# Nuclease Activity of Oxo-bridged Diiron Complexes†

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The dinuclear oxo-bridged complexes $[Fe(salen)]_2O$, $[Fe(salen)]_2O\cdot X$ (where X = pyridine, perchlorate) cleave plasmid pBR322 DNA in concert with $H_2O_2$.

The design of DNA and RNA specific agents capable of controlled chemical cleavage are of paramount importance due to their potential use as drugs, regulators of gene expression and tools of molecular biology.[1–3] Metal complexes are attractive reagents for the cleavage of nucleic acids due to their inherently diverse structure and reactivity. Among the complexes which cleave DNA through an oxidative pathway are $[Cu(phen)_2]^+$ (phen = 1,10-phenanthroline),[4] $[Fe(edta)]^{2-}$ (edta = ethylenediaminetetraacetic acid),[5] Fe-BLM (BLM = bleomycin),[6] metalloporphyrins,[7] Ru, Rh and Re complexes of polypyridyl ligands,[8] Ni-azamacrocycles,[9] Cu-desferal,[10] $Mn^{III}$-salen [salen = *N*,*N*′-ethylenebis (salicylidene aminato)].[11]

The disadvantage of oxidative cleavage agents is that they produce diffusible radicals which give rise to multiple cleavage sites by modifying the deoxyribose moiety, which results in fragments that cannot be re-ligated. On the other hand, agents that promote the hydrolytic cleavage of the phosphodiester backbone of DNA do not suffer from these drawbacks.

Reagents shown to promote efficient hydrolysis of phosphodiester bonds include $Ce^{III}$ salts,[12] macrocyclic complexes of lanthanides,[13] tetramine complexes[14,15] of $Co^{III}$ and $Ir^{III}$ and copper(II) 1,4,7-triazacyclononane dichloride $[Cu([9]aneN_3)Cl_2]$.[16] Recently bi- and tri-metallic systems such as tetrakis(methyl imidazole)$M^{II}_2$ (M = Cu, Ni, Zn),[17] dinuclear $Mn^{IV}$ complex of 1,4,7-trimethyl-1,4,7-triazacyclononane,[18] trinuclear $Cu^{II}$ complex of 2-aminoethyl amine,[19] and $[Fe_2(HPTB)OHNO_3]$ (HPTB = *N*,*N*,*N*′*N*′-tetrakis-(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane) in the presence of $H_2O_2$[20] have also been shown to lead to hydrolytic cleavage. This motivated us to examine whether multinuclearity can contribute specifically and advantageously to the catalytic cleavage of DNA.

In the present work we report on the nucleolytic activity of some oxo/hydroxo bridged diiron complexes which include $[Fe(salen)]_2O$ **1**; $[Fe(salen)]_2O\cdot 2py$ **2**; $[Fe(salox)_2OH]_2$ **3** and $[Fe(salen)]_2O\cdot ClO_4$ **4** (salox = salicylaldoximate; py = pyridine). These complexes have been advocated as structural analogues of non-heme diiron containing metalloproteins.[21]

From the DNA cleavage experiment shown in Fig. 1 it is observed that at micromolar concentrations, in the presence of $H_2O_2$, all complexes except **3** cleave DNA as evidenced by the disappearance of Form I (supercoiled) of the plasmid and the appearance of a well-defined band for the linear DNA (Form III). It is observed that only the oxo-bridged diiron-(III, III) and -(III, IV) complexes are able to cleave DNA, indicating that the oxo group is involved in the cleavage process. The probable mechanism for the phosphodiester hydrolysis by the present dimers is the attack of the metal bound hydroperoxide nucleophile on the phosphorus giving rise to a trigonal bipyramidal phosphorus intermediate,[22] which finally leads to DNA cleavage. Formation of such peroxo intermediates through the interaction of $[Fe_2O(CH_3CO_2)(mep)_2]$ [mep = *N*,*N*′-dimethyl-*N*,*N*′-bis(2-pyridylmethyl)ethane-1,2-diamine] with $H_2O_2$ has recently been reported by Nishida *et al.*[23] A similar mechanism was proposed by Schnaith *et al.* for $[Fe_2(HPTB)OHNO_3]$ in the presence of $H_2O_2$;[20] while a similar intermediate was proposed by Menage *et al.*[24] for the hydrolysis of phosphodiesters by $[Fe_2O(phen)_4(OH_2)_2(NO_3)_4]$. Complex **3** does not relax supercoiled DNA to the same extent as complexes **1**, **2**

