Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 871–874

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ORGANOMETALLIC PHOTONUCLEASES: A NOVEL CLASS OF DNA-CLEAVING AGENTS†

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Received 3 November 1997; accepted 5 March 1998

Abstract: The first demonstration of DNA cleavage by an organic radical generated via homolysis of a metal–alkyl bond in a Cp-metal complex is presented. Irradiation of $CpW(CO)_3CH_3$ (1.5 molecules/base pair) produced methyl radical, giving single-strand cleavage of pBR322 DNA. This process was inhibited by the general radical trap cysteine and by TEMPO, which traps carbon radicals but not oxygen-centered radicals.

Metal complexes are biologically important species that are crucial to a wide variety of processes including oxygen transport,[1] digestion,[2] and gene transcription.[3] For example, zinc-finger proteins regulate gene expression by complexing DNA,[3] and some naturally-occurring metal complexes have been exploited as DNA-cleaving medicinal agents.[4] Most of the work involving the interactions of metal complexes with DNA has focused on inorganic substrates containing amines or carboxylates as chelators, yielding systems that are highly useful for obtaining nucleic acid structure and binding information as well as cleaving, alkylating, or crosslinking DNA.[5,6] In contrast, fewer studies have employed organometallic species, which usually probe or modify oligonucleotides via complexation of DNA components to the metal center without direct participation of the organic ligands.[7] We found in the literature only one example of an organic ligand serving as the active species in DNA cleavage, through photolytic generation of a carbon-based radical by homolysis of a metal–carbon sigma bond in an alkyl cobalt cyclam complex.[8]

In an effort to develop organometallic compounds as new agents for the modification of oligonucleotides, we have begun to study the reactivity of cyclopentadienyl (Cp) metal complexes towards DNA. Representing a large subset of organometallic compounds, these complexes offer a diverse array of reactivities and their many spectroscopic characteristics allow one to monitor their reactions and binding to DNA. Their Cp ligands are tightly bound,[9] providing a location to attach DNA-recognition elements without perturbing the reactivity of the metal center. Furthermore, the photolability of the metal-alkyl sigma bond in Cp metal alkyl complexes can be used to generate highly reactive carbon-centered radicals. The cleavage of DNA by such radical intermediates is currently the subject of intense investigation in the fields of chemistry, biology, and medicine, as evidenced by the impressive efforts directed toward the enediyne anticancer antibiotics and their analogs[10,11] and by the development of simpler but still highly effective organic radical sources.[12]

For initial studies, we chose to investigate the DNA cleavage behavior of $CpW(CO)_3CH_3$ (**1**) because its photochemical behavior has been well studied and has been reported to produce methyl radical via metal-alkyl bond homolysis at wavelengths greater than 300 nm (eq 1),[13,14] conditions which do not degrade DNA.

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0960-894X/98/$19.00
PII: S0960-894X(98)00127-9

Additionally, It has been reported to be air- and water-stable and can be synthesized in one step from commercially available materials.[15] Despite such well-characterized methods for generating highly reactive radicals, **1** and similar compounds have never been developed as biologically active agents.

$$\underset{\mathbf{1}}{CpW(CO)_3CH_3} \xrightarrow[-CO]{h\nu} CpW(CO)_2CH_3 \longrightarrow \overset{\bullet}{CpW}(CO)_2 + \overset{\bullet}{C}H_3 \quad (1)$$

To test the hydrogen atom abstraction ability of the photogenerated methyl radical, the photolysis of **1** (3.3 M in THF-d_8) through a Pyrex filter was conducted (eq 2). The deuterated THF was used to model the sugar portion of the DNA backbone, since similar experiments with DNA would require deuterated DNA and because the concentrations at which the DNA cleavage experiments are conducted are typically too low to allow for the unambiguous detection of the radical-derived products. Preliminary results indicated that the prevailing methyl radical-derived products detected by GC-MS were methane and methane-d_1 (in approximately a 2:1 ratio), a result which is in accord with the production of methyl radical followed by hydrogen or deuterium atom abstraction from the Cp ring[14] or THF-d_8. A control experiment conducted in nondeuterated THF showed

$$\underset{\mathbf{1}}{CpW(CO)_3CH_3} \xrightarrow{h\nu,\ THF\text{-}d_8} [CpW(CO)_3]_2 + CH_4 + CH_3D \quad (2)$$

only negligible amounts of a species with an *m/z* of 17. Since the production of non-deuterated methane can be attributed to a cage effect, the detection of CH_3D suggests that methyl radical should be able to abstract hydrogens from the sugar-phosphate backbone of DNA. Although abstraction from the Cp ring competes with that from THF-d_8 in this simple photolysis experiment, the cage effect apparently does not prevent DNA cleavage (*vide infra*), in which the radical is produced in the highly structured environment of DNA.

