Thymidine-Specific Depyrimidination of DNA by Oxopolypyridylruthenium(IV) Complexes

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Recently, there has been significant effort directed toward the selective oxidation of DNA by metal complexes.[1-3] Significant progress has been made in achieving selectivity by altering the *binding* specificity of the complex by appropriate tailoring of the ligands, leading to shape-selective cleavage.[4] In contrast, little effort has been directed toward achieving selectivity by altering the *reactivity* of the metal complex. Burrows and Rokita have developed a nickel complex which, in the presence of persulfate, can oxidize guanine bases, leading to guanine-selective cleavage in single-stranded DNA.[5] We have been interested in determining whether altering the reactivity of complexes that cleave DNA via sugar oxidation could lead to an increase in the specificity of cleavage of double-stranded DNA. We have been studying the stoichiometric and electrocatalytic oxidation of DNA by oxopolypyridyl complexes of ruthenium(IV) based on $Ru(tpy)(bpy)O^{2+}$ (**1**) and $Ru(bpy)_2(py)O^{2+}$ (**2**) (tpy = 2,2′,2″-terpyridine, bpy = 2,2′-bipyridine).[6] These complexes oxidize DNA efficiently to generate the corresponding aquaruthenium(II) forms, which are readily reoxidized to the active Ru(IV)O forms. We report here on studies of related complexes, $Ru(bpy)_2(EtG)O^{2+}$ (EtG = 9-ethylguanine) and $Ru(bpy)_2(dmap)O^{2+}$ (dmap = 4-(dimethylamino)pyridine), which exhibit a startling specificity for oxidation of thymidine sugars.

We have reported previously that oxidation of calf thymus DNA by **1** leads to release of free base as detected by HPLC with significant amounts of all four bases released.[7] The release of nucleic acid bases is indicative of chemistry occurring via abstraction of a hydrogen atom from one of several positions on the deoxyribose ring.[8] Quantitation of the released bases accounts for 10% of the total ruthenium concentration.[9] Identical results are observed for oxidation of calf thymus DNA by **2**.

We have reported the synthesis of $Ru(bpy)_2(EtG)OH_2^{2+}$,[10] which we have been studying as a model for covalent binding reactions.[11] This complex can be oxidized to the reactive Ru(IV)O form, $Ru(bpy)_2(EtG)O^{2+}$ (**3**), at a potential of $E_{1/2}(IV/II)$ = 0.43 V vs SSCE, which is 210 mV lower than that required for **1**. Complex **3** is significantly less reactive than **1**; for example, **1** is an efficient oxidant of 2-propanol,[12] while solutions of **3** are stable in the presence of 2-propanol. Thus far, we have not been able to isolate **3** in the solid state, so studies of DNA oxidation reactions have been performed electrocatalytically, by electrochemical oxidation at 0.8 V of solutions of $Ru(bpy)_2(EtG)OH_2^{2+}$ in the presence of calf thymus DNA. Analysis of HPLC of these oxidations performed in argon-degassed solution provides the results shown in Figure 1.[13] *Surprisingly, the only nucleic acid base released is thymine.* Quantitation of the thymine released reveals a 12% efficiency for sugar oxidation. Thus, for both **1** and **3**, the yield of base release is the same, but only thymidine sugars are oxidized by **3**, while all four sugars are oxidized by **1**, at least via the pathway that leads to immediate base release.

