A Ferryl(v) Pathway in DNA Cleavage induced by Fe^{II}(haph) with O_2 or H_2O_2†

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The mechanism of DNA cleavage induced by both Fe^{II}(haph)–O_2 and Fe^{III}(haph)–H_2O_2 is shown by the dimethyl sulfoxide scavenging procedure to occur *via* ferryl(v) intermediates.

Fe^{II} and Cu^{II} complexes of haph† have recently been prepared as modification analogues of the bleomycin (BLM) metal binding site.[1,2] The crystal structure of [Cu(haph)]-$ClO_4 \cdot 1.6H_2O$[3] shows that the ligation is similar to that of the Cu^{II}(P-3A) biosynthetic precursor of BLM.[4] The active iron–oxygen species of BLM which cleaves DNA for antitumour action is best described as a ferryl $Fe^{III}O$(BLM) complex which is formed in the minor groove of DNA and which abstracts the C-4H position of an adjacent deoxyribose sugar moiety of DNA.[5–15] Fe^{II}(BLM)–O_2 promotes preferential G-C (5′ → 3′) asnd G-T (5′ → 3′) scissions of DNA.[5] The DNA cleavage produced by Fe^{II}(haph)–O_2 is relatively sequence-nonspecific, possibly owing to the absence of the bithiazole–tripeptide–S component of BLM. In spite of this, Fe^{II}(haph)–O_2 is only ten-fold less efficient than Fe^{II}(BLM)–O_2 in DNA cleavage,[3] but exceeds the activity of other contemporary synthetic models including M(amphis),[16,17]

† haph = *N*-(2-imidazol-3-ylethyl)-6-[(2-imidazol-3-ylethylamino)-methyl]pyridine-2-carboxamide

M(pyml)[18,19] and M(pma)[20,21] (M = Fe^{II} or Cu^{II}).‡ An essential difference in the Fe^{II}(haph) complex from other current synthetic models is the presence of imidazole functionalities both in-plane [as for Cu(P-3A) or authentic BLM] and as the axial donor *trans* to O_2. This may explain the advantage of Fe^{II}(haph) in O_2 activation *vs.* pyml, amphis and pma.[1-3,22]

In order to assess the mechanism of O_2 activation and DNA cleavage by Fe^{II}(haph)–O_2 we have used the methods of Hecht *et al.*[23] and Repine *et al.*[24] to identify the presence of a dominant ferryl-promoted DNA cleavage pathway of both

