Inorg. Chem. **2007**, *46*, 8120–8122

Formal DNA Hydrolysis by Mono- and Dinuclear Iron Complexes

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Received June 22, 2007

The complexes $CpFe(CO)_2Ph$ and $[CpFe(CO)_2]_2$ cleave DNA in the presence of H_2O_2 or organic peroxides to give products resulting from the formal hydrolysis of the phosphodiester groups.

Synthetic agents that cleave DNA in a hydrolytic manner are increasingly important for a number of reasons.[1] They may function as artificial restriction enzymes in molecular biology, offering the possibility of designing systems with different sequence specificities than natural protein endonucleases. This capability may also be useful for targeting DNA sequences to block gene expression at the molecular level in chemotherapy or for studying nucleic acid conformations. While most synthetic nucleases, inspired by the active sites of natural enzymes, include two or more metal atoms, only a limited number of mononuclear species have demonstrated the ability to cause the hydrolysis of phosphodiesters. These include the aqueous ions and chelates of a number of transition metals;[2] however, there are no examples of DNA hydrolysis by a mononuclear iron species.

Therefore, we now describe the formal hydrolysis[3] of plasmid DNA by $CpFe(CO)_2Ph$ (**1**) and $[CpFe(CO)_2]_2$ (**2**) in the presence of peroxides. While our previously reported studies implicated radicals in DNA cleavage by **1** and H_2O_2,[4] further investigations have since shown that strand scission also occurs via a formal hydrolysis under these conditions. Thus, incubation of DNA with **1** and H_2O_2 (Figure 1, lanes 2−3) or with **1** and unpurified THF[5] (lanes 4−5) resulted in cleaved DNA (lanes 2 and 4) that could be religated by T4 DNA ligase (lanes 3 and 5). Unlike radical-induced oxidative cleavage, which results in small molecule products and cleaved DNA without 3′-OH termini,[6] hydrolytic DNA cleavage results in strands with either 5′-OH and 3′-PO_4 or 3′-OH and 5′-PO_4 termini, which can be enzymatically manipulated. Since T4 DNA ligase catalyzes the formation of a phosphodiester bond only between 3′-OH and 5′-PO_4 termini,[7] the successful religation confirms that cleavage of the phosphodiesters occurs in addition to the previously observed[4] radical-mediated oxidative strand scission.

Because the $CpFe(CO)_2$ radical is a potential intermediate in the photolysis of **1**[4] and can be produced by irradiation of **2**,[8] the DNA cleaving behavior of **2** in the presence of peroxides was examined (Figure 2). The combination of **2** (5 μM) and H_2O_2 (250 μM) resulted in strand scission (lane 6). Control experiments demonstrated that both **2** (lane 5) and H_2O_2 (lane 4) were needed to cause significant amounts of cleavage at the concentrations used in this experiment. Strand scission decreased dramatically when the amount of either **2** or H_2O_2 was reduced (lanes 7 and 8, respectively). Further indication of the necessity of H_2O_2 was provided by the lack of cleavage when 10 mM Tris buffer was used in place of water (results not shown), presumably because H_2O_2 is consumed by reacting with Tris to give its *N*-oxide in a known reaction.[9] In addition, irradiation is not required for strand scission but may enhance it (lane 2 vs lane 6).

Interestingly, unpurified THF[5] (Figure 3, lanes 4−13) was also effective as a peroxide source, causing strand scission in the presence of **2** at concentrations as low as 1.4 μM. Again, light was not necessary for cleavage but did enhance it (lane 2 vs lane 5).

