Methyl Radical-Initiated DNA Cleavage Facilitated by a Discrete Organometallic Complex

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Metal complexes have shown great utility as tools in molecular biology for recognizing and understanding DNA structure,[1] as metalloproteins which regulate gene expression by binding to DNA,[2] and as medicinal agents.[3] Inorganic metal complexes have been employed in the majority of these studies[4] with less attention given to organometallic complexes.[5] The organometallics studied have used the carbon moieties (Cp, Cp*) solely as spectator ligands. In contrast, we have begun a program aimed at exploiting organometallic complexes with reactive metal–carbon σ-bonds as sources of carbon radicals. While there are various methods for generating carbon radicals, organometallic complexes with reactive alkyl/aryl groups are uniquely attractive probes for the study of carbon-centered radical–DNA reactions. These species possess numerous spectroscopic handles, which facilitate characterization and kinetic monitoring of reactions and are capable of liberating radicals photolytically, thermally, or chemically. The metal complexes provide the opportunity to observe equilibrium binding (electrostatic, intercalative, groove) to nucleic acids either via the inherent physical properties of the species or by tethering oligonucleotides to the ligand periphery imparting sequence specificity. In this manner, one may establish and, ideally, systematically evaluate the generation site of the carbon radical by determining the binding site(s) of the organometallic complex. Carbon-centered radical degradation of DNA is of significant current interest. The metabolism of hydrazine and its derivatives is thought to involve free-radical intermediates. Ortiz de Montellano and co-workers have characterized organometallic and radical intermediates in the oxidation of hydrazines by hemoproteins.[6] Phenelzine ((2-phenethyl)-hydrazine), an antidepressant, when metabolized by microsomes produces the phenethyl radical, which has been directly implicated in DNA strand scission.[7] Of particular relevance, 7-methylguanine and 8-methylguanine are products of methyl radical DNA alkylation during methylhydrazine oxidation by horseradish peroxidase.[6e] Additionally, the mechanism of action of the anticancer, antibiotic enediynes is thought to proceed through double-stranded DNA scission via hydrogen atom abstraction initiated by a 1,4-benzenoid diradical.[8]

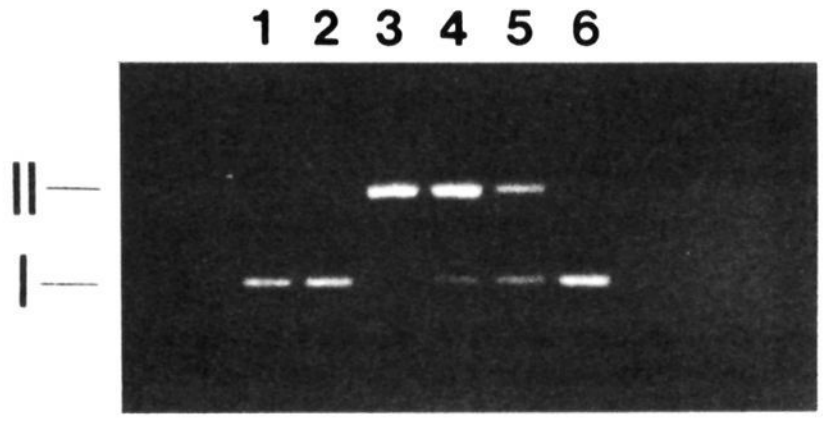

Figure 1. 0.8% agarose gel showing the results of electrophoresis of *pBluescript* II KS(–) DNA (1.5 mM per base pair) after 2 h of visible irradiation (except lane 2) in the presence of (1) DNA alone; (2) **1** (2 mM) with no irradiation; (3) **1** (2 mM); (4) **1** (500 μM); (5) **1** (100 μM); (6) **1** (2 mM) which was first irradiated for 12 h and then added to DNA and further irradiated for 2 h. Form I is supercoiled DNA and form II is nicked circular DNA.

$Co(cyclam)Me(H_2O)(ClO_4)_2$ (**1**) provides an excellent entry into these studies due to its stability to both water and oxygen and its well-characterized photochemistry.[9,10] Photolysis has been shown to generate methyl radicals via rate-limiting Co–Me bond homolysis (eq 1).[9] Aerobic photolysis of aqueous solutions of **1**

$$Co^{III}(cyclam)(H_2O)Me^{2+} \overset{h\nu}{\rightleftharpoons} Co^{II}(cyclam)(H_2O)^{2+} + Me^{\bullet} \quad (1)$$

and plasmid DNA with ordinary room light for 2 h results in conversion of supercoiled DNA to the nicked circular form (Figure 1, lane 3).[11] The extent of DNA modification is dependent on the concentration of **1** (lanes 3, 4, and 5). Longer photolysis times result in increased yields of nicked DNA. Importantly, in the *absence of light*, **1** is incapable of modifying supercoiled DNA (lane 2). Control experiments show that the cobalt-containing product of photolysis, the peroxo dimer $[(H_2O)Co(cyclam)]_2O_2$, does not alter DNA under photolytic conditions (lane 6).

To implicate radicals in the DNA modification, several radical scavengers were used. Cysteine, which we view as a general radical scavenger capable of reacting with radicals via hydrogen atom

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(10) cyclam = 1,4,8,11-tetraazacyclotetradecane; TEMPO = 2,2,6,6-tetramethyl-1-piperdinyloxy.

