jasco J815 spectropolarimeter (Jasco International Co. Ltd, Hachioji, Japan) equipped with a Jasco temperature controller (model PFD 425L/15) interfaced with a HP PC at 20±0.5 °C using the instrument parameters reported previously.

Synthesis of [Fe$_2$(phen)$_2$Cl$_2$(NO$_3$)$_2$] (1)

1,10-Phenanthroline (0.09912 g, 0.5 mmol) was added to a stirring solution of anhydrous FeCl$_3$ (0.0821 g, 0.5 mmol) in 20 mL acetic acid-water (60:40) mixture and then ammonium ceric nitrate (0.5480 g, 1.0 mmol) in solid state was added to the above solution with continuous stirring on a magnetic stirrer. The solution was kept on a magnetic stirrer for about 30 min and then filtered. The filtrate was kept for slow evaporation in open air. After about two weeks brown small crystals were obtained. Yield: 0.8 34 g (40%); Anal. (%) Calc. for Ca$_4$H$_2$N$_{12}$O$_{13}$Cl$_2$Fe$_2$: C, 55.23; H, 3.09; N, 10.72. Found: C, 55.16; H, 3.14; N, 10.62. IR (KBr pellet):1384 (v$_{NO3}$), 852 (v$_{ox}$) cm$^{-1}$. UV-vis ($\lambda$, nm): 232, 318.

X-ray diffraction study

Single crystals of (1) suitable for X-ray crystallographic analysis were selected following examination under a microscope. Diffraction data at 150 K for (1) were collected on a Bruker P4 CCD diffractometer using Mo-K$\alpha$ radiation ($\lambda$=0.71073 Å). The crystal data and data collection parameters are listed in Table 1. The compound was identified as

Table 1 – Crystal data and structure refinement for (1)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emperical formula</td>
<td>Ca$<em>4$H$<em>2$N$</em>{12}$O$</em>{13}$Cl$_2$Fe$_2$</td>
</tr>
<tr>
<td>Formula wt</td>
<td>1167.46</td>
</tr>
<tr>
<td>Temp. (K)</td>
<td>150</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pbn</td>
</tr>
<tr>
<td>a (Å)</td>
<td>10.619(2)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>17.975(4)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>26.788(6)</td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
<td>89.938(7)</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>89.998(6)</td>
</tr>
<tr>
<td>$\gamma$ (°)</td>
<td>90.074(6)</td>
</tr>
<tr>
<td>Vol. (Å$^3$)</td>
<td>5113.2(18)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>$\rho$ (g cm$^{-3}$)</td>
<td>1.517</td>
</tr>
<tr>
<td>$\mu$ (mm$^{-1}$)</td>
<td>5.380</td>
</tr>
<tr>
<td>F(000)</td>
<td>4225</td>
</tr>
<tr>
<td>Crystal size (mm)</td>
<td>0.20 × 0.14 × 0.09</td>
</tr>
<tr>
<td>$R$ (reflections)</td>
<td>0.0532 (1333)</td>
</tr>
<tr>
<td>wR2 (reflections)</td>
<td>0.1344 (1639)</td>
</tr>
</tbody>
</table>
of \textit{Pbcn} space group. Of the 1639 unique reflections for the complex, 1333 with $I>2\sigma(I)$ was used for structure solution. The structure was solved by direct method, and the structure solution and refinement were based on $|F|^2$. The final differences Fourier map showed the maximum and minimum peak heights at -0.31 and 0.53 eÅ$^{-3}$ for (1) with no chemical significance. All calculations were carried out using SHELXL-97$^{24}$ and ORTEP-32$^{25}$.

**DNA cleavage**

DNA cleavage by (1) was monitored by agarose gel electrophoretic technique. The cleavage efficiency of DNA as a function of metal complex concentration was examined by their ability to convert supercoiled DNA (Form I) to open circular (Form II) and linear forms (Form III). The cleavage experiments were carried out in the absence and presence of activating agent hydrogen peroxide. (1) (20 µM, 40 µM, 60 µM and 80 µM) was incubated with pUC 18 in the absence and presence of hydrogen peroxide (2 µM) for 2 h at 37 ºC. A loading buffer containing 0.25% bromophenol blue, 40% (w/v) sucrose and 0.5 M EDTA was added and the electrophoresis of the DNA cleavage products was performed on agarose gel containing ethidium bromide. The gels were run at 100 V for 1 h in tris-boric acid-ethylenediamine tetra acetic acid (TBE) buffer at pH 7.4. The cleavage of DNA was monitored using 0.8% agarose gel electrophoresis containing 0.5 µg/mL ethidium bromide. The bands were viewed by placing the gel on UV illuminator and were photographed using gel documentation system.

