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The hydrogen peroxide induced enhancement of DNA cleavage in the ambient light photolysis of $CpFe(CO)_2Ph$: A potential strategy for targeting cancer cells

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Abstract—DNA strand scission is produced by the ambient light photolysis of $CpFe(CO)_2Ph$ and H_2O_2, a result that shows potential as a means of targeting tumors, due to the high levels of hydrogen peroxide in cancer cells. This cleavage process is dependent on the concentration of both $CpFe(CO)_2Ph$ and H_2O_2, and preliminary experiments implicate both carbon-centered radicals and reactive oxygen species.

For years, moderately indiscriminate cytotoxic agents have formed the basis of conventional cancer chemotherapy; however, their lack of selectivity for diseased cells and the resultant undesired side effects are major drawbacks to their use. More recently, a number of molecular approaches have been developed to target tumor cells, including gene therapy, antibody therapeutics, and angiogenesis inhibition;[1,2] while others, such as radiation sensitization[3] and photodynamic agents,[4] rely on localized irradiation to produce cell death. Also promising are strategies that exploit physiological features that are unique to tumor cells, such as their low cytoplasmic pH,[2,5] hypoxic intracellular environment,[6] and higher peroxide (H_2O_2) concentrations[7,8] relative to normal cells.

Interestingly, H_2O_2 itself is poorly reactive toward lipids, DNA, and most proteins until it is converted to the reactive hydroxyl radical (•OH) by transition metal ions or ultraviolet light.[9,10] Due to this reactivity, organisms have evolved a number of strategies to minimize the potential oxidative damage of hydroxyl radical in normal tissues, including enzymatic pathways to reduce the amount of H_2O_2, non-enzymatic antioxidant defenses,[11] and mechanisms to sequester transition metal ions into non-catalytic forms.[9] In contrast, malignant cells show increased production of H_2O_2,[7,12] as well as an impaired ability to reduce H_2O_2 to water.[13] Therefore, an agent that can be triggered to cause the conversion of H_2O_2 to •OH may represent a potential new treatment paradigm, because it would take advantage of not only a physiological characteristic unique to cancer cells but also an activation method to localize damage only to these cells.

Therefore, we now report the ambient light induced activation of $CpFe(CO)_2Ph$ (**1**) and hydrogen peroxide to cause DNA damage. We have previously employed the photolysis of unfunctionalized and substituted complexes with the general formula $CpM(CO)_nR$ to generate carbon-centered radicals that cause single- and/or double-strand cleavage of plasmid DNA.[14,15] These compounds are easily synthesized, and their DNA cleaving activity is triggered by high intensity visible light irradiation.[16]

The initial indication of the DNA cleaving ability of $CpFe(CO)_2Ph$ and H_2O_2 with room light irradiation was obtained in a plasmid relaxation assay, which monitors the conversion of intact circular supercoiled DNA (form I) to relaxed circular, or nicked, DNA (form II). Gel electrophoresis (Fig. 1) showed that at H_2O_2 concentrations at or above 250 μM, DNA cleavage occurred at concentrations of **1** as low as 25 μM (lane 4) or 0.83 mol/bp. Control experiments have demonstrated that both the organometallic compound (lane 3) and H_2O_2 (lane 2) were necessary to cause significant

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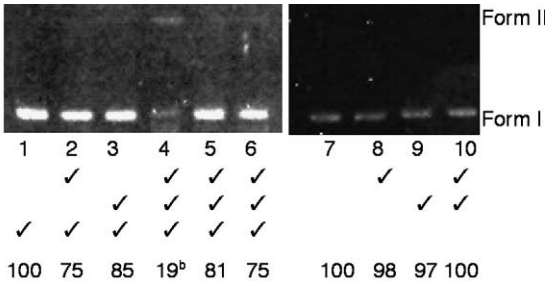


Figure 1. Ambient light induced cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by $CpFe(CO)_2Ph$ and H_2O_2. Lanes 1 and 7, DNA alone; lane 2, DNA and $CpFe(CO)_2Ph$ (25 μM); lane 3, DNA and H_2O_2 (250 μM); lanes 4–6, DNA, $CpFe(CO)_2Ph$ (25, 2.5, and 25 μM, respectively), and H_2O_2 (250, 250, and 25 μM, respectively); lane 8, DNA and $CpFe(CO)_2Ph$ (500 μM); lane 9, DNA and H_2O_2 (10 mM); lane 10, DNA, $CpFe(CO)_2Ph$ (500 μM), and H_2O_2 (10 mM). Samples in lanes 1–6 were incubated on a benchtop for 30 min; and mixtures in lanes 7–10 were prepared, incubated, and subjected to electrophoresis in a dark environment. [a]Total amount of DNA in each lane as compared to control (lane 1 or 7). [b]Sum of forms I (10%) and II (9%).

cleavage and that strand scission decreased dramatically when the concentration of either the iron complex or H_2O_2 was reduced (lanes 5 and 6, respectively).

