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Light-Induced Cleavage of DNA by Metal Complexes

Sir:

Single-strand cleavage of the sugar–phosphate backbone of double-stranded DNA ("nicking") can be induced by several groups of compounds, most prominent of which are bleomycins,[1] neocarzinostatin,[2] daunomycin,[3] and mitomycin.[4] Some metal complexes are capable of oxidatively cleaving DNA, including methidium-tethered Fe(II)–EDTA complexes[5] and bis(1,10-phenanthroline)copper(I) $[Cu(phen)_2]^+$.[6] A metal cofactor (presumably ferrous ion in living systems[7]) and molecular oxygen are required to activate bleomycin toward DNA strand scission. In fact, ferrous ion in the presence of oxygen will cleave DNA in the absence of bleomycin, but the cleavage is less efficient. Several metals are useful cofactors of bleomycins in vitro, including Fe(II),[8-12] Co(III),[13,14] Mn(II),[15-17] and Cu(I).[18] In addition to the requirement of a metal, activation of the cleavage process requires oxidant (e.g., molecular oxygen), or in the case of Co(III), Mn(II), or Fe(III), ultraviolet light. Recently, we reported the ultraviolet-light-induced DNA strand scission in vitro by tris(1,10-phenanthroline)cobalt(III) ($Co(phen)_3^{3+}$) and tris(4,7-diphenylphenanthroline)cobalt(II) ($Co(dip)_3^{3+}$) complexes.[19] In this paper, we wish to report some preliminary findings on the properties and diversity of photoactivated cleavers of DNA. We will show that a range of simple octahedral inorganic complexes are capable of inducing single-strand scissions by photoexcitation of different absorption bands. Moreover, we will show that this cleavage can be induced by metal complexes known to bind in different manners to DNA.

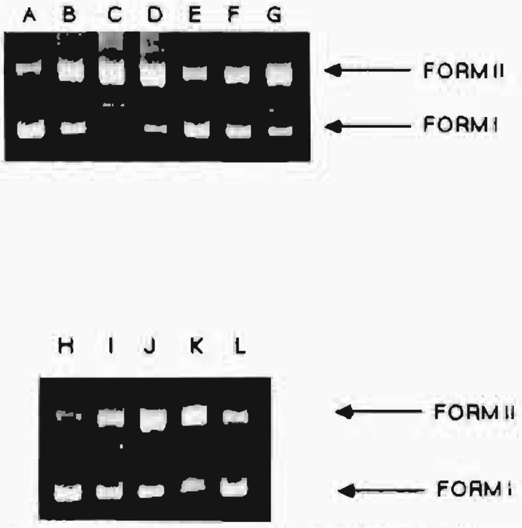

Figure 1. Photoactivated cleavage of 100 μM pBR322 DNA with 10 μM complex after 5-min irradiation at 325 nm (Liconix He/Cd Laser) in 10 μL of 50 mM Tris, 18 mM NaCl, and pH 7.0 solution with A = no complex, B = 1,10-phenanthroline, C = $Rh(phen)_3^{3+}$,[34] D = $Co(phen)_3^{3+}$,[35] E = $Co(bpy)_3^{3+}$,[36] F = $Co(en)_3^{3+}$, and G = $Co(NH_3)_6^{3+}$ and after 15-min irradiation at 442 ± 15 nm (Hg/Xe lamp, Oriel) with H = no complex, I = $Co(NH_3)_6^{3+}$, J = $Co(phen)_3^{3+}$, K = $Ru(phen)_3^{2+}$,[37] and L = $Ru(bpy)_3^{2+}$. Samples were subjected to electrophoresis on 1% agarose gels followed by staining with ethidium bromide.