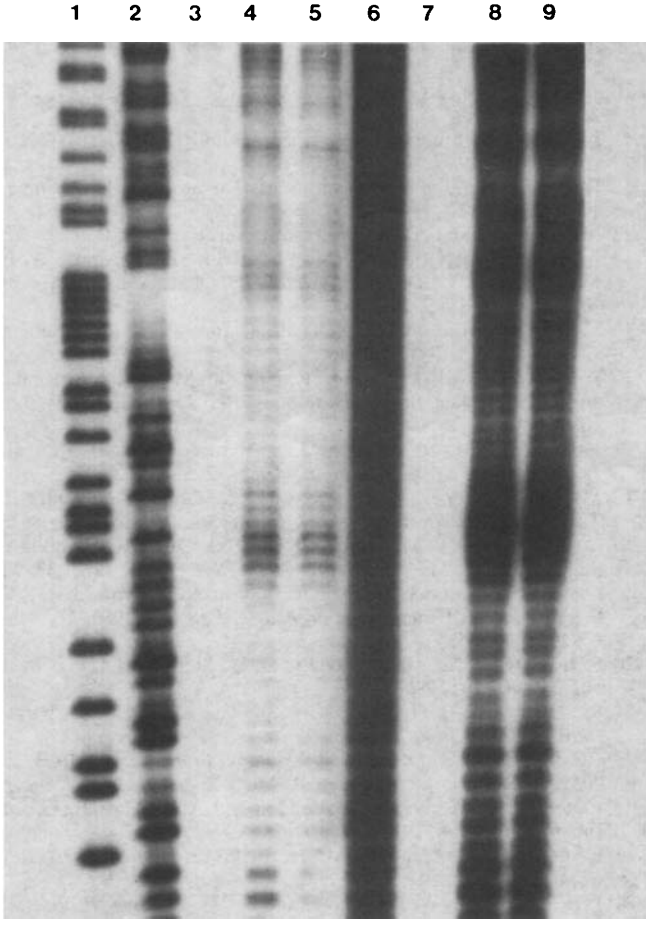


Fig. 1 DNA cleavage by Fe^{II}(haph) and $Fe^{II}(edta)^{2-}$. A 111 base pair singularly end-labelled DNA fragment was obtained by treatment of EcoRI cleaved plasmid pGEM4Z (Promega, Madison, WI) with alkaline phosphatase, polynucleotide kinase and γ ^{32}P-ATP and isolated by standard methods.[3,28] The 111 bp fragment (*ca.* 100 ng) was mixed with 10 μg of sonicated salmon sperm DNA and divided into 10 reaction tubes. Total final volumes were 100 μl containing 50 mmol dm^{-3} Tris buffer (pH 8.0) and specified components. Following incubation for 20 min (room temp.), the reactions were quenched with 10 μg t-RNA and 10 μl of 3 mol dm^{-3} sodium acetate followed by standard isolation procedures. Samples were electrophoresed through 8% urea–acrylamide sequencing gels for 4 h at 1200 V. The gel lanes were exposed to X-ray film for 5 days at −70 °C. Lanes 1 and 2 are A + G and C + T sequencing reactions as markers;[28] Lane 3; 4.0 μmol dm^{-3} haph alone; Lane 4 Fe^{II}(haph) 2 μmol dm^{-3}, H_2O_2 290 μmol dm^{-3}, Lane 5 Fe^{II}(haph) 2 μmol dm^{-3}, H_2O_2 290 μmol dm^{-3}, dmso 280 mmol dm^{-3}; Lane 6 $Fe(edta)^{2-}$ 50 μmol dm^{-3}, H_2O_2 290 μmol dm^{-3}; Lane 7 $Fe(edta)^{2-}$ 50 μmol dm^{-3}, H_2O_2 290 μmol dm^{-3}, dmso 280 mmol dm^{-3}; Lane 8 Fe^{II}(haph) 10 μmol dm^{-3}, DTT 20 μmol dm^{-3}; Lane 9 Fe^{II}(haph) 10 μmol dm^{-3}, DTT 20 μmol dm^{-3}, dmso 280 mmol dm^{-3}; haph 20 μmol dm^{-3}, H_2O_2 280 μmol dm^{-3} was the same as Lane 3.

‡ amphis = methyl 2-(2-aminoethyl)aminoethylpyridine-6-carbamoylhistidinate; pyml = *N*-[6-({[(*S*)-2-amino-2(carbamoyl)-ethyl]-amino}methyl)pyridine-2-carbamoyl]-L-histidinate; pma = *N*-[2-aminomethyl(2-ethylanine)]-4-carbamoyl-(2-ethyl-5-imidazole)-5-bromopyrimidine.

Fe^{II}(haph)–O_2 and Fe^{III}(haph)–H_2O_2. In these procedures 0.28 mol dm^{-3} dimethyl sulfoxide (dmso) is used to quench the diffusable HO· oxidant pathways,[23,24] as was done previously to establish the presence of the bound ferryl $Fe^{III}O$(BLM) species as the active agent for DNA cleavage by Fe^{II}(BLM).[23]

The results of the DNA cleavage experiments are shown in Fig. 1. Only a trace of background cleavage is seen in Lane 3 with 4.0 μmol dm^{-3} haph alone, with O_2 as the oxidant and adventious iron sources. Lane 6 shows the anticipated strong, random cleavage[25] of the 50 μmol dm^{-3} $Fe^{II}(edta)^{2-}$–H_2O_2 (H_4 edta = ethylenediaminetetraacetic acid) reagent in the absence of dmso. Lane 7 shows the $Fe^{II}(edta)^{2-}$–H_2O_2 cleavage reactions with 0.28 mol dm^{-3} dmso added. Virtually all of the cleavage is quenched by the presence of 0.28 mol dm^{-3} dmso in agreement with the data of Repine *et al.*[24] The same treatment of DNA with 2.0 μmol dm^{-3} Fe^{II}(haph)–H_2O_2 is shown in Lanes 4 and 5. The presence of 0.28 mol dm^{-3} dmso suppresses at most 30% of the reactions proceeding through oxidation of Fe^{II}(haph) by H_2O_2. The minor HO· component may be in the first turnover forming Fe^{III}(haph). The important feature is that a very little decrease in DNA cleavage in the presence of dmso is observed for the Fe^{II}(haph)–H_2O_2 in contrast to the quenching of $Fe^{II}(edta)^{2-}$–H_2O_2