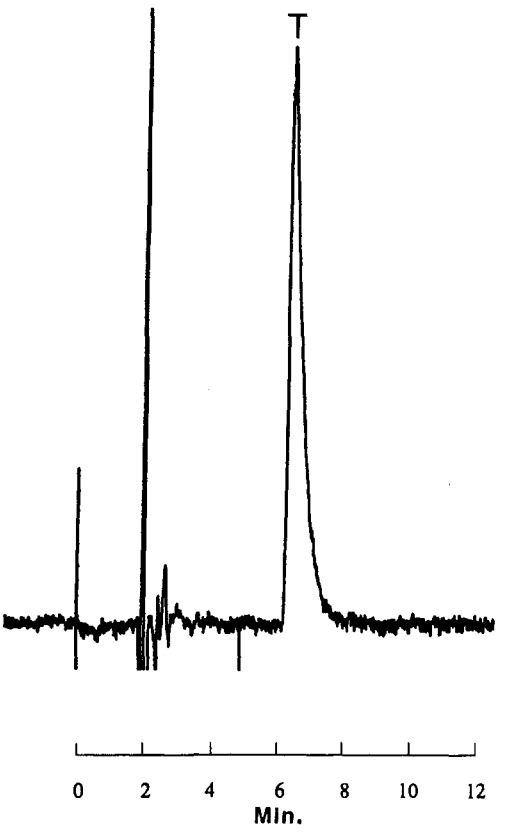

Figure 1. HPLC of the products of the electrocatalytic reaction of **3** with DNA at an applied potential of 0.8 V. Chromatography was performed on a Rainin Microsorb-MV "Short One" C18 column with 0.1 M ammonium formate buffer, pH 7. The retention times of the other bases under these conditions are cytosine, 2.2 min; guanine, 4.33 min; and adenine, 11.5 min.

To rule out specific recognition by the ethylguanine ligand as the mechanism of thymidine specificity, the complex $Ru(bpy)_2$-

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(9) We cannot yet quantitatively account for the remainder of the Ru(IV)O; however, base oxidation or sugar oxidation at sites that do not lead to base release or to products detectable under our chromatographic conditions may also occur. In any case, the present HPLC analysis allows us to monitor accurately at least one sugar-oxidation pathway exclusive of any base oxidation pathways or other sugar-oxidation pathways. We are confident that no base propenals[8] or propenoic acids (see Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303) are formed. No change in the HPLC results in Figure 1 is observed upon base treatment.
(10) Grover, N.; Welch, T. W.; Fairley, T.; Cory, M.; Thorp, H. H. *Inorg. Chem.*, submitted for publication. $Ru(bpy)_2(EtG)(OH_2)^{2+}$ is the aquation product of $[Ru(bpy)_2(EtG)(NCMe)](PF_6)_2$. Anal. Calcd for $[(bpy)_2(EtG)Ru(NCMe)](PF_6)_2 \cdot 3H_2O$: C, 34.6; H, 3.70; N, 14.4. Found: C, 34.6; H, 3.27; N, 13.7. A complete preparation is given in the supplementary material.
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Table I. Influence of Redox Potential on Base Release from DNA Oxidation by $M(IV)O^{2+}$ Complexes

complex	$E_{1/2}$(IV/II) V vs SSCE	bases released
$(tpy)(bpy)RuO^{2+}$ (1	0.56	A, T, G, C
$(bpy)_2(py)RuO^{2+}$ (2)	0.48	A, T, G, C
$(bpy)_2(EtG)RuO^{2+}$ (3)	0.43	T
$(bpy)_2(dmap)RuO^{2+}$ (4)	0.46	T
$(tpy)(bpy)OsO^{2+}$ (5)	0.23	none

$(dmap)OH_2^{2+}$ was prepared.[14] The spectral and electrochemical properties are nearly identical to those of the thymidine-specific complex **3**, but the structure differs from that of the nonspecific complex **2** only by the addition of a single dimethylamino group, which would not be expected to exhibit special DNA-recognition properties. Analogous reactions using $Ru(bpy)_2(dmap)O^{2+}$ (**4**) lead to the release of only thymine, as seen with **3**. No base release was detected upon reaction with DNA of $Os(tpy)(bpy)O^{2+}$ (**5**) for which $E_{1/2}(IV/II) = 0.23$ V (Table I).[15]

The thymidine-specific reaction does not lead to frank strand scission. Oxidation of the self-complementary hexanucleotide $d(CAGCTG)_2$ by **3** leads to the release of thymine, again in 10% yield based on the concentration of **3**; however, no deoxyguanosine 5′-monophosphate (5′-dGMP) was detected concomitantly with release of thymine.[16] The thymine-specific oxidation therefore produces a relatively stable depyrimidinated lesion, which would be consistent with oxidation of the DNA at one of the several positions on the deoxyribose ring, including oxidation at the 1′ position to yield a stable 2-deoxyribonolactone residue.[8,17]