Low-spin d^6 transition-metal complexes (e.g., Co(III), Rh(III), and Ru(II)) show several types of electronically excited states including ligand field (LF), intraligand (IL), and charge-transfer (CT) states.[20] Representative examples of each of these types of d^6 metal complexes were analyzed in the presence of supercoiled plasmid (form I) DNA (pBR322) to determine the amount of photoactivated strand scission induced by the complex (amount of form II DNA produced). In terms of complexation with DNA, we were also interested in distinguishing among complexes with a ligand capable of intercalating between base pairs of the DNA (e.g., phen),[21,22] those involved in weaker electrostatic binding to

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Table I. Photoactivated Cleavage of DNA Induced by Metal Complexes

species	wavelength, nm	excited state[a]	log ε	relative quantum yield for form II DNA formation[b]	cleavage enhancement in D_2O[c]	effect of histidine on cleavage[d]
$Co(phen)_3^{3+}$	325	IL	3.8	1	no	none
	442	LF	2.3	1	yes	decrease
$Co(NH_3)_6^{3+}$	325	LF	2.0	60	no	none
	442	LF	1.8	1.2	no	NA
$Co(en)_3^{3+}$	325	LF	1.7	10	no	none
$Co(bpy)_3^{3+}$	325	IL	4.1	0.2	no	increase
$Rh(phen)_3^{3+}$	325	^{3}IL	3.7	1.5	no	none
$Ru(phen)_3^{2+}$	442	^{3}MLCT	4.4	0.017	yes[e]	decrease
$Ru(bpy)_3^{2+}$	442	^{3}MLCT	4.2	0.003	yes	NA
$Cr(phen)_3^{3+}$	325	IL	4.0	0		
	442	LF	3.0	0		
phen	325		2.6	20	yes	decrease

[a] IL = intraligand, LF = ligand field, MLCT = metal-to-ligand charge transfer. See ref 20 for assignments. [b] Quantum yields relative to $Co(phen)_3^{3+}$ (assigned a value of 1.0 for 442 nm and 1.0 for 325 nm) were determined by using a laser densitometer (LKB) interfaced to an IBM PC to scan the form I and form II bands of pBR322 DNA after gel electrophoresis and ethidium bromide staining. Absolute quantum efficiency for cleavage per plasmid at 325 nm is 10^{-7} and at 442 nm is roughly 10^{-4}, although variations with DNA concentration and superhelical density have been evident. Values relative to $Co(phen)_3^{3+}$ were, however, all obtained under identical conditions. The values are corrected for absorptions (without DNA) and are within ±5%. All irradiations were performed as indicated in Figure 1 and involve subtraction of appropriate light controls. [c] Cleavage enhancement in D_2O (>90% D_2O in the sample during irradiation) is reported here only for enhancements of greater than a factor of 2. In some cases, a D_2O enhancement is observed after long irradiation times. [d] Samples were made 1.2 mM in histidine, pH 8, immediately before irradiation and were ethanol-precipitated immediately after irradiation. [e] Danishefsky, A. T. Ph.D. Thesis.

the DNA (e.g., bipyridyl (bpy)),[21a] and those hydrogen bonded (groove bound) to the helix (e.g., amines).[23] In cases where the excited-state populations depend on the excitation wavelength, we distinguished between the amount of cleavage induced by two different wavelengths (325 and 442 nm).

Shown in Figure 1 is a pictorial representation of the results of these experiments conducted with a constant concentration of metal complex. In this figure is illustrated the comparison and practical application of these DNA photocleavers. Under identical conditions, but without light, none of these metal complexes induce strand scission. Table I includes the same results quantitated. Here the quantum yield represents the efficiency of strand scission per photon absorbed. One can see in Table I, for example, that $Co(NH_3)_6^{3+}$ shows a significantly higher quantum efficiency for cleavage than does $Co(phen)_3^{3+}$. However, in Figure 1 it is apparent that $Co(NH_3)_6^{3+}$ is a comparable or slightly poorer photocleaver compared to $Co(phen)_3^{3+}$ at the same concentration owing to its lower absorptivity at 325 nm. It is also noteworthy that whereas several types of d^6 metal complexes induce single-strand scissions in supercoiled DNA upon near-UV or visible irradiation (Co(III), Rh(III), and Ru(II)[24]), the d^3 complex $Cr(phen)_3^{3+}$ appears to be inert under similar conditions. The prevalence of photosubstitution over photoredox processes for $Cr(phen)_3^{3+}$ may be important in accounting for this result.[25] Especially noteworthy is the induction of cleavage with $Co(phen)_3^{3+}$ through excitation in both the IL and LF bands.