An even more dramatic effect is observed for Fe^{II}(haph)–O_2-induced DNA cleavage (Lanes 8 with no dmso and Lane 9 with 0.28 mol dm^{-3} dmso.) Virtually identical DNA cleavage is induced by the Fe^{II}(haph)–O_2 system. The DNA cleavage is not suppressed by 0.28 mol dm^{-3} dmso for Fe^{II}(haph)–O_2. Therefore the dominant mechanism for Fe^{II}(haph)-induced DNA cleavage for *bound* Fe^{II}(haph) must involve other than generation of a freely diffusable HO·. Since it is known from prior DMPO spin-trapping experiments that Fe^{II}(haph) is oxidized in homogeneous solution by either O_2 or H_2O_2 with the generation of free HO·,[1,2] the absence of this pathway for O_2–Fe^{II}(haph)-induced cleavage is particularly significant. The same behaviour is known for Fe^{II}(BLM)–O_2 where, in the absence of DNA, HO· is detected by spin-trapping in homogeneous solution.[4,5] However, the DNA cleavage mechanism requires a bound ferryl BLM species.[4,5,22] This phenomenon is consistent with two parallel pathways for the break-up of an iron(III)–bleomycin–peroxo precursor complex of the DNA cleavage-active $Fe^{III}O$(BLM) complex.[4,5] The same sequence may be envisaged for Fe^{II}(haph) in the presence of O_2 and dithiothreitol (DTT) (L = haph) [reactions (1)–(5)]. In the presence of H_2O_2, $Fe^{III}O$(haph) forms *via* steps (6), (7) and (3).

$$Fe^{II}L + O_2 \rightleftharpoons Fe^{III}(O_2^-)L \quad (1)$$

$$Fe^{III}(O_2^-)L \xrightarrow[(Fe^{II}\text{ or DDT})]{e^-, H^+} Fe^{III}(O_2H^-)L \quad (2)$$

$$Fe^{III}(O_2H^-)L \xrightarrow{H^+} Fe^{III}O(L) + H_2O \quad (3)$$

$$Fe^{III}O(L) \xrightarrow{DNA} \text{strand scissions} + Fe^{III}L \quad (4)$$

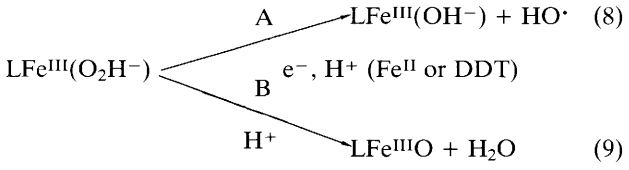
$$Fe^{III}L \xrightarrow{DTT} Fe^{II}L \quad (5)$$

$$Fe^{II}L + H_2O_2 \rightarrow Fe^{III}L + OH^- + HO\cdot \quad (6)$$

$$Fe^{III}L + H_2O_2 \xrightarrow{H_2O} Fe^{III}(O_2H^-)L + H_3O^+ \quad (7)$$

Although EPR methods have previously identified an iron(III)–peroxo complex as a transient in the autoxidations of Fe^{II}(pyml) and other BLM metallobinding site analogues which convert to stable ferric Fe^{III} species,[4a,19c] this evidence is insufficient to establish the identity of the nature of the species which oxidizes DNA. Two pathways [reactions (8) and (9)] may be available for conversion of the peroxo precursor to

an active form (L = BLM or BLM analogue). The results reported in the present communication show that for Fe^{II}(haph) the active cleavage pathway is predominantly through the ferryl route (B), which is not quenched by the presence of 0.28 mol dm^{-3} dmso.



Additional evidence that Fe^{II}(haph) must bind in the minor groove to achieve significant amounts of DNA cleavage were provided by experiments (not shown) with a 180 base pair (bp) fragment of pT181 plasmid DNA. In this study 4.0 μmol dm^{-3} Fe^{II}(haph)–O_2 again showed no difference in the amount of DNA cleavage in the presence or absence of 0.28 mol dm^{-3} dmso. When 300 μmol dm^{-3} distamycin was added prior to the addition of Fe^{II}(haph) a 75 ± 5% reduction in cleavage occurred. The amount of attenuation was the same in the presence or absence of 0.28 mol dm^{-3} dmso. This result shows that distamycin, which binds in the minor groove of the DNA helix, will block the binding of Fe^{II}(haph) and protect the DNA from Fe^{II}(haph)–O_2-induced cleavage.

The binding constant of distamycin for calf-thymus DNA is 1.16×10^6 dm^3 mol^{-1}.[26] The binding constant for Fe^{II}(BLM) is estimated at *ca.* 1.2×10^5 dm^3 mol^{-1} with almost all of the affinity attributed to the bithiazole–tripeptide–S region.[27] Therefore, the metallo-head group of BLM or small molecule analogues such as Fe^{II}(haph) must bind with $K_f \leqslant 10^3$ dm^3 mol^{-1}. Fe^{II}(haph) at 4.0 μmol dm^{-3} cannot compete for minor groove DNA sites with distamycin at ⩾ 300 μmol dm^{-3}. A nearly constant suppression (*ca.* 80%) was obtained with a 50 bp fragment of pT181 plasmid DNA when [distamycin] was in the range 300–1200 μmol dm^{-3}. These studies indicate that the predominant site of action of $Fe^{III}O$(haph) formed *via* the O_2 reaction with Fe^{II}(haph) is in the minor groove of the DNA helix. An additional 20% of cleavage action of $Fe^{III}O$(haph) must occur from other sites of the major groove which are unprotected by up to 1200 μmol dm^{-3} distamycin.

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