With a reduction potential of −0.5 V[10] for the Fe(I)/Fe(II) couple of the $CpFe(CO)_2$ radical and $[CpFe(CO)_2$-

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(3) The term "hydrolysis" is used not in the mechanistic sense here but to describe the overall conversion (the addition of water across a P−O bond of a phosphodiester).
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10.1021/ic701231d CCC: $37.00
Published on Web 09/05/2007

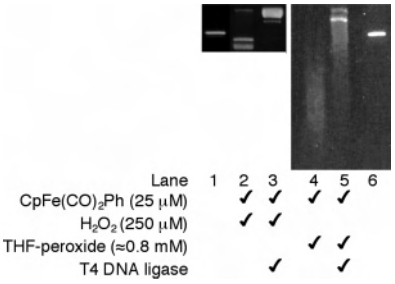

Figure 1. Religation studies of the cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by **1** with H_2O_2 or THF peroxide. All samples were incubated under ambient conditions for 30 min. After concentration and removal of **1** by ultrafiltration, aliquots of the reaction mixtures in lanes 2 and 4 were removed, concentrated, and treated with T4 DNA ligase (lanes 3 and 5).

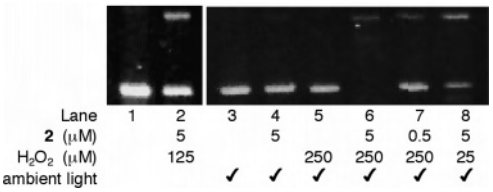

Figure 2. Cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by **2** and H_2O_2. Samples in lanes 1 and 2 were prepared and incubated in the dark, and the mixtures in lanes 3−8 were incubated under ambient conditions for 30 min.

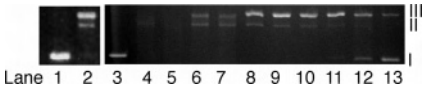

Figure 3. Cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by **2** and THF peroxide: lanes 1 and 3, DNA alone; lanes 2 and 4−13, DNA, **2** (50.0, 55.0, 36.7, 24.5, 16.3, 10.9, 7.3, 4.8, 3.2, 2.1, and 1.4 μM, respectively) and THF peroxide (∼0.8 mM). Samples in lanes 1 and 2 were prepared and incubated in the dark, and the mixtures in lanes 3−13 were incubated in ambient light for 30 min.

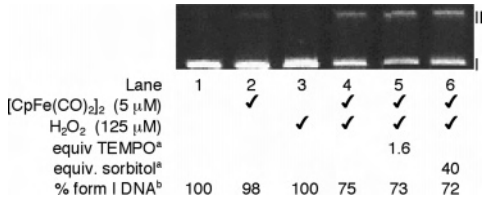

Figure 4. Radical trapping studies of the cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by **2** and H_2O_2. All samples were incubated under ambient conditions for 30 min.

$(NCCH_3)]^+$, it is possible that $CpFe(CO)_2$ reduces H_2O_2 (0.46 V) in Fenton-like chemistry to make a hydroxide ion and a hydroxyl radical, a well-known DNA cleaver. To assess this possibility, radical-trapping experiments using TEMPO or sorbitol were undertaken (Figure 4). TEMPO is a nitroxide species that selectively traps carbon-[11] and metal-centered radicals,[12] but not oxygen-based radicals, which react with sorbitol.[13] Surprisingly, neither TEMPO (lane 5) nor sorbitol (lane 6) effectively inhibited DNA cleavage by **2** with H_2O_2. This finding indicates that strand scission does not result primarily from diffusible radical species (in contrast to **1**,

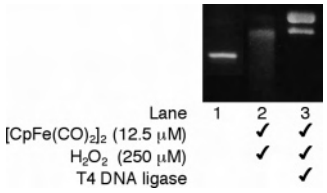

Figure 5. Religation studies of the cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by **2** and H_2O_2. Samples were incubated under ambient light for 30 min. After concentration and removal of **2** by ultrafiltration, an aliquot of the sample in lane 2 was treated with T4 DNA ligase (lane 3).

