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 H₂O₂, 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 H₂O₂, and preliminary experiments implicate both carbon-centered radicals and reactive oxygen species.

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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, while others, such as radiation sensitization and photodynamic agents, 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, hypoxic intracellular environment, and higher peroxide (H₂O₂) concentrations relative to normal cells.

Interestingly, H₂O₂ 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. 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 H₂O₂, non-enzymatic antioxidant defenses, and mechanisms to sequester transition metal ions into non-catalytic forms. In contrast, malignant cells show increased production of H₂O₂, as well as an impaired ability to reduce H₂O₂ to water. Therefore, an agent that can be triggered to cause the conversion of H₂O₂ 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 (I) 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. These compounds are easily synthesized, and their DNA cleaving activity is triggered by high intensity visible light irradiation.

The initial indication of the DNA cleaving ability of CpFe(CO)₂Ph and H₂O₂ 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 H₂O₂ concentrations at or above 250 μM, DNA cleavage occurred at concentrations of I as low as 25 μM (lane 4) or 0.83 mol/bp. Control experiments have demonstrated that both the organometallic compound (lane 3) and H₂O₂ (lane 2) were necessary to cause significant...
cleavage and that strand scission decreased dramatically when the concentration of either the iron complex or \( \text{H}_2\text{O}_2 \) 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 \( \text{I} \) and \( \text{H}_2\text{O}_2 \) (500 \( \mu \)M and 10 mM, respectively) in the dark samples were much higher than in previous experiments in which cleavage was observed (e.g., 25 \( \mu \)M and 250 \( \mu \)M for \( \text{I} \) and \( \text{H}_2\text{O}_2 \), 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 \( \text{I} \) with ambient irradiation but no \( \text{H}_2\text{O}_2 \) was further examined. Mixtures of DNA with varying concentrations of \( \text{CpFe(CO)}_2\text{Ph} \) 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 \( \text{I} \) of 250 \( \mu \)M or greater (lane 4), the conditions are not as efficient as when 250 \( \mu \)M hydrogen peroxide is present (only 10% of intact DNA at 25 \( \mu \)M of \( \text{I} \), lane 4 in Fig. 1) or when high intensity irradiation alone is used (no intact plasmid at 22.5 \( \mu \)M of \( \text{I} \)).

In contrast to our previously published work with \( \text{CpFe(CO)}_3\text{Ph} \) alone, in which carbon-centered radicals were implicated, 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 \( \text{I} \) and \( \text{H}_2\text{O}_2 \), radical trapping experiments using TEMPO or sorbitol were conducted (Fig. 3). TEMPO is a nitroxide species that selectively traps carbon-centered radicals and metal-centered radicals, but not oxygen-based radicals, which react with sorbitol. 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 \( \text{CpFe(CO)}_2 \text{ radical, are not expected to contribute to strand scission by hydrogen atom abstraction}^{21} \) since such a process is disfavored both thermodynamically (as predicted by relative bond dissociation energies of metal hydrides and hydrocarbons) and kinetically. However, the participation of \( \text{CpFe(CO)}_2 \) 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 \( \text{H}_2\text{O}_2 \) and iron species suggests that Fenton or Fenton-like chemistry may be taking place on photolysis of \( \text{I} \). The general Eq. 1 shows the central step of the most commonly proposed mechanism for such reactions, which typically involve \( \text{Fe(II)} \)

\[
\text{L}_\text{m Fe}^+ + \text{H}_2\text{O}_2 \rightarrow \text{L}_\text{m Fe}^{+1} + \text{HO}^- + \text{HO}^-
\]

(1)

species with \( \text{Fe(III)/Fe(II)} \) reduction potentials lower than 0.46 V versus NHE, a value that corresponds to the potential for the one-electron reduction of \( \text{H}_2\text{O}_2 \) to

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**Figure 1.** Ambient light induced cleavage of pBR322 DNA (30 \( \mu \)M/bp in 10% THF/water) by \( \text{CpFe(CO)}_2\text{Ph} \) and \( \text{H}_2\text{O}_2 \). Lanes 1 and 7, DNA alone; lane 2, DNA and \( \text{CpFe(CO)}_2\text{Ph} \) (23 \( \mu \)M); lane 3, DNA and \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M); lanes 4–6, DNA, \( \text{CpFe(CO)}_2\text{Ph} \) (25, 25, and 25 \( \mu \)M, respectively), and \( \text{H}_2\text{O}_2 \) (250, 250, and 25 \( \mu \)M, respectively); lane 8, DNA and \( \text{CpFe(CO)}_2\text{Ph} \) (500 \( \mu \)M); lane 9, DNA and \( \text{H}_2\text{O}_2 \) (10 mM); lane 10, DNA, \( \text{CpFe(CO)}_2\text{Ph} \) (500 \( \mu \)M), and \( \text{H}_2\text{O}_2 \) (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. *Total amount of DNA in each lane as compared to control (lane 1 or 7). †Sum of forms I (10%) and II (9%).

**Figure 2.** Ambient light induced cleavage of pBR322 DNA (30 \( \mu \)M/bp in 10% THF/water) by \( \text{CpFe(CO)}_2\text{Ph} \) without \( \text{H}_2\text{O}_2 \). Lane 1, DNA alone; lanes 2–6, DNA and \( \text{CpFe(CO)}_2\text{Ph} \) (500, 500, 250, 125, and 50 \( \mu \)M, 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.

**Figure 3.** Radical trapping studies of the cleavage of pBR322 DNA (30 \( \mu \)M/bp in 10% THF/water) by \( \text{CpFe(CO)}_2\text{Ph} \) and \( \text{H}_2\text{O}_2 \). Lane 1, DNA alone; lane 2, DNA and \( \text{CpFe(CO)}_2\text{Ph} \) (25 \( \mu \)M); lane 3, DNA and \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M); lane 4, DNA, \( \text{CpFe(CO)}_2\text{Ph} \) (25 \( \mu \)M), and \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M); lanes 5–6, DNA, \( \text{CpFe(CO)}_2\text{Ph} \) (25 \( \mu \)M), \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M), and TEMPO (1.0 or 0.1 mM respectively); lanes 7–8 DNA, \( \text{CpFe(CO)}_2\text{Ph} \) (25 \( \mu \)M), \( \text{H}_2\text{O}_2 \) (250 \( \mu \)M), and sorbitol (10.0 or 1.0 mM, respectively). All samples were incubated on a benchtop under ambient light for 30 min. *versus \( \text{CpFe(CO)}_2\text{Ph} \) or 10 \( \mu \)M \( \text{H}_2\text{O}_2 \) relative to lane 1.
hydroxyl radical and hydroxide/water.26 Clearly, in our system, the identity of L₅nFe⁶⁺ 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)₃CH₃, the only complex whose photochemistry has been extensively studied.27 In this case, it has been proposed that CpW(CO)₂CH₃ (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 = PPh₃, CH₃CN, THF, or H₂O); and when M = W and L = PPh₃, 6 produces methyl radicals upon further photolysis.

Our observation that CpFe(CO)₂Ph (1) itself does not react directly with H₂O₂ to produce radicals is consistent with the reduction potential of the Fe(III) species [CpFe(CO)₂]⁺, which is expected to be similar to that measured for [CpFe(CO)₂]⁺ (1.47 V28). Likewise, other photochemically generated Fe(II) complexes, such as 6, exhibit unsuitable potentials,20 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(NCCCH₃)]⁺, has been reported as −0.5 V,20 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).

In conclusion, the presence of H₂O₂ 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 nM8), 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.

References and notes

17. For gel band quantitation data, see Supporting Information.