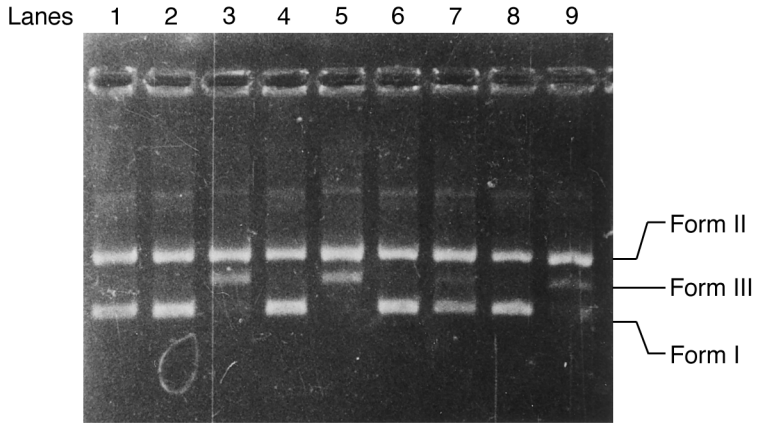


**Fig. 1** 1% agarose gel electrophoresis showing results of plasmid pBR322 DNA (~4.3 kb) cleavage by diiron complexes with (%) formation of Form III (linear) as measured using UVP gel documentation system GDS 2000. *Reaction conditions*: DNA (300 ng); diiron complex (100 μM) in DMF, $H_2O_2$ (18 μM); Incubation time: 37 °C for 30 min. Sterile deionised water was added to make final volume to 20 μl. Lane 1: DNA + $H_2O_2$; Lane 2: DNA + **1**; Lane 3: DNA + **1** + $H_2O_2$ (48); Lane 4: DNA + **2**; Lane 5: DNA + **2** + $H_2O_2$ (50); Lane 6: DNA + **3**; Lane 7: DNA + **3** + $H_2O_2$ (34); Lane 8: DNA + **4**; Lane 9: DNA + **4** + $H_2O_2$ (36). The commercial pBR322 used in our experiments contained 50% Form I (supercoiled) and 54% Form II (nicked circular)

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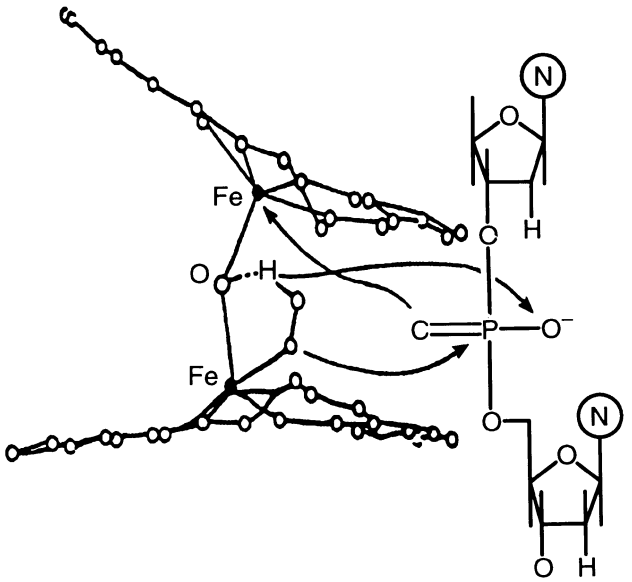


**Fig. 2**

and **4**. Complex **3** is coordinatively saturated and therefore a nucleophilic attack by $H_2O_2$ is prevented and hence no cleavage results. In the other complexes the oxo-bridge is flexible and therefore forms the phosphorus trigonal bipyramidal intermediate which results in cleavage.

It is interesting to note that the bridged 'oxo' group in the dimeric complexes **1** and **2** maintains an Fe–Fe distance of 3.39–3.50 Å which is comparable with the distance between successive base pairs in B-DNA (Fig. 2).[25] This is thought to facilitate the cleavage process as evidenced by the appearance of prominent bands in lanes 3 and 5 in Fig. 1. Complex **3** does not cleave DNA under the present experimental conditions probably due to the compression of the Fe–O(H) angle[21] by about 20° thus preventing the formation of the hydroperoxide intermediate. Under the same experimental conditions mononuclear complexes such as $[Fe(phen)_3]^{2+/3+}$ and $[Fe(bipy)_3]^{2+/3+}$ in the presence of $H_2O_2$ completely degrade plasmid DNA by an oxidative mechanism generating hydroxyl radicals by the well known Fenton reaction.

Our work suggests that dinuclear iron complexes containing $\mu$-oxo bridges are efficient DNA cleaving agents probably acting by the hydrolytic mechanism involving the formation of a unidentate peroxide adduct. This hypothesis is clearly very important to the design of efficient diiron chemical nucleases and will be tested further by cleaving experiments with other dinuclear complexes containing chloro, sulfido and carboxylato bridges.

## Experimental

All common chemicals and solvents were purchased from BDH (Mumbai). The supercoiled pBR322 DNA (Bangalore Genei, Bangalore) was used as received. Agarose and ethidium bromide were purchased from Bio-rad. Deionized, triply distilled water was used for preparing buffers. The metal complexes $[Fe(salen)]_2O$[26] **1**; $[Fe(salen)]_2O{\cdot}2py$[27] **2**; $[Fe(salox)_2(OH)]_2$[28] **3**; $[Fe(salen)]_2O{\cdot}ClO_4$ **4**; $[Fe^{2+/3+}(phen)_3]$[29] and $[Fe^{2+/3+}(bipy)_3]$[29] were prepared and their microanalytical, magnetic and spectroscopic data were found to be consistent with literature values. The gel electrophoresis experiments using supercoiled pBR322 DNA ~4.3 kb were carried out as reported previously[30] and gel documentation using UVP system GDS 2000.

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