(11) $Co(cyclam)Me(H_2O)(ClO_4)_2$ was prepared according to Endicott's procedure[9c] and characterized by ^1H NMR and electronic spectroscopy (λ_{max} = 370, ϵ = 101 M^{-1} cm^{-1}; 476, ϵ = 80 M^{-1} cm^{-1}). *pBluescript* KS(–) was extracted from *Escherichia coli* XL1-Blue, ϵ_{260} = 12 300 M^{-1} cm^{-1} according to: Sambrook, J.; Fritsch; Maniatis *Molecular Cloning, A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989. All DNA experiments were run in TE-buffered solutions, pH = 7.4. The agarose gels were stained with ethidium bromide and photographed under ultraviolet light.

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abstraction from the thiol,[12] decreases the yield of nicked DNA.[13] At sufficiently high cysteine concentrations, [cys]/[1] = 40, only supercoiled DNA is observed. To differentiate between activity derived from the photogenerated Co^{II} such as metal superoxo, peroxo, etc., and the methyl radical, we sought a scavenger which would react selectively with Me•. TEMPO,[10] a stable nitroxyl radical, is used extensively in organometallic chemistry to selectively trap alkyl radicals generated upon metal–carbon bond homolysis.[14] It has been used effectively for other Co alkyl complexes, most notably B_{12} derivatives.[15] 4-Hydroxy-TEMPO, HTEMPO, a water-soluble derivative, proved suitable for the current studies. Photolysis of **1** and DNA in the presence of increasing concentrations of HTEMPO results in decreasing yields of nicked DNA.[13] With HTEMPO in large excess, [HTEMPO]/[1] = 40, the cleavage reaction is almost completely inhibited. These results strongly implicate methyl radicals or reactive intermediates mechanistically downstream from methyl radicals as the competent species in DNA modification. Methyl radicals react with oxygen at diffusion-controlled rates (eq 2).[16] However,

$$CH_3^{\bullet} + O_2 \xrightarrow{10^9\ M^{-1}\ s^{-1}} CH_3O_2^{\bullet} \qquad (2)$$

it has been argued that the resulting methylperoxy radical is a poor hydrogen atom abstractor.[17] Anaerobic experiments are underway to evaluate the role of oxygen and alkylperoxo intermediates in this system.

Metal-mediated oxidation of phenylhydrazine leading to DNA damage is a complex process involving hydroxyl radicals in addition to phenyl radicals, the former of which are highly efficient species in DNA degradation.[18] Yamamoto and Kawanishi[6f] found hydroxyl radicals more active in DNA modification than phenyl radicals. To rule out the involvement of a hydroxyl radical pathway in our system, ethanol was used as a hydroxyl radical scavenger.[6f,19] Even at 0.2 M EtOH, **1** (2 mM) completely converts supercoiled DNA to the nicked form.[13] The lack of an inhibitory effect by EtOH argues against the existence of hydroxyl radicals.

Product analysis by HPLC of the photolysis of **1** with either fish sperm DNA or the single-stranded oligonucleotide 5′-TGAATTCGGATATCAGT shows the liberation of all four free bases, C, G, T, and A.[20] In each case, the total concentration of free base represents approximately 1–2% of the concentration of **1**. Base release is indicative of nucleic acid oxidation occurring via hydrogen atom abstraction from the deoxyribose ring.[21] Production of all four free bases suggests that the methyl radical is highly reactive and incapable of discriminating among the nucleotides. This result is consistent with the large bond dissociation energy for CH_3–H (104 kcal/mol) providing a sufficient driving force for H-atom abstraction from any one of the positions on the sugar.[3c,21] The relatively low yield of base production suggests that most of the methyl radicals do not react with the DNA and/or react at other positions which do not result in immediate base release (such as base methylation).[6e] The binding affinity of **1** as estimated from polyelectrolyte theory is almost certainly $\leq 500\ M^{-1}$.[22] This low binding constant and the weak photolysis source probably contribute to making the efficiency appear lower than its actual value.[23] Experiments designed to quantify the efficiency of the DNA modification reactions using a controlled light source are underway.

The present results provide the first example of utilizing the reactive metal–carbon σ-bonds of an organometallic complex to modify DNA and suggest that cobalt alkyl complexes and perhaps other organometallics are well suited to serve as carbon-centered radical "carriers" in the study of radical–DNA interactions. This underexplored route to carbon radicals has proved successful in generating the radicals under the mild conditions of room light. Importantly, complex **1** is capable of modifying different nucleic acid structures including single- and double-stranded DNA and plasmids. The release of all free bases suggests that the methyl radical accesses H-atom abstraction pathways similar to those accessed by the hydroxyl radical and, therefore, may be suitable for footprinting experiments, particularly in systems which are difficult to evaluate in the presence of EDTA.[1] We also are preparing derivatives of **1** capable of generating radicals such as $Pr^{i\bullet}$, which are both larger and have a weaker thermodynamic driving force than Me• for H-atom abstraction, in hopes of imparting selectivity.

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Supplementary Material Available: Figures showing the electrophoresis gels of photolyses of **1** and DNA with radical traps and the HPLC chromatogram of the products of fish sperm DNA oxidation by **1** (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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