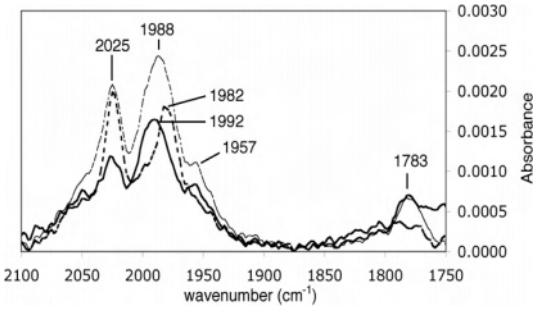

Figure 6. ATR-IR studies of the reaction of **2** with H_2O_2 in 10% THF/water. The solid trace shows the spectrum of **2** (40 μM) 2 min after dissolution. The long dash trace (− −) is the spectrum of **2** (40 μM) and H_2O_2 (260 μM) obtained 2 min after mixing, and the short dash trace (---) is the same sample after 10 min.

which also gives radical-induced cleavage)[4] but rather via phosphodiester cleavage, in which case the strand termini should be subject to religation. Indeed, such ligation was observed in mixtures resulting from extensive DNA cleavage by **2** with H_2O_2 (Figure 5, lane 2) after subsequent treatment with T4 ligase (lane 3).

With these indications that phosphodiester bond cleavage was the predominant manner of DNA strand scission by **2** in the presence of H_2O_2, the obvious question concerned the nature of the active DNA cleaving species. To investigate its structure, attenuated total reflectance IR (ATR-IR) spectroscopy was employed. The carbonyl region of the spectrum of **2** two minutes after dissolution in 10% peroxide-free THF/water (Figure 6, solid trace) exhibits ν(CO) absorbances for **2** at 1783, 1957, and 1992 cm^{-1}. The latter peak overlaps with another at 1982 cm^{-1}, which, along with a signal at 2025 cm^{-1}, is consistent with literature data for mononuclear species of the formula $CpFe(CO)_2L^2$ or $[CpFe(CO)_2L^2]^+X^-$ (**5**), in which L^2 is THF or water.[14,15] When H_2O_2 is present (dashed lines), the signals from **2** are decreased relative to those for the mononuclear species, suggesting that H_2O_2 facilitates the conversion of **2** to the mononuclear complex. After 10 min, **2** is gone in samples with H_2O_2, and no other dinuclear species (e.g., **3** or **4**) is present, as evident from the lack of an absorbance in the range of 1940−1960 cm^{-1}.[16]

While these studies are far from conclusive, they suggest that a mononuclear iron complex may be responsible for strand scission because **2** is converted by H_2O_2 to a mononuclear species much more rapidly than DNA is

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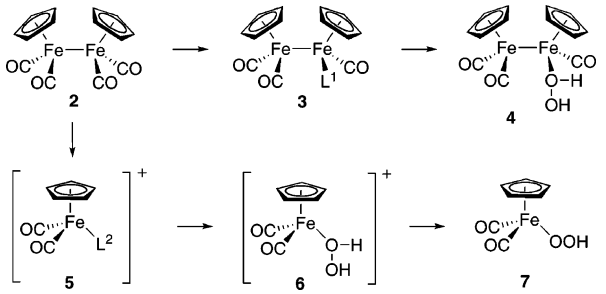
Scheme 1