**Results and Discussion**

**Description of crystal structure**

An Oak Ridge Thermal Ellipsoid Plot (ORTEP) representation of (1) is shown in Fig. 1. The coordination geometry around each iron(III) center in [Fe$_2$O(phen)$_4$Cl$_2$(NO$_3$)$_2$] (1) is best described as distorted octahedron with FeN$_4$OCl chromophore. The four N (N1, N2, N3 and N4) atoms are from two phenanthroline units. Considering bond angle and bond distance data (Table 2) it is assumed that one of the polypyridinic N atoms (N3) and

![Fig. 1](image)

**Table 2 – Selected bond lengths (Å) and bond angles (°) for (1)**

<table>
<thead>
<tr>
<th>Bond distances (Å)</th>
<th>Bond angles (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(1)-O(7) 1.7769(14) Fe(1)-N(2) 2.190(7)</td>
<td>O(7)-Fe(1)-N(4) 94.5(2) N(1)-Fe(1)-N(3) 90.1(2)</td>
</tr>
<tr>
<td>Fe(1)-N(4) 2.140(7) Fe(1)-N(3) 2.243(7)</td>
<td>O(7)-Fe(1)-N(2) 92.9(3) N(2)-Fe(1)-N(3) 79.0(2)</td>
</tr>
<tr>
<td>Fe(1)-N(1) 2.173(6) Fe(1)-Cl(1) 2.313(2)</td>
<td>N(4)-Fe(1)-N(1) 161.9(3) O(7)-Fe(1)-Cl(1) 102.14(19)</td>
</tr>
<tr>
<td>Fe(1)-N(2) 2.190(7) Fe(1)-Cl(1) 2.313(2)</td>
<td>O(7)-Fe(1)-N(2) 92.9(3) N(4)-Fe(1)-Cl(1) 93.77(18)</td>
</tr>
<tr>
<td>Fe(1)-N(3) 2.243(7) Fe(1)-Cl(1) 2.313(2)</td>
<td>N(4)-Fe(1)-N(2) 92.7(3) N(1)-Fe(1)-Cl(1) 94.4(2)</td>
</tr>
<tr>
<td>Fe(1)-N(4) 2.140(7) Fe(1)-Cl(1) 2.313(2)</td>
<td>N(1)-Fe(1)-N(2) 75.3(3) N(2)-Fe(1)-Cl(1) 163.10(19)</td>
</tr>
<tr>
<td>N(2)-Fe(1)-N(3) 90.1(2)</td>
<td>O(7)-Fe(1)-N(3) 165.53(19) N(3)-Fe(1)-Cl(1) 87.76(17)</td>
</tr>
<tr>
<td>N(3)-Fe(1)-Cl(1) 166.01(1)</td>
<td>N(3)-Fe(1)-N(3) 74.1(3)</td>
</tr>
</tbody>
</table>
the bridging O atoms are in the axial part and the remaining N1, N2, N4 and Cl1 are in the equatorial plane. The Fe-N/O/Cl bond distances range are in the 2.313(2)-1.7769(14) Å and the difference Δ between the longest and shortest bond is found to be 0.537 Å.

Magnetic properties

The magnetic susceptibility of (I) decreased linearly (Fig. 2) from 0.93 cm$^3$ mol$^{-1}$ K at 300 K until 50 K approximately, reaching a plateau at a value of 0.05 cm$^3$ mol$^{-1}$ K and dropping softly till 0.02 cm$^3$ mol$^{-1}$ K at 2.0 K. The shape of the graph indicates strong antiferromagnetic coupling between the two high spin Fe(III) ions, as has been observed elsewhere$^{14-20}$. Experimental data were fitted using the van Vleck equation for a dinuclear $S = 5/2$ system and the spin Hamiltonian $H = -2JS_1 S_2$. Paramagnetic impurities were added into the equation since they appear to be common in this type of compounds causing the slight variations from absolute zero of $\chi_M T$ at the lowest temperatures. The best fit of the data (red line in Fig. 2) was obtained for $J = -104\pm2$ cm$^{-1}$, $p = 0.8\%$ and $TIP = 280\times10^{-6}$ cm$^{-1}$, fixing $g = 2.00$. Generally, the exchange coupling expected for µ-oxo diiron(III) complexes is in the range$^{26}$ of $-90$ to $-110$ cm$^{-1}$. In addition, this data match perfectly with the value of $J$ obtained from the angular and radial overlap model of Weihe and Güdel$^{26,27}$ ($-105.3$ cm$^{-1}$). The correlation between $J$ and Fe-O-Fe angle is well established$^{28}$. The oxobridge angle in (I) and its $J$ value are in good conformity with the literature values$^{29}$.

