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Relative Efficiencies of $CpM(CO)_nCH_3$ and $CpM(CO)_nPh$ (M = Cr, Mo, W, and Fe) Complexes in Photoinduced DNA Cleavage

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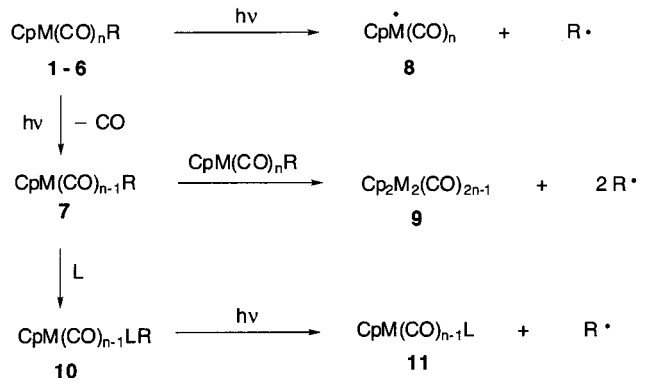
Abstract: The relative efficiencies of photoinduced DNA cleavage by complexes of the type $CpM(CO)_nR$ (M = Cr, Mo, or W, n = 3, R = CH_3 or Ph; M = Fe, n = 2, R = CH_3 or C_6H_5) have been investigated using a plasmid relaxation assay. Only the tungsten and iron complexes reproducibly caused single strand scission, in addition to which the iron systems efficiently gave double strand cleavage. The iron complexes gave strand scission at lower concentrations than the corresponding tungsten systems, with the phenyl complexes producing more damage than the methyl systems.

In developing agents for use in biological systems, two primary concerns are efficiency and selectivity, whether the application occurs in a therapeutic or laboratory setting. For example, the enediyne anticancer antibiotics incorporate both recognition elements and triggering devices, to target DNA for damage by the active radical species.[1] As part of an effort to exploit simple and readily available metal complexes as photoactivatable sources of organic radicals for the modification of biomolecules, we have optimized the DNA cleaving behavior of substituted analogues of $CpW(CO)_3R$ (R = CH_3, **1**[2] or C_6H_5, **2**) for nonrandom double-strand scission[3] and for sequence-specificity.[4] However, the effect of changing the metal center has not been assessed; therefore, we now report studies of the cleavage of plasmid DNA by $CpCr(CO)_3CH_3$ (**3**), $CpMo(CO)_3CH_3$ (**4**), $CpW(CO)_3C_6H_5$ (**2**), $CpFe(CO)_2CH_3$ (**5**), and $CpFe(CO)_2C_6H_5$ (**6**).

Aside from their obvious similarity to $CpW(CO)_3CH_3$, the above complexes were chosen because each has been easily prepared by literature methods and has been reported to be stable to the aqueous aerobic conditions required by DNA cleavage experiments. Most importantly, for each complex, there was some reported evidence for the desired photochemical production of carbon-centered radicals,[5,6,7,8] a general scheme for which is shown below. It is generally accepted that the primary



photoprocess for complexes **1**−**6**, in which R = CH_3 or C_6H_5, involves loss of carbon monoxide (to give **7**), which may be accompanied by homolysis of the metal−methyl or metal−aryl bond to yield the metal-based radical **8** 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.[9,10] In this case, it has been proposed that $CpW(CO)_2CH_3$ (**7**) reacts with another molecule of starting material to produce the metal−metal bonded species **9** and two methyl radicals. Furthermore, it has been demonstrated that the 16 electron species $CpW(CO)_2CH_3$ (**7**) can coordinate a variety of ligands (e.g., L = PPh_3, CH_3CN, THF, or H_2O), and when $CpW(CO)_2(PPh_3)CH_3$ (either purified or produced in situ during the photolysis of **1** in the presence of PPh_3) is photolyzed, methyl radicals are formed. It is such carbon-centered radicals that have been implicated as the active species leading to DNA strand scission.[2,4]

The DNA cleaving activity of each of the complexes was determined using a plasmid relaxation assay to monitor the conversion of circular supercoiled DNA (form I) to relaxed circular (form II) and linear DNA (form III). Each compound was photolyzed through a Pyrex filter in the presence of pBR322 DNA, and the amounts of double- and single-strand scission was assessed via agarose gel electrophoresis (Figure 1). Quantitation[11] of the bands in these gels indicated that form II DNA resulting from single strand cleavage was present at complex concentrations of 22.5, 11.3, and 2.8 μM for $CpW(CO)_3C_6H_5$ (a, lane 8), $CpFe(CO)_2CH_3$ (b, lane 9), and $CpFe(CO)_2C_6H_5$ (c, lane 11), respectively. Additionally, form III DNA (presumably arising from random, proximal single strand cuts) was observed for the photolysis of $CpFe(CO)_2CH_3$

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(11) Densitometry was accomplished with the NIH ImageJ software program. The amount of supercoiled DNA was multiplied by a factor of 1.22 to account for reduced ethidium bromide intercalation into the form I plasmid DNA.