To examine the DNA-cleaving ability of complex **1**, it was irradiated at various concentrations in the presence of pBR322 DNA (Figure 1). This resulted in the conversion of circular supercoiled DNA (form I) to circular relaxed DNA (form II) via single-strand cleavage (lanes 4–9). Control experiments showed that both light and **1** were required to effect cleavage (lanes 2 and 3). The cleavage occurred in a concentration dependent manner at as few as 1.5 molecules/base pair, a surpisingly low ratio considering that the complex does not contain a DNA binding element and that most synthetic enediynes (which generate diradicals) without

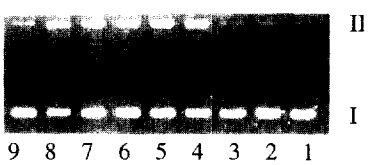

Figure 1. Photo-induced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by $CpW(CO)_3CH_3$ (**1**). Lane 1, DNA alone; lane 2, DNA alone, irradiated; lane 3, DNA + complex (720 mM), no irradiation; lanes 4 through 9, DNA + complex (720, 360, 180, 90, 45, and 23 μM, respectively). Reactions in lanes 4–9 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 minutes.

recognition devices exert this behavior at 100-1000 molecules/base pair.[10] The use of other organic solvents (EtOH, dioxane, THF) to solubilize the complex gave cleavage at similar molecule/base pair ratios as DMSO. This result represents the first demonstration of DNA cleavage by homolysis of a metal-alkyl bond in a Cp-metal complex.[16]

To determine whether methyl radicals participate in the cleavage process, additional experiments were conducted in which radical scavengers were added to the reaction mixtures before photolysis. When 80 equivalents of cysteine, which can function as a general radical trap,[17] is present in the reaction mixture (Figure 2), no cleavage is observed, as expected. To discriminate between methyl radicals and oxygen-centered radicals,

Figure 2. Inhibition by cysteine and TEMPO of cleavage of pBR322 DNA (44.1 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by $CpW(CO)_3CH_3$ (**1**). Lane 1, DNA alone; lane 2, DNA + complex (0.9 mM); lanes 3–7, DNA + complex (0.9 mM) + cysteine (72, 36, 18, 9, and 0.9 mM, respectively); Lanes 8–12, DNA + complex (0.9 mM) + TEMPO (72, 36, 18, 9, and 0.9 mM, respectively). Reactions in all lanes except 1 were irradiated as described above.

cleavage experiments were conducted in the presence of 2,2,6,6-tetramethyl-1-piperdinyloxy (TEMPO), a stable nitroxide which can be used to selectively trap carbon radicals.[18] Thus, when any amount of TEMPO was present, the amount of form II DNA was decreased. While this argues against the involvement of oxygen-centered radicals, it does not preclude the participation of metal-based radicals, since nitroxides have been shown to react with a similar metal-centered radicals.[19] However, the contribution of such radical species to the cleavage is expected to be minimal, because the metal-hydrogen bond strength in similar systems are typically too low[20] to energetically favor hydrogen abstraction from DNA by such radicals. Interestingly, when the photolysis of $[CpW(CO)_3]_2$ (which generates $CpW(CO)_3$ radical but obviously no methyl radical) is conducted in the presence of DNA, a very small amount of cleavage was seen only at concentrations greater than 6 molecules/base pair and was shown to be inhibited by the presence of TEMPO. Therefore, although an active metal species formed in the reaction cascade may contribute a minor amount, these results implicate methyl radicals or reactive intermediates mechanistically downstream from methyl radicals as the active species giving rise to most of the DNA cleavage resulting from photolysis of **1**.

In summary, we have reported the first example of DNA cleavage by the homolysis of metal–alkyl bond in a Cp-metal complex, as an initial step in developing such compounds as agents for the modification of nucleic acids. Current work is directed towards the further elucidation of the mechanism of cleavage as well as the attachment of DNA recognition elements to such complexes.

Acknowledgement. We gratefully acknowledge financial support provided by West Virginia University and the American Cancer Society (IRG-204).

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