VOLUME 115, NUMBER 11
JUNE 2, 1993

JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY

Binding and Kinetics Studies of Oxidation of DNA by Oxoruthenium(IV)

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Received November 2, 1992

Abstract: The binding to DNA of complexes based on $Ru(tpy)(L)OH_2^{2+}$ (tpy = 2,2′,2″-terpyridine; L = bpy, 2,2′-bipyridine; phen, 1,10-phenanthroline; or dppz, dipyridophenazine) has been studied by viscometry, thermal denaturation, and absorbance hypochromism along with the kinetics of oxidation of DNA by the analogous $Ru(tpy)(L)O^{2+}$ complexes. These studies show that very weak binding occurs when L = bpy; however, when L = dppz, ΔT_m is larger than that for ethidium bromide. Viscometry studies of the dppz complex show that the dppz complex does lengthen DNA, as occurs with intercalative binding. The slope of the viscometry plot is identical to that for ethidium bromide, and neighbor exclusion binding is observed for both, with saturation occurring between 0.2 and 0.25 small molecules per nucleotide phosphate. The $Ru(tpy)(dppz)OH_2^{2+}$ complex also unwinds DNA by 17 ± 2°, as determined using a topoisomerase assay. For L = bpy, no evidence of DNA lengthening was obtained. The kinetics of the oxidation of DNA by the $Ru(IV)O^{2+}$ forms of these complexes occurs in two phases. The first phase involves oxidation of DNA by $Ru(IV)O^{2+}$ that is bound at time zero. The second phase occurs when a reduced $Ru(II)OH_2^{2+}$ complex dissociates and another $Ru(IV)O^{2+}$ complex binds from solution. The kinetics of this phase are governed by dissociation of the reduced complex, which allows the relative dissociation rates of the L = bpy, phen, and dppz complexes to be determined. These experiments show that the dissociation rate for the dppz complex is an order of magnitude slower than those for bpy and phen, which is also consistent with an intercalative interaction for dppz. The cleavage reaction is shown to lead to the release of nucleic acid bases, implicating sugar oxidation as the reaction pathway.

Development of metal complexes that cleave DNA has been pursued with a variety of goals.[1-4] Recently, a growing emphasis has been placed on mechanistic studies aimed at determining the site of reaction of the metal complex with the DNA[5-7] and, in some cases, the kinetic isotope effects of cleavage reactions.[8] To date, direct kinetic studies on DNA cleavage reactions have been difficult, because most cleavage reactions are either photolytic or initiated by treatment with an external oxidant or reductant, which complicates the mechanism on the metal complex side of the reaction. We report here on a stoichiometric DNA cleavage

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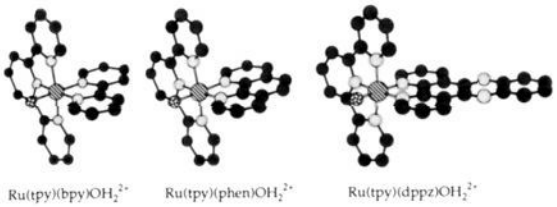
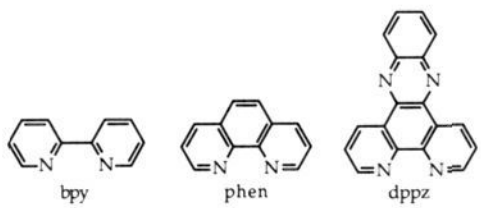


Figure 1. Structures of metal complexes used in this study.

reaction where the metal complex oxidant and products have well-defined optical spectra, allowing for the first direct kinetic study of DNA cleavage by a metal complex. This allows important aspects of metal–DNA reactivity to be addressed, such as whether binding or cleavage is rate-limiting and the effects of the binding affinity on the overall efficiency. Clearly, an understanding of these issues is important in developing new DNA cleavage agents.

