Oxidative and Random Cleavage of DNA by the Novel Iron(II) Complex Capable of Yielding an Iron(III) Hydroperoxide Intermediate

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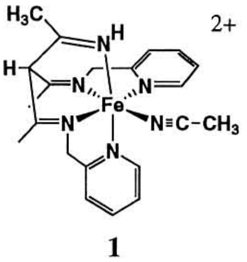
A novel Fe(II) complex and H_2O_2 system causes sequence non-specific double-strand breaks of duplex DNAs at micromolar concentrations and the DNA cleavage is independent of the presence of hydroxyl radical scavengers. The same system can also oxidize hydrocarbons and the active species in the system is likely to be a Fe(III) hydroperoxide complex supported by characteristic purple absorption and EPR signals.

Fe(II) chelates such as Fe(II)-edta[1] and Fe(II)-methidium-propyl-edta [2] are widely used in the DNA footprinting method,[3] which serves to identify the sequence specificity of binders to DNA.[4] These chelates produce hydroxyl radicals in the presence of hydrogen peroxide, and the hydroxyl radicals cleave DNA with sequence non-specificity, which is essential property for the footprinting. The Fe(II)-edta-H_2O_2 system, however, is disturbed by the presence of hydroxyl radical scavengers such as glycerol, which is generally used in the isolation and storage of DNA-binding proteins.[5] Therefore, the synthesis and the application of iron complexes producing an alternative active species, *e.g.*, a Fe(III) hydroperoxide one, must be valuable and effective for the footprinting. We herein describe an efficient double-strand cleavage of duplex DNAs with non-specificity by a novel Fe(II) complex, **1**, -H_2O_2 system which yields a Fe(III) hydroperoxide intermediate as evidenced by its characteristic purple color and EPR signals . Compound **1**, (acetonitrile){3-(1-iminoethyl)-2,4-propanediylidenebis(2-pyridylmethanamine)} iron(II) perchlorate, has been recently reported to be prepared by a reaction of a precursor Fe(II) complex of a tetradentate ligand with acetonitrile.[6] A variety of derivatives can be prepared by choosing particular nitriles to modulate its biological activity.



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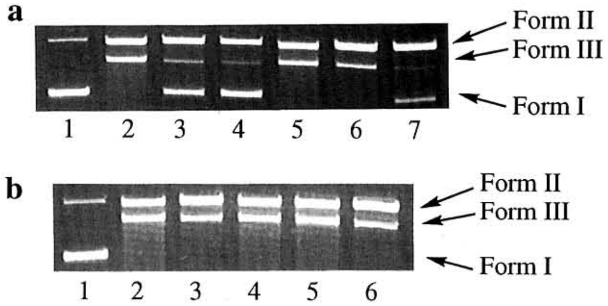

Figure 1. 1% agarose gel electrophoresis of cleavage reaction of DNA by **1** with H_2O_2. Cleavage conditions: 0.2 μg pUC19 plasmid DNA; 3 mM H_2O_2; 20 mM tris-borate buffer (pH 8.0); reaction time, 0.5 h at 20 °C. (a) Lane 1: DNA alone. Lanes 2-4: DNA + 0.5, 0.1, and 0.05 μM Fe(II)-BLM. Lanes 5-7: DNA + 10, 5, and 1 μM **1**. (b) Lane 1: DNA alone. Lane 2: DNA + 10 μM **1**. Lanes 3-6: DNA + 10 μM **1** + 280 mM dmso, 280 mM 1-propanol, 280 mM glycerol, and 280 mM thiourea.

Figure 2. Chemiluminescent detection of the sequence of a DNA fragment cleaved by **1**-H_2O_2. The 293 bp 5'-biotinylated fragment of pUC19 plasmid DNA was used. Lanes 1-4: A, C, G, and T. Lane 5: 5 μM **1** + 3 mM H_2O_2. Lane 6: 2.5 μM **1** + 3 mM H_2O_2.

The DNA cleaving ability of **1**-H_2O_2 system was evaluated with degradation of pUC19 plasmid DNA by incubation at pH 8.0 and 20 °C for 0.5 h and compared with that of Fe(II)-bleomycin(BLM)[7](prepared *in situ*)-H_2O_2. These results are shown in Figure1a. Since the conversion of form I (supercoiled DNA) to form II (open circular DNA) did not proceed in the absence of **1**, the Fe(II) complex is indispensable to activate H_2O_2 and cleave DNA. The complete conversion of form I to form II or form III (linear DNA) is observed at concentrations as low as 5 μM (lane 6), though **1** is less efficient in DNA cleavage than Fe(II)-BLM. The DNA cleavage experiments with 10 μM **1** and 3 mM H_2O_2 in the presence of a large excess of hydroxyl radical scavengers were carried out.[8] Since the extent of DNA strand scission is little reduced by the presence of 280 mM dimethylsulfoxide, 1-propanol, glycerol and thiourea (lanes 3, 4, 5 and 6) as is shown in Figure1b, the dominant active species generated by **1**-H_2O_2 in DNA cleavage is not diffusible hydroxyl radical produced by a Fenton or Haber-Weiss mechanism.