Since all the reaction mixtures in lanes 1–6 were handled under typical laboratory lighting, additional experiments were conducted to assess the possible contribution of this level of irradiation to DNA cleavage; and the mixtures in lanes 7–10 were prepared and incubated in a darkroom. Interestingly, despite the fact that concentrations of **1** and H_2O_2 (500 μM and 10 mM, respectively) in the dark samples were much higher than in previous experiments in which cleavage was observed (e.g., 25 μM and 250 μM for **1** and H_2O_2, respectively, in lane 4), no strand scission or loss of DNA was evident without exposure to light (lane 10).

For comparison with experiments containing hydrogen peroxide, the efficiency of DNA cleavage by **1** with ambient irradiation but no H_2O_2 was further examined. Mixtures of DNA with varying concentrations of $CpFe(CO)_2Ph$ were incubated on a lab bench for 30 min and then analyzed by electrophoresis (Fig. 2). While room light alone is sufficient to cause significant production (43%) of nicked DNA at concentrations of **1** of 250 μM or greater (lane 4),[17] these conditions are not as efficient as when 250 μM hydrogen peroxide is

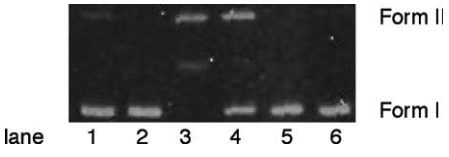


Figure 2. Ambient light induced cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by $CpFe(CO)_2Ph$ without H_2O_2. Lane 1, DNA alone; lanes 2–6, DNA and $CpFe(CO)_2Ph$ (500, 500, 250, 125, and 50 μM, respectively). Samples in lanes 1 and 3–6 were incubated on a benchtop for 30 minutes; and the mixture in lane 2 was prepared and incubated in a dark environment.

present (only 10% of intact DNA at 25 μM of **1**, lane 4 in Fig. 1) or when high intensity irradiation alone is used (no intact plasmid at 22.5 μM of **1**).[14]

In contrast to our previously published work with $CpFe(CO)_2Ph$ alone, in which carbon-centered radicals were implicated,[14] the inclusion of hydrogen peroxide in the current experiments raises the possibility of the involvement of reactive oxygen species. Therefore, to assess the potential roles of carbon- and/or oxygen-centered radicals in the reaction that leads to DNA cleavage by the photolysis of **1** and H_2O_2, radical trapping experiments using TEMPO or sorbitol were conducted (Fig. 3). TEMPO is a nitroxide species that selectively traps carbon-[18] and metal-centered radicals,[19] but not oxygen-based radicals, which react with sorbitol.[20] Interestingly, both TEMPO and sorbitol inhibited, but did not completely suppress, DNA cleavage (lanes 5–8), suggesting that both carbon- and oxygen-centered radicals may be involved in the pathway(s) leading to strand scission. Although TEMPO also scavenges metal-centered radicals, species, such as $CpFe(CO)_2$ radical, are not expected to contribute to strand scission *by hydrogen atom abstraction*,[21] since such a process is disfavored both thermodynamically (as predicted by relative bond dissociation energies of metal hydrides[22] and hydrocarbons[23]) and kinetically.[24] However, the participation of $CpFe(CO)_2$ radical in other pathways leading to DNA damage cannot be ruled out (vide infra).

The observed production of reactive oxygen species from a solution containing H_2O_2 and iron species suggests that Fenton or Fenton-like chemistry may be taking place on photolysis of **1**. The general Eq. 1 shows the central step of the most commonly proposed mechanism for such reactions,[25] which typically involve Fe(II)

$$L_m\ Fe^n + H_2\ O_2 \rightarrow L_m\ Fe^{n+1} + HO^{\cdot} + HO^- \qquad (1)$$

species with Fe(III)/Fe(II) reduction potentials lower than 0.46 V versus NHE, a value that corresponds to the potential for the one-electron reduction of H_2O_2 to

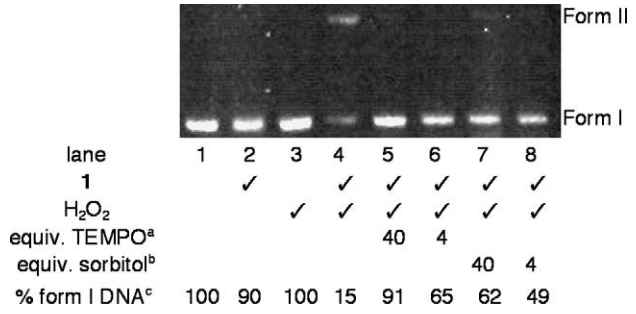
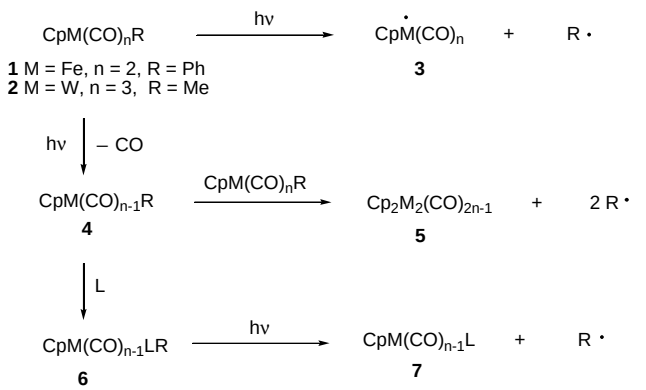


