DNA Cleavage by Photogenerated $Rh_2(O_2CCH_3)_4(H_2O)_2^+$

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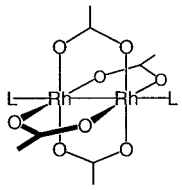
Metal complexes possessing reactive excited states that bind nucleic acids have been the subject of intense investigation since, upon light activation, they can act as reporters of DNA structure and environment,[1−4] promote photocleavage,[5,6] and photorepair thymine dimers.[5,7] The $Rh_2(O_2CCH_3)_4$ complex (structure shown in Figure 1) is known to bind duplex DNA and to inhibit DNA replication.[8] In addition, the related $Rh_2(O_2CR)_2(N\text{-}N)_2^{2+}$ (R = PhCH(OH), $CH_3CH(OH)$; N-N = 1,10-phenanthroline, 2,2′-bipyridine) systems have recently been shown to act as antibacterial agents and to exhibit cytostatic activity against human oral carcinoma.[9] The recent discovery of a relatively long-lived excited state of the $Rh_2(O_2CCH_3)_4(L)_2$ (L = H_2O, py, PPh_3) systems (py = pyridine, PPh_3 = triphenylphosphine) with lifetimes ranging from 3.5 to 5.0 μs[10] led us to investigate the photoreactivity of the complexes with duplex DNA.

Dirhodium tetraacetates, $Rh_2(O_2CCH_3)_4(L)_2$, with various axial ligands, L, have long been known,[11] and their molecular and electronic structure, bonding, and reactivity have been extensively investigated.[12,13] Various model systems of the binding of nucleic acids to the $Rh_2(O_2CCH_3)_4$ core have been recently synthesized and crystallographically characterized, where complexes possessing both axial and bridging nucleobases were isolated.[14] Substituted adenines and adenosines typically bind the complex as axial ligands, whereas guanines have been shown to replace two cis-bridging ligands across the rhodium−rhodium bond.[14] Furthermore, $Rh_2(O_2CCH_3)_4$ was recently shown to bind as dirhodium bis-acetate units to GG and AA sites on single-stranded oligonucleotides.[15] Although dirhodium complexes containing nucleic acids have been isolated and coordination of the dirhodium core to single-stranded oligonucleotides has been observed, the mode of binding of $Rh_2(O_2CCH_3)_4$ to double-helical DNA remains unknown.



$Rh_2(O_2CCH_3)_4(L)_2$

Figure 1. Structure of the $Rh_2(O_2CCH_3)_4(L)_2$ complexes (L = H_2O, py, PPh_3).

The imaged ethidium bromide stained agarose gel in Figure 2a reveals that the excited state of $Rh_2(O_2CCH_3)_4(H_2O)_2$ itself does not cleave DNA.[16] Lane 1 (Figure 2a) shows that 100 μM (bases) pUC18 plasmid alone is found mostly as a supercoil (form I) with a small amount of nicked, circular DNA (form II). Relative to lane 1, irradiation ($\lambda_{irr} \geq 395$ nm) of 100 μM plasmid alone (lane 2) or in the presence of 40 μM $Rh_2(O_2CCH_3)_4(H_2O)_2$ does not result in DNA cleavage. Excitation of $Rh_2(O_2CCH_3)_4(H_2O)_2$ with visible light in the presence of electron acceptors, such as 3-cyano-1-methylpyridinium tetrafluoroborate (py^+),[17] results in the formation of the one-electron-oxidized complex, $Rh_2(O_2CCH_3)_4(H_2O)_2^+$.[10] Lane 6 (Figure 2a) shows the efficient cleavage of the plasmid by 40 μM $Rh_2(O_2CCH_3)_4(H_2O)_2$ irradiated with $\lambda_{irr} \geq 395$ nm (10 min) in the presence of 2 mM py^+, evidenced by the disappearance of form I and the formation of form II DNA. This cleavage is not observed when the mixture is kept in the dark (lane 5). Furthermore, irradiation of 100 μM plasmid with 2 mM py^+ (lane 4) does not result in DNA cleavage.

To ensure that the cationic electron acceptor was not taking part in the cleavage, the anionic 1,8-anthraquinone disulfonate (AQ^{2-}) was utilized,[17] whose negative charge precludes its binding to the polyanionic double helix. Cleavage of 100 μM plasmid by photoproduced ($\lambda_{irr} \geq 450$ nm, 15 min) $Rh_2(O_2CCH_3)_4(H_2O)_2^+$ (25 μM) in the presence of 20 mM AQ^{2-} is shown in Figure 2b, lane 4, with the dark control shown in lane 2. Irradiation of 20 mM AQ^{2-} with 100 μM plasmid (lane 3) does not result in DNA cleavage. Similar results were observed when 200 μM Ag^+ and 200 μM Fe^{3+} were utilized as the electron acceptors.