To test the possibility that photoinduced cleavage involves the formation of singlet oxygen, which is known to react with guanine residues at neutral pH,[26,27] the cleavage was tested in the presence of D_2O. Singlet oxygen would be expected to induce more strand scissions in D_2O than in H_2O due to its longer lifetime in the former solvent.[28] As can be seen in Table I, several of the examples, including $Ru(phen)_3^{2+}$ and $Co(phen)_3^{3+}$ (for LF excitation) show such an enhancement. Indeed, phenanthroline itself induces strand scissions with a marked D_2O enhancement, suggesting that photoexcitation of phenanthroline leads to oxygen sensitization.[29] The cleavage with $Ru(bpy)_3^{2+}$, a known oxygen sensitizer (producing singlet oxygen),[30] shows the expected enhancement in D_2O. For the IL states of $Co(phen)_3^{3+}$ and $Rh(phen)_3^{3+}$, cleavage is not enhanced in the presence of D_2O, suggesting that a different mechanism is involved for this type of excitation. Moreover, studies with the singlet oxygen quencher histidine[31] (see Table I) indicate that for those examples where an enhancement in the amount of single-strand cleavage is observed in D_2O, a parallel decrease in the amount of strand scissions occurs in the presence of histidine. Furthermore, no inhibition was observed for those examples that showed no D_2O effect. These observations are consistent with at least two operational mechanisms for the light-induced strand scission reaction, one of which appears to involve the production of singlet oxygen.[32] Although the reactivity of singlet oxygen with DNA is generally low,[27] if generated locally, its reactivity becomes considerably higher.[33]

Different binding modes to the helix may be examined as well. While $Co(phen)_3^{3+}$ and $Ru(phen)_3^{2+}$ intercalate into double-stranded DNA, $Co(bpy)_3^{3+}$ and $Ru(bpy)_3^{2+}$ only electrostatically bind.[21a] Thus, in spite of their similar electronic structures (and presumably similar cleavage mechanisms), the bipyridyl complexes cleave DNA to a much lesser extent than the corresponding phenanthroline complexes. In fact the ratio of quantum yields for cleavage of the bipyridyl complex to the phenanthroline complex for Co(III) and Ru(II) is identically 1:5 and likely reflects the relative DNA binding affinity of bipyridyl and phenanthroline complexes.

In comparing a series of cobaltic polyamine complexes, we see variations both in binding modes and mechanisms for cleavage. With $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$, no IL or low-lying CT states

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are available as with the ligand π to metal e_g* states of $Co(phen)_3^{3+}$ and $Co(bpy)_3^{3+}$. Both $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ possess two LF bands. Photoexcitation of either LF band leads to a high degree of DNA cleavage. Clearly neither $Co(NH_3)_6^{3+}$ nor $Co(en)_3^{3+}$ bind to DNA via intercalation. Indeed these complexes are known to maintain intimate hydrogen-bonding interactions with the helix.[23] Again, coupling inefficient photoreactions to DNA binding of all sorts may yield useful photocleavers. These photoactivated cleavage reactions, in particular those with $Co(NH_3)_6^{3+}$, might be helpful in mapping and photofootprinting experiments.

These results indicate that light-induced cleavage of DNA by a range of d^6 metal complexes can occur from CT, LF, and IL states of simple inorganic complexes, albeit via different mechanisms. Cleavage occurs with complexes having positive charges of either 2+ or 3+. The complexes cleave DNA in spite of possessing different binding modes to the DNA (intercalative, hydrogen bonded, and electrostatic). It may therefore be possible to couple the binding mode of interest with the photoactivated cleavage process of the metal complexes to give a variety of site-specific DNA cleavers. Photoactivated cleavage of DNA with simple coordination complexes may be considered a general phenomenon. Moreover, since these reagents are activated by near-UV and visible light, they may provide useful probes for mapping experiments in vivo.

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Photoaquation of the Iodopentaamminechromium(III) Ion in Acidic Aqueous Solution

Sir:

The acidopentaamminechromium(III) complexes[1-8] $[Cr(NH_3)_5X]^{2+}$ where X = Cl^-, Br^-, and F^-, together with some of their analogues containing ethylenediamine in place of four ammonias,[8-11] have figured importantly in studies of ligand labilization and stereochemistry of the photosubstitution reactions of d^3 complexes.