DNA binding studies

A broad spectrum in the range 250–280 nm is shown in the UV-vis spectrum of the complex (Fig. 3). The higher energy band in the spectrum is indicative of oxo-to-Fe(III) charge transfer transition$^{30,31}$. After addition of CT-DNA to the solution of Fe(III) complex in tris-buffer, it is clearly observed that the absorption peak at 267 nm undergoes a significant increase in molecular absorption (hyperchromic effect) with no detectable shift in the absorption wavelength. Intercalative interaction of metal-phenanthroline complexes with DNA is well documented$^{15-20}$. In the case of intercalation, the complex undergoes bathochromism as the $\pi^*$ orbital of the intercalated ligand couple with the $\pi$ orbital of the DNA base pairs, thus lowering the $\pi-\pi^*$ transition energy$^{32}$. No detectable changes in the energy of the ligand field bands of the complex were observed, ruling out the possibility of binding of these complexes with DNA bases$^{7,33}$. As no bathochromism is observed, the possibility of intercalation is excluded, and more indicative of groove binding mode$^{33}$. Groove binding molecules contain unfused aromatic ring systems connected by bonds with torsional freedom in order to adopt appropriate conformation that closely matches the helical turn of DNA grooves, while fused polyaromatic systems containing protonated nitrogen atoms or having protonated side chains attached to the ring system are typically intercalators$^{34}$. As the present compound
contains fused aromatic heterocycles in the ligand framework, partial intercalation of these molecules cannot be ruled out.32

The binding constant, $K_b$ for the complex has been determined from the linear plot of $[\text{DNA}] / (\epsilon_A - \epsilon_F)$ vs $[\text{DNA}]$ and found to be $1.49 \times 10^3 \text{ M}^{-1}$ ($R = 0.99922$ for five points).

The fluorescence spectral method using ethidium bromide (EB) as a reference was used to determine the relative DNA binding properties of the complex to the CT-DNA in tris-buffer (5 mM, pH 8.0). DNA concentrations were determined spectrophotometrically using the reported data35 for a molar absorption coefficient of $6600 \text{ M}^{-1}\text{cm}^{-1}$ at 260 nm. Fluorescence intensities of EB in DNA were measured at different complex concentrations. Reduction in the emission intensity (Fig. 4) was observed with increasing complex concentrations. This result is also in favour of groove binding of the complex to the CT-DNA.36

The relative binding tendency of the complex with the CT-DNA was determined from the comparison of the slope of the lines in the fluorescence intensity versus complex concentration plot.

From the slope of the regression line in the derived plot of $I_0/I$ versus [complex], the Stern-Volmer quenching constant $K_{sv}$ value for the complex was found to be $2.02 \times 10^3$; ($R = 0.99917$ for five points) indicating a strong affinity of the complexes to CT-DNA.

The binding was further tested from optical thermal melting studies.35 Double stranded CT-DNA under the conditions of the experiment had a melting temperature ($T_m$) of 78 °C (Fig. 5). The melting temperature of the native DNA enhanced in the presence of the complex and at saturation the increase in melting temperature value of 6 °C was observed indicating high stabilization of the DNA helix with (1) is essentially due to the strong binding of (1) with the DNA either in the grooves or by intercalation.

To further probe the binding mode, viscosity of the DNA solution was measured in presence of increasing concentrations of (1) and the change in relative viscosities with varying inputs of (1) was estimated.38 The relative specific viscosity of the CT-DNA–I complex increased slowly but steadily as the $D/P$ (drug/DNA molar ratio) increased and ultimately attained saturation at $D/P = 0.6$ (Fig. 6), suggesting the partial intercalation of the diiron complex into the double helical organization of the CT DNA.