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(16) The $Rh_2(O_2CCH_3)_4(L)_2$ (L = CH_3OH, py, PPh_3) complexes were prepared by published methods (see refs 10, 12, 13). The pUC18 plasmid was purchased from Bayou Biolabs and desalted using a Concert Miniprep System from Life Technology. The gels were imaged on a GelDoc 2000 (BioRad) transilluminator.
(17) The 3-cyano-1-methylpyridinium was prepared from the reaction of 3-cyanopyridine with CH_3I. The tetrafluoroborate salt (py^+) was obtained through the precipitation of AgI after treatment of an aqueous solution of the iodide compound with $AgBF_4$. All the reagents were purchased from Aldrich, and AQ^{2-} was manufactured by Tokyo Chemical Industries.

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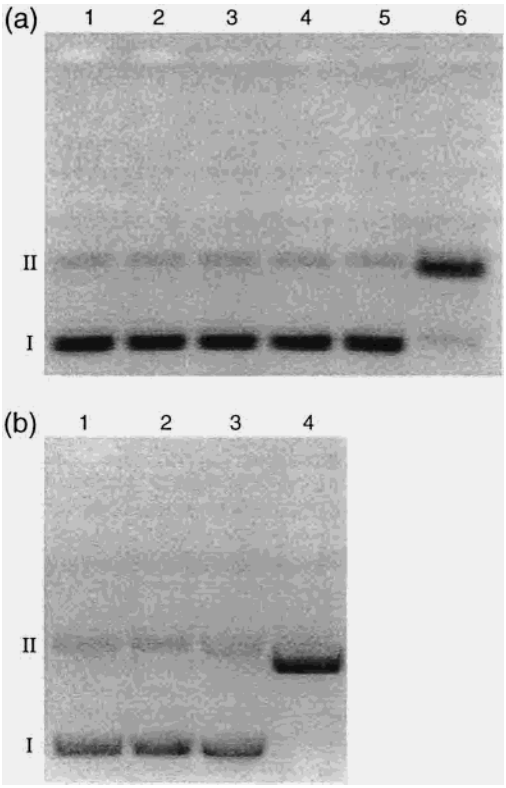

Figure 2. Imaged agarose gels showing the photocleavage of 100 μM pUC18 plasmid (5 mM Tris buffer, pH = 7.5) by Rh_2 ($Rh_2 = Rh_2(O_2CCH_3)_4(H_2O)_2$) in the presence of (a) 2 mM py^+, 40 μM Rh_2, 10 min irradiation, $\lambda_{irr} \geq 395$ nm, and (b) 20 mM AQ^{2-}, 25 μM Rh_2, 15 min irradiation, $\lambda_{irr} \geq 450$ nm. (a) Lane 1: plasmid only, dark. Lane 2: plasmid only, irradiated. Lane 3: plasmid + Rh_2, irradiated. Lane 4: plasmid + py^+, irradiated. Lane 5: plasmid + Rh_2 + py^+, dark. Lane 6: plasmid + Rh_2 + py^+, irradiated. (b) Lane 1: plasmid only, dark. Lane 2: plasmid + Rh_2 + AQ^{2-}, dark. Lane 3: plasmid + AQ^{2-}, irradiated. Lane 4: plasmid + Rh_2 + AQ^{2-}, irradiated.

The photoinduced DNA cleavage by $Rh_2(O_2CCH_3)_4(H_2O)_2$ in the presence of electron acceptors is more effective under N_2 atmosphere than in air, consistent with the quenching of the excited state of the rhodium complex by O_2 resulting in a decreased production of the $Rh_2(O_2CCH_3)_4(H_2O)_2^+$ electron transfer product. In addition, the wavelength dependence shows that the cleavage remains efficient up through $\lambda_{irr} \geq 590$ nm, and a significant amount of cleavage is also observed for $\lambda_{irr} \geq 610$ nm. No photocleavage is evident at $\lambda_{irr} \geq 630$ nm, consistent with the electronic absorption spectrum of the dirhodium complex.[10,12,13]

No cleavage of 100 μM pUC18 plasmid was observed for 10 μM $Rh_2(O_2CCH_3)_4(L)_2$ with py and PPh_3 axial ligands, L, up to 30 min irradiation time ($\lambda_{irr} > 395$ nm) in the presence of 200 μM Ag^+. These results are important because they point at the necessity of a labile axial ligand, such as H_2O,[18] for the DNA cleavage to take place. The need for a labile axial ligand may indicate that the presence of an open coordination site is required either for the reactivity of the complex or for its binding to DNA. The radical $Rh_2(O_2CCH_3)_4(H_2O)_2^+$ complex may be able to abstract a hydrogen atom from the DNA backbone, thus resulting in the observed cleavage. Alternatively, hydroxide ions or hydroxyl radicals formed by $Rh_2(O_2CCH_3)_4(H_2O)_2^+$ may participate in the DNA cleavage and cannot be ruled out at this time. Guanine oxidation ($E_{1/2}(G^{+/0}) \approx 1.3$ V vs NHE)[19] by $Rh_2(O_2CCH_3)_4(H_2O)_2^+$ ($E_{1/2}(Rh_2^{+/0}) = 1.14$ V vs NHE)[20] is slightly unfavorable; however, it cannot be ruled out as a means for initiation of the DNA cleavage at this time.

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