The results summarized in Table I show strikingly that simple alteration of the reactivity of the M(IV)O oxidant dramatically alters the specificity of sugar oxidation. The binding affinity of $Ru(bpy)_2(EtG)OH_2^{2+}$ ($K_B = 2300\ M^{-1}$)[18] is modest and is between those of $Ru(tpy)(bpy)OH_2^{2+}$ ($K_B = 660\ M^{-1}$) and $Ru(tpy)(phen)OH_2^{2+}$ ($K_B = 3700\ M^{-1}$);[7] oxidation of calf thymus DNA by $Ru(tpy)(phen)O^{2+}$ ($E_{1/2}(IV/II) = 0.56$ V) gives results identical to those for **1**. Addition of a single dimethylamino group to the pyridine ligand of **2** converts the complex from a nonspecific oxidant to a specific oxidant. Thus, it seems certain that the lower oxidation potential is the source of the specificity. We are exploring two mechanisms for the thymidine specificity. First, the sugar oxidation may occur at the 1′-position,[8a,17] where the reactivity would be strongly influenced by the nature of the coordinated base. Second, the structure of thymidine sugars may permit close approach of the oxo group to sugar hydrogens, and the low oxidation potential may necessitate a close approach in order for oxidation to occur. In either case, it appears that the combination of new advances in control of binding[1] with the ability to tune the reactivity of the metal complexes may provide an increasingly diverse repertoire of selective DNA damage agents.

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Supplementary Material Available: Synthesis and binding isotherm for $Ru(bpy)_2(EtG)OH_2^{2+}$ (3 pages). Ordering information is given on any current masthead page.

(13) Oxidation reactions using $RuOH_2^{2+}$ and $OsOH_2^{2+}$ complexes (0.05 mM) and calf thymus DNA (1.0 mM) were performed in pH 7 phosphate buffer at an ionic strength of 50 mM. Reactions were performed in a three-compartment cell with a reticulated vitreous carbon working electrode at an applied potential of 0.8 V. Release of all four bases is observed with **1** and **2** in both electrocatalytic and stoichiometric oxidations.

(14) $Ru(bpy)_2(dmap)(OH_2)^{2+}$ is the aquation product of $[Ru(bpy)_2(dmap)(NCMe)](PF6)_2$, which was synthesized by analogy to $[Ru(bpy)_2(py)(OH_2)](PF_6)_2$. Moyer, B. A.; Meyer, T. J. *Inorg. Chem.* **1981**, *20*, 436–444. Anal. Calcd for $[Ru(bpy)_2(dmap)(NCMe)](PF_6)_2$: C, 40.2; H, 3.37; N, 11.31. Found: C, 39.7; H, 3.22; N, 10.61.

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(16) The purified d(CAGCTG) hexamer was purchased from Genosys. Oxidations were performed by treating the oligonucleotide with an equal concentration of **3** that had been generated by electrochemical oxidation. Analysis was performed on a Rainin Microsorb 300-Å, 4.6- × 250-mm C18 column eluted with 0.1 M ammonium formate and a 0–10% linear CH_3CN gradient (20 min). The retention times were 9.75 min, thymine; 8.90 min, 5′-dGMP; and 21.56 min, d(CAGCTG). Analysis of the untreated oligonucleotide gave only the peak at 21.56 min. Oxidation with **3** gave only the thymine peak (9.75 min) and the d(CAGCTG) peak (21.56 min). Chromatography was performed using a Waters system with a diode array detector that allowed all species to be identified both by retention time and by UV spectrum. Heating the reaction mixture at 90 °C for 20 min or treating the sample with 0.1 M NaOH with heating for 20 min also did not lead to release of 5′-dGMP; however, these conditions may not have been capable of labilizing the lesion formed upon thymine release at these oligonucleotide concentrations.[17]

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(18) The binding isotherm for $Ru(bpy)_2(EtG)OH_2^{2+}$ is given in the supplementary material.