Fe-BLM is known to cleave DNA with sequence specificity (preferentially at 5'-GC-3' and 5'-GT-3'), while 5 or 2.5 μM **1** and 3 mM H_2O_2 (lanes 5 and 6) cleaves a 293 base pair fragment of pUC19 DNA with non-selectivity,[9] as shown in Figure 2. The reason for the non-selective DNA strand scission by **1** is possibly due to the absence of any group to recognize a specific base sequence such as the bithiazole moiety of BLM and the non-selectivity is favorable for the DNA footprinting.

Compound **1** is capable of oxidizing hydrocarbons with 50 equiv. H_2O_2 in acetonitrile.[10] When cyclohexane was used as the substrate, cyclohexanol (125%) and cyclohexanone (89%) (yield based on the mol of **1**) were obtained. With cyclohexene, cyclohexene oxide (161%), 2-cyclohexen-1-ol (123%), and 2-cyclohexen-1-one (115%) were produced. The addition of 50 equiv. H_2O_2 to an orange acetonitrile solution of the low-spin Fe(II) complex **1** generated a transient purple species (λ_{max} = 580 nm, $\varepsilon = 710\ M^{-1}cm^{-1}$) at 25 °C and the signals with g value of 2.16, 2.10 and 1.98 were exhibited in the EPR spectrum at -196 °C (Figure 3).[11] The transient purple absorption and the g-values indicate that the newly formed species is a low-spin Fe(III) complex, probably a Fe(III) hydroperoxide one as reported by Que et al.[12] The dominant active species in DNA cleavage and oxidation of hydrocarbons is possibly the Fe(III) hydroperoxide one, **1**-OOH, and hydrogen atom abstraction of sugar moiety of DNA or cyclohexane by **1**-OOH may be the initial step in their oxidative processes.

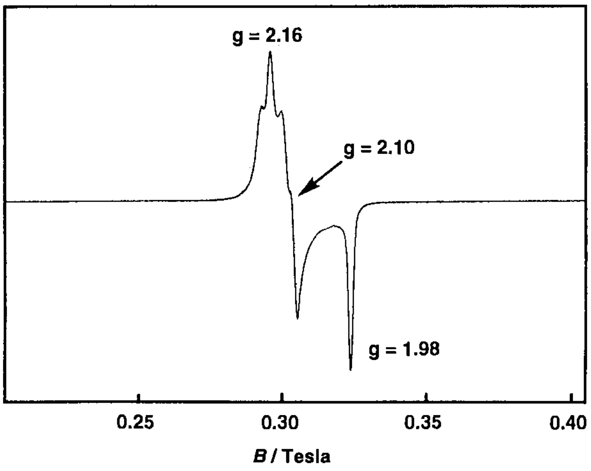

Figure 3. X-band EPR spectrum of **1** with 50 equiv. H_2O_2 in acetonitrile at 77 K

Taken together, the novel Fe(II) complex **1** with H_2O_2 yields a Fe(III) hydroperoxide intermediate and cleaves DNA, and hence **1** has potential as a new "Fe(III) hydroperoxide DNA footprinting" reagent on the basis of non-selectivity in DNA cleavage. Further studies about the stability and reactivity of **1**-OOH and the introduction of a variety of functional group to enhance interaction with DNA into **1** are in progress.

References and Notes

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9 A 293 bp 5'-biotinylated double strand DNA was amplified by polymerase chain reaction (PCR) with 5'-biotinylated primer 1 (5'-[BT]GTTTTCCCAGTCACGAC-3', New England Biolabs, Inc.) and primer 2 (5'-TTGGCCGATTCATTAATGCA-3', custom synthesis), using the pUC19 plasmid as a template. The PCR product was purified by 0.8% agarose gel electrophoresis. DNA cleavage experiments were performed by mixing 0.5 μg of the 293 bp fragment with 5 or 2.5 μM **1** and 3 mM H_2O_2 in 20 mM Tris-HCl buffer (pH 7.5) for 10 min at 37 °C. After loading buffer [95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol] was added to the reaction mixture, the samples were heated at 95 °C for 3 min, then cooled on an ice-bath, loaded on a 6% Long Ranger polyacrylamide (FMC Bioproducts) denaturing (7 M urea) gel and electrophoresed at 35 W for 4 h. The DNA was electrotransfered onto Biodyne B nylon membrane (Pall BioSupport Corp.) and 5'-biotinylated DNA bands were detected by Phototope-Star Detection Kit (New England Biolabs, Inc.). Sequence ladder was generated with primer 1 using pUC19 as a template in dideoxy sequencing reactions.

10 Reactions were performed in CH_3CN under argon at 25 °C for 5min: **1** (0.02 mmol), H_2O_2 (30% aqueous, 1.0 mmol), cyclohexane (10.0 mmol) or cyclohexene (10.0 mmol).

11 Spectrometer settings: microwave frequency, 8.99 GHz; microwave power, 1.83 mW; modulation frequency, 100 kHz; modulation width, 0.63 mT.

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