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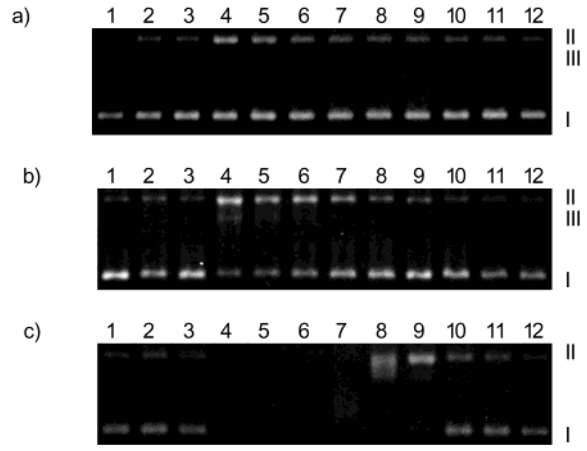

FIGURE 1. Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/20 mM Tris buffer, pH 8) by $CpW(CO)_3C_6H_5$ (a), $CpFe(CO)_2CH_3$ (b), and $CpFe(CO)_2C_6H_5$ (c). Lane 1, DNA alone; lane 2, DNA + complex (360 μM), no irradiation; lane 3, DNA alone, irradiated; lanes 4 through 12, DNA + complex (360, 180, 90, 45, 22.5, 11.3, 5.6, 2.8, and 1.4 μM, respectively). Mixtures in lanes 3−12 were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 20 min.

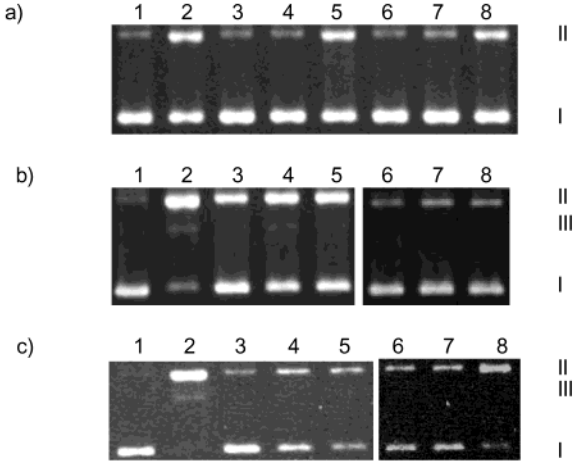

FIGURE 2. Effects of radical trapping agents on the photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/20 mM Tris buffer, pH 8) by 360 μM $CpW(CO)_3C_6H_5$ (a), 45 μM $CpFe(CO)_2CH_3$ (b), and 24 μM $CpFe(CO)_2C_6H_5$ (c). Lane 1, DNA alone; lane 2, DNA + complex; lanes 3−5, DNA + complex + cysteine (100, 10, and 1 equivalents vs metal complex, respectively); lanes 6−8, DNA + complex + TEMPO (100, 10, and 1 equivalents, respectively). Mixtures in lanes 2−8 were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 20 min.

and $CpFe(CO)_2C_6H_5$ at 45 and 11.3 μM, respectively. These latter values correspond to ratios of 1.5 and 0.38 molecules/bp and are similar to the ratio reported for the photoinduced double-strand cleavage of DNA by the natural enediyne dynemicin (0.75 molecules/bp[12]). These results, coupled with our previous finding that $CpW(CO)_3CH_3$ cleaves DNA in a single-stranded manner at 45 μM, show two trends. The iron complexes are more efficient, giving single-strand cleavage at lower concentrations than the corresponding tungsten systems and producing double-strand scission (which is not observed for either tungsten complex). Furthermore, for each metal, the phenyl complex is active at lower concentrations than the methyl system. In all cases, control experiments (lanes 2 and 3) show that both light and the complex are necessary for strand scission to occur.

To investigate the potential involvement of carbon-centered radicals in the mechanism leading to strand scission, the photolysis of **2**, **5**, and **6** was conducted in the presence of cysteine, a general radical trap,[13] or a nitroxide species, which traps carbon-[14] and metal-centered radicals,[15] but not oxygen-based radicals. The results of these trapping experiments are shown in Figure 2. In all cases, the presence of either trapping agent suppressed strand scission, thus implicating either a metal- or carbon-centered radical in the mechanistic pathway ultimately resulting in strand scission. The involvement of metal-based radicals of the formula **8** was ruled out by generating them via the photolytic homolysis of the metal−metal bond[16] in the dimers $[CpW(CO)_3]_2$ and $[CpFe(CO)_2]_2$ in the presence of DNA (Figures S1 and S2, Supporting Information). With these complexes, much higher organometallic concentrations than those used for the metal−alkyl and −aryl complexes were necessary to cause cleavage. For example, while $CpW(CO)_3R$ concentrations of 45 μM (R = CH_3) or 22.5 μM (R = C_6H_5) led to single-strand scission, 900 μM $[CpW(CO)_3]_2$ was required to give only a minor amount of cleavage. For the minimal activity exhibited by the bimetallic systems, the mechanism is not expected to involve hydrogen atom abstraction by the metal radical,[17] since this process is disfavored both thermodynamically (as indicated by the relative bond dissociation energies of the metal hydrides[18] and hydrocarbons[19]) and kinetically.[20] Therefore, it is unlikely that these metal radicals are the primary active species responsible for strand scission in the photolysis of $CpM(CO)_nR$. These findings are consistent with our previous implication of a carbon-centered radical in the mechanistic pathway leading to strand scission by $CpW(CO)_3CH_3$.[2]