**Circular dichroism studies**

The CD spectra of the DNA duplex displayed a canonical B-form conformation characterized by a positive band in the 280 nm and a negative band around 246 nm. These bands are caused due to the stacking interactions between the base pairs and the helical structure of the duplex that provide

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**Fig. 4** – Emission spectrum of the CT-DNA-EB system in tris–HCl buffer based on the titration of the title complex. $[\epsilon_{\text{ex}} = 522 \text{ nm}; [\text{DNA}] = 1.0 \times 10^{-5}; [\text{Complex}]: (a) 0.0; (b) 1.10 \times 10^{-5}; (c) 2.04 \times 10^{-5}; (d) 3.06 \times 10^{-5}; (e) 4.08 \times 10^{-5}; (f) 5.1 \times 10^{-5} \text{ mol L}^{-1}$. The arrow indicates the increase of the complex concentration.

**Fig. 5** – Optical thermal melting profiles of CT DNA (●), and, CT-DNA-I (▪).
asymmetric environment for the bases. To record the diiron complex induced changes in the DNA conformation, the CD spectra in the 225–350 nm regions were recorded (Fig. 7) in presence of increasing concentration of diiron complex. The intrinsic CD contribution of the ligand was subtracted in each case. The ellipticity of the long wavelength positive band decreased in ellipticity as the interaction progressed with a slight red shift in the wavelength maximum. Moderate changes were also observed in the negative CD band at 246 nm. Besides, as the interaction progressed a positive peak gradually developed at the 262 nm region.

DNA cleavage

(1), when incubated with plasmid DNA, did not bring about any DNA cleavage as can be seen from the Fig. 8 (lane b). In the presence of external additive, viz., hydrogen peroxide, the complex brought about DNA cleavage in a concentration-dependent manner (lanes c-f). Results show that (1) brought about oxidative cleavage of DNA. With increasing concentration of (1) (20 μM, 40 μM and 60 μM), the supercoiled (SC) form of DNA (Form I) was converted into the nicked circular (NC) form (Form II) and a small amount of linear form of DNA (Form III). With further increase in the concentration of the complex (80 μM) both Form II and Form III of DNA were seen clearly. In the presence of DMSO, a scavenger for hydroxyl radical, the DNA cleavage by the metal complex and hydrogen peroxide was inhibited (lane g). This confirms the oxidative cleavage of DNA by the Fe(III) complex (1) where the hydroxyl radical plays the role of reactive oxygen.

Conclusions

In the present investigation, we have reported the synthesis, single crystal X-ray structural characterization and variable temperature magnetic behavior of an oxobridged diiron(III) complex with 1,10-phenanthroline as ligand. Interaction of the compound with CT-DNA has also been reported. The binding constant for the complex is found to be 1.49×10^3 M⁻¹. The linear linear Stern-Volmer quenching constant was determined as 2.02×10^3.
(R = 0.99917 for five points). The diiron complex (1) enhanced the thermal stabilization of native DNA by 6 °C, clearly suggesting strong stabilization effects. Viscosity measurements provided evidence for partial intercalation of (1) into the DNA double helix. Conformational changes of DNA within the B-form induced by the diiron complex further testified to the interaction of the diiron complex with double stranded DNA. The present studies indicate a mixed mode of interaction involving a groove binding along with partial intercalative interaction. Furthermore, (1) was found to cleave DNA in an oxidative manner to both of its nicked circular and linear forms. With increasing concentration of the metal complex, DNA was cleaved up to 75% of the former (nicked circular) and 25% of the latter (linear) forms.

Supplementary Data
CCDC 757110 contains the supplementary crystallographic data for (1). 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 Email: deposit@ccdc.cam.ac.uk.

Acknowledgement
Financial support by the Department of Science & Technology, New Delhi, India (F. No. SR/FT/CS-83/2010 dt. 11-02-2011) is gratefully acknowledged by RG. MM and AB are respectively thankful to The University of Burdwan and the University Grants Commission, Govt. of India, respectively for their fellowships. NAA thanks Ministerio de Educación y Ciencia (CTQ2009-06959/BQU) for the grant. Also, the work performed at the Universitat de Barcelona by NAA was supported by ICREA (Institució Catalana de Recerca i Estudis Avançats).

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