cleaved (Figure S1) and because **2** without peroxide does not cleave DNA (e.g., Figure 4, lane 2). Therefore, we propose an iron peroxide species such as **7** to be the active DNA-cleaving species and to arise from the reaction of H_2O_2 with **5**. The iron(II) aquo complex **5** ($L^2 = H_2O$) has been shown to result from the oxidation of **2** by O_2 in aqueous acetone,[15] and it is this or a similar species (e.g., L^2 = THF) that gives the ν(CO) absorbances at 2025 and 1982 cm^{-1}. In addition, nucleophilic ligands are known to replace the coordinated water in **5**.[15,17] In our system, H_2O_2 is the likely oxidant,[18] as well as the nucleophile. The observation that solutions of the aquo complex **5** (synthesized with a BF_4 counterion as in the literature[15]) do not cleave DNA (Figure S2, lane 3), unless H_2O_2 is added (lanes 4 and 5), is also consistent with the involvement of a species derived from both **5** and H_2O_2 in the pathway leading to DNA cleavage.The cleavage of biomacromolecules by complexed peroxides is known.[19−21] Another mononuclear iron−peroxo complex has been proposed in the formal hydrolysis of peptide bonds in proteins by an EDTA-Fe chelate in the presence of ascorbate and H_2O_2.[20] In this case, the protein-cleaving species also results from the replacement of a water ligand by the peroxide, in a manner similar to the reaction of this aquo complex with other nucleophiles.[22] The hydrolysis of proteins under these conditions is especially interesting becausechelated iron systems such as Fe-EDTA are widely used in the footprinting of nucleic acids[23] and because these reactions have proven to be radical-based.[24]

More relevantly to our work, DNA hydrolysis by the diiron complex Fe(HPTB)(OH)$(NO_3)_4$ with H_2O_2 or O_2 has been reported[19] and likely involves a (μ-1,2-peroxo)diiron species.[25] The involvement of similar intermediates in DNA cleavage by **2** is unlikely, given the lack of evidence for any persistent dinuclear species in the IR spectra discussed previously. In addition, μ-1,2-peroxo complexes with bonds between the peroxo-bridged metals are unknown. A number of other μ-1,2-peroxo dinuclear species (without metal−metal bonds) have been reported,[25,26] but no reasonable mechanism for the conversion of **2** to an analogous compound with no Fe−Fe bond can be written. Its formation from two mononuclear complexes such as **5**, **6**, or **7** is also unlikely under the very dilute conditions used in the DNA cleavage experiments. A metal−metal-bonded dinuclear compound with a nonbridging peroxide such as $Cp_2Fe_2(CO)_3(H_2O_2)$ **4** could conceivably be produced from the aforementioned complex $Cp_2Fe_2(CO)_3(L^1)$, but because of the reduced electron density on the coordinated oxygen, this species is not expected to be nucleophilic enough to cleave DNA without Lewis acid activation of the phosphodiester. In most bimetallic nucleases, one metal fulfills this function, while the other delivers the nucleophile; however, the likelihood of the second iron center in $Cp_2Fe_2(CO)_3(H_2O_2)$ losing a carbonyl to act as a Lewis acid is very small. Further, to form the neutral deprotonated form of **4** (which might cleave DNA without Lewis acid activity), one Fe must be oxidized, but the oxidation typically cleaves the Fe−Fe bond, as seen for **2**.[15]

In the case of phosphodiester cleavage by $CpFe(CO)_2Ph$ (**1**) in the presence of H_2O_2, intermediates similar to **5** and **6** could also be envisioned, although the mechanism of their formation is even less clear. Unfortunately, preliminary attempts to study the reaction of **1** with H_2O_2 have been complicated by decomposition processes that are presumably induced by the competing radical pathways indicated previously in scavenging studies with **1**.[4]

In summary, although the exact mechanism and active species in the cleavage of DNA by **1** or **2** in the presence of peroxides remain to be elucidated, both reactions give the formal hydrolysis of phosphodiester bonds, as evidenced by enymatic ligation studies. While **2** appears to act via a mononuclear intermediate, **1** most likely does.

Acknowledgment. Financial support from the NSF-REU program (CHE-0353807), James Madison University, and Emory University is gratefully acknowledged.

Supporting Information Available: Gels for additional experiments, quantitation data for selected gels, and detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) For example, $Cp_2Fe_2(CO)_3(L^1)$ (**3**, Scheme 1) in which L^1 is H_2O or THF, shows ν(CO) at 1967, 1943, and 1726 cm^{-1}. Dixon, A. J.; George, M. W.; Hughes, C.; Poliakoff, M.; Turner, J. J. *J. Am. Chem. Soc.* **1992**, *114*, 1719−1729.
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