Because there is little data in the literature on the quantum yields, partitioning between the two primary photoprocesses, or kinetics of later reactions, explaining the DNA cleaving trends is difficult. The reported quantum yields for the disappearance of $CpW(CO)_3CH_3$ ($\Phi_{366} \sim 0.40$[10]) and $CpFe(CO)_sCH_3$ ($\Phi_{366} = 0.70$[21]) in the

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presence of external ligands are in line with the observed cleaving activities, and it is not unreasonable to expect the phenyl complexes to follow the same trend. Additionally, the higher activity of the phenyl complexes over the methyl systems is consistent with the greater reactivity of phenyl radical in hydrogen atom abstraction, as predicted by C−H bond dissociation energies.[19]

In contrast to the highly efficient strand scission exhibited by the tungsten and iron complexes, the photolysis of neither $CpCr(CO)_3CH_3$ nor $CpMo(CO)_3CH_3$ gave reproducible evidence of DNA cleavage. In the case of the molybdenum complex, this result is surprising in light of the reported observation of methane[6] and spin-trapping of methyl radical.[7] For the chromium complex, however, methyl radical has not been detected upon irradiation;[7] although homolysis of the chromium−methyl bond has been inferred from the photolytic production of $[CpCr(CO)_3]_2$ and methane.[6,8]

In summary, DNA cleavage has been demonstrated to occur upon photolysis of complexes of the formula $CpM(CO)_nR$, in which M = W, Fe and R = CH_3, C_6H_5, but not when M = Cr or Mo. The iron complexes gave strand scission at lower concentrations than the corresponding tungsten molecules, in addition to giving double strand cleavage. The R group also affected activity, with the phenyl complexes cleaving more efficiently than the methyl systems.

Experimental Section

General. Sodium cyclopentadienide, $CpFe(CO)_2I$, MeLi, $Cr(CO)_6$, $W(CO)_6$ $[CpW(CO)_3]_2$, and MeI were used as obtained. Molybdenum hexacarbonyl and $[CpFe(CO)_2]_2$ were purchased and used without further purification. THF and Et_2O were distilled from sodium benzophenone ketal just prior to use. Zinc(II) chloride was purchased and purified by melting under vacuum to remove residual HCl. The complexes $CpCr(CO)_3CH_3$[22] and $CpMo(CO)_3CH_3$,[23] were prepared from the tricarbonylcyclopentadienylmetal anions and iodomethane, while $CpFe(CO)_2CH_3$ was synthesized from while $CpFe(CO)_2I$ and MeLi.[24] The synthesis of $CpW(CO)_3C_6H_5$[25] employed the corresponding metal anion and diphenyliodonium chloride. The preparation of $CpFe(CO)_2C_6H_5$ was accomplished by a palladium-catalyzed arylation of $Zn[CpFe(CO)_2]_2$.[26]

DNA Cleavage Studies. General. Purified, deionized water was obtained by filtration with a four cartridge apparatus and was used for all aqueous reactions and dilutions. Plasmid pBR322 DNA (3461 bp) was obtained from New England Biolabs. High strength analytical grade agarose was used. Gel electrophoresis was carried out with 1% agarose gels and 90 mM TBE buffer. The concentrated loading buffer for agarose gels consisted of 35% (w/v) sucrose solution containing 0.20% bromophenol and 0.20% xylene cyanol FF.

Plasmid Relaxation Assays. A DMSO solution was made of the compound of interest and serial dilutions were made. The appropriate DMSO solution was added to a 1.5 mL plastic centrifuge tube containing 9 times the volume of a solution containing 33.3 μM/bp DNA (pBR322) in 20 mM Tris-HCl reaction buffer pH 8 (final concentration = 30.0 μM/bp). The tubes were then strapped to the outside of a water-jacketed reaction vessel for a photolysis apparatus with a Pyrex filter and irradiated with light from a 450 W medium-pressure mercury arc lamp for 20 min. After the irradiation, 5 μL of loading buffer was added to each tube, and the contents of the tube were loaded onto a 1% agarose gel and electrophoresed for 12 h at 30 V. The gel was then stained in a dilute solution of ethidium bromide (~0.5 μg/mL) for 10 min and then destained with water. The DNA was visualized with UV light and photographed using a Polaroid DS34 camera with black and white Polaroid 667 film.

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Supporting Information Available: Plasmid assay gel quantitation data and gels for control experiments with $[CpFe(CO)_2]_2$ and $[CpW(CO)_3]_2$. This material is available free of charge via the Internet at http://pubs.acs.org/.

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