Figure 3. Radical trapping studies of the cleavage of pBR322 DNA (30 μM/bp in 10% THF/water) by $CpFe(CO)_2Ph$ and H_2O_2. Lane 1, DNA alone; lane 2, DNA and $CpFe(CO)_2Ph$ (25 μM); lane 3, DNA and H_2O_2 (250 μM); lane 4, DNA, $CpFe(CO)_2Ph$ (25 μM), and H_2O_2 (250 μM); lanes 5–6, DNA, $CpFe(CO)_2Ph$ (25 μM), H_2O_2 (250 μM), and TEMPO (1.0 or 0.1 mM respectively); lanes 7–8 DNA, $CpFe(CO)_2Ph$ (25 μM), H_2O_2 (250 μM), and sorbitol (10.0 or 1.0 mM, respectively). All samples were incubated on a benchtop under ambient light for 30 min. [a] versus $CpFe(CO)_2Ph$ or [b]H_2O_2 [c]relative to lane 1.

hydroxyl radical and hydroxide/water.[26] Clearly, in our system, the identity of L_mFe^n has not been determined, but previously reported studies with complexes of the type $CpM(CO)_nR$ offer some ideas (Scheme 1).

It is generally accepted that the primary photoprocess for these complexes involves loss of carbon monoxide (to give **4**), which may be accompanied by homolysis of the metal-methyl or metal-aryl bond to yield the metal-based radical **3** along with methyl or phenyl radical. However, radical formation may occur by multiple pathways, as has been suggested for the photolysis of $CpW(CO)_3CH_3$, the only complex whose photochemistry has been extensively studied.[27] In this case, it has been proposed that $CpW(CO)_2CH_3$ (**4**) reacts with another molecule of starting material to produce the metal–metal bonded species **5** and two methyl radicals. For both **1** and **2**, the predominant organometallic product is the metal–metal bonded dimer, $[CpM(CO)_n]_2$, implicating a similar mechanism for both metals. Furthermore, it has been demonstrated that the 16 electron species **4** containing either iron or tungsten can coordinate a variety of ligands (e.g., L = PPh_3, CH_3CN, THF, or H_2O); and when M = W and L = PPh_3, **6** produces methyl radicals upon further photolysis.

Our observation that $CpFe(CO)_2Ph$ (**1**) itself does not react directly with H_2O_2 to produce radicals is consistent with the reduction potential of the Fe(III) species $[CpFe(CO)_2Ph]^+$, which is expected to be similar to that measured for $[CpFe(CO)_2Me]^+$ (1.47 V[28]). Likewise, other photochemically generated Fe(II) complexes, such as **6**, exhibit unsuitable potentials,[28] and the intermediate **4** is probably even more difficult to oxidize. Provocatively, however, the reduction potential for the Fe(I)/Fe(II) couple, $CpFe(CO)_2$ (**3**) and $[CpFe(CO)_2(NCCH_3)]^+$, has been reported as −0.5 V,[29] indicating that **3** may be able to reduce hydrogen peroxide, as in Eq. (1); although the formation of **3** in the photolysis of **1** is still a matter of debate. Its production is not the predominant photoprocess;[27] and the metal-centered radical **3** has not been observed in either ESR or UV–visible studies of the photolysis of **1**.[30] This fact could be ascribed to its instability, except that the radical $CpFe(CO)_2$ (**3**) has been detected in the photolysis of $[CpFe(CO)_2]_2$.[31] Intriguingly, however, TEMPO is known to trap radicals similar to **3**,[19] which could account for our observation that TEMPO is a more effective inhibitor of DNA damage than sorbitol (Fig. 3).



Scheme 1. General mechanism of the photolysis of complexes of the type $(CpMCO)_nR$.

In conclusion, the presence of H_2O_2 enhances the ambient light induced cleavage of DNA by $CpFe(CO)_2Ph$. Although the preliminary evidence indicates that this process may involve both reactive oxygen species and carbon-centered radicals, further studies are required to establish the exact mechanism(s) and active species. While the concentration of hydrogen peroxide required to cause strand scission in this system (250 μM) is much higher than that reported in the intracellular environment of cancer cells (typically 1–100 nM[8]), this strategy nevertheless represents a first step in a new approach to targeting tumors.

Supplementary data

Quantitation data for all gels and detailed experimental procedures. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.06.102.

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