For the large majority of such complexes the photochemistry is well accounted for by the angular overlap ligand field model[12,13] of Vanquickenborne and Ceulemans (hereafter VC). For the fluoro compound particularly the theory gave a better description of the photosubstitution modes than Adamson's rule[14] although the stereochemical results required some extensions.[15] As has been pointed out,[16] the results for the bromo pentaammine compound are somewhat equivocal since the calculated excited-state bond strengths for the Cr–Br and Cr–N bonds are nearly equal, but the molecule efficiently loses mainly ammonia at all wavelengths.

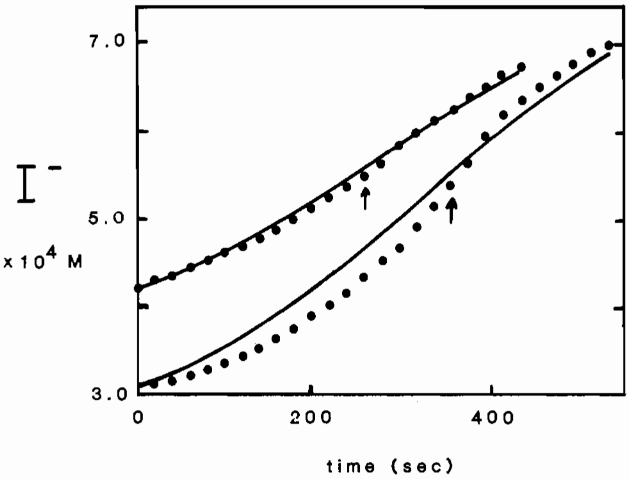

Figure 1. Time dependence of $[I^-]$ during and after 546-nm photolysis of $[Cr(NH_3)_5I]^{2+}$ in acidic aqueous perchlorate medium at 7 °C: points, experimental data; solid line, theoretical fit (see text). Two data sets are shown corresponding to the best and worst fits found, one displaced 1.2 × 10^{-4} M upward for clarity. Photolysis begins at zero and ends at the arrow.

Surprisingly the iodo compound has never been studied photochemically, although it has been reported and its thermal aquation studied polarographically.[17] Probably its lability has deterred photochemical study, the half-life for iodide aquation in 25 °C aqueous solution being only 17 min.

It is important, however, to fill this gap in our knowledge and to take advantage of the potential offered by this molecule to provide a useful test of the VC theory. Using as ligand field parameters, for ammonia, $E_\sigma = 0.7183\ \mu m^{-1}$ and, for iodide, $E_\sigma = 0.4583\ \mu m^{-1}$ and $E_\pi = 0.065\ \mu m^{-1}$, the VC theory predicts, for the ^4E_g lowest excited quartet state, bond energies of 0.72, 0.82, and 1.21 μm^{-1} for the Cr–I, Cr–NH_3(ax), and Cr–NH_3(eq) bonds, respectively, unambiguously implying dominant photochemical loss of iodide ion in contrast to the Adamson rule prediction of ammonia loss. We have therefore chosen to study this molecule, recognizing that the problems deriving from the thermal lability of the complex would limit the precision of any results and probably obscure beyond recovery the finer details of the wavelength and temperature dependence of the yields as well as the photostereochemistry.

$[Cr(NH_3)_5I]I_2$ was prepared as previously described[17] and characterized by its visible spectrum, which showed bands at 532 nm (38.1 L mol^{-1} cm^{-1}) and 472 (36.4), compared with the literature[17] values 530 (40) and 472 (40). Chromium analysis by oxidation with boiling alkaline hydrogen peroxide and spectrophotometric determination of chromate gave 10.3 ± 0.25% (five runs), consistent with theory, 10.04%.

Irradiation at 8 °C and 546 nm, into the first ligand field quartet band, of 5 × 10^{-3} M solutions in 1.319 × 10^{-3} M $HClO_4$/0.04 M $KClO_4$ aqueous medium led to a rise in pH, demonstrating photoaquation of ammonia. Reineckate actinometry together with absorbance measurements and standard additions of H^+ determined a quantum yield of 0.28 ± 0.04 (13 runs) for this process. The thermal lability of the compound caused unusually large scatter in these data and, more importantly, precluded reliable

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