We have been studying the cleavage reactions of complexes based on $Ru(tpy)(L)O^{2+}$ (tpy = 2,2′,2″-terpyridine),[9-12] where L = bpy, phen, or dppz (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, and dppz = dipyridophenazine). These reactive



cleavage agents can be generated electrochemically or chemically via oxidation of the corresponding $Ru(tpy)(L)OH_2^{2+}$ complexes (Figure 1). These complexes present a number of advantages for studying the mechanism of cleavage reactions from the point of view of the metal complex. One of these is that the cleavage reactions are readily studied by electrochemical techniques.[11] Another advantage of our system is that the oxidized forms can be isolated and used for kinetic studies by optical spectroscopy.[9]

We report here on kinetic studies of the oxidation of DNA by $Ru(tpy)(L)O^{2+}$. We show that the oxidation reaction leads to the release of the nucleic acid bases, implicating the sugar functionality as the site of oxidation. A complete kinetic analysis of the cleavage reactions is only possible when the binding of the metal complexes is thoroughly understood. We therefore present results on the viscometry, binding affinity, thermal denaturation, and absorbance hypochromism of the family of complexes. Viscometry studies show that when L = dppz, binding of the metal complex lengthens DNA in a manner that is quantitatively identical to that observed for the classical intercalator ethidium bromide. Finally, these binding results are used to develop a complete kinetic model for the oxidation of DNA by oxoruthenium(IV).

Experimental Section

Metal Complexes. $[Ru(tpy)(bpy)OH_2](ClO_4)_2$, $[Ru(tpy)(phen)OH_2](ClO_4)_2$, and $[Ru(tpy)(dppz)OH_2](ClO_4)_2$ were prepared as described previously.[11-13] The complex $Ru(tpy)(bpy)O^{2+}$ was prepared by oxidation of $Ru(tpy)(bpy)OH_2^{2+}$ according to the method of Takeuchi et al.,[13] except Cl_2 was used in place of Br_2 as the oxidant. The complex $Ru(tpy)(phen)O^{2+}$ was prepared similarly, as previously described.[11] The complex $Ru(tpy)(dppz)O^{2+}$ was prepared by electrochemical oxidation of a solution of $Ru(tpy)(dppz)OH_2^{2+}$ just prior to use, as reported.[12]

Binding Studies. Thermal denaturation and viscometry measurements were made as described previously.[14] Thermal denaturation studies were performed in 5.0 mM, pH 7.55, Tris-HCl buffer containing 50 μM sonicated DNA phosphate, 5 μM metal complex, 50 μM EDTA, and 5% DMSO. Viscometric titrations were performed in 2 mM, pH 6.4, MES buffer containing 1 mM EDTA and 1 mM ammonium fluoride. Absorbance hypochromicity studies were performed in 50 mM, pH 7, phosphate buffer with 20 μM metal complex and 7 mM calf thymus DNA. A Cary 14 spectrophotometer modified by On-Line Instruments Systems was used to collect the spectra. Topoisomerase studies were performed in 5 mM phosphate buffer (pH 7) containing 10 mM NaCl, 1 mM $MgCl_2$, and 60 μM pBR322 plasmid DNA. Unwinding angles were determined from concentrations of bound metal complex required to achieve complete unwinding, according to published procedures.[15] Concentrations of bound metal complex were determined using the binding affinities given in Table I. Viscometry and unwinding experiments with $Ru(tpy)(phen)OH_2^{2+}$ were performed up to the limit of solubility of the metal complex. For product analysis, solutions of calf thymus DNA (1.0 mM) were treated with $Ru(tpy)(bpy)O^{2+}$ (0.05 mM) for 12 h in pH 7 phosphate buffer (50 mM). Products were analyzed by HPLC using a Rainin Microsorb-MV "Short-One" C_{18} column with 0.1 M ammonium formate buffer (pH 7) at a flow rate of 1.0 mL/min. Bases were identified by coelution with standards.

Binding affinities were determined using an emission titration involving quenching of the excited state of $Pt_2(pop)_4^{4-}$ (pop = $P_2O_5H_4^{2-}$).[16] The complete details of this method for determining binding constants of the present complexes and many others will be published shortly. Titration curves and fits for the three complexes studied here are given in the supplementary material.

Oxidation Kinetics. Spectra were collected on an OLIS-modified Cary 14 spectrophotometer. At each DNA/metal ratio studied, initial sets of spectra were collected to determine the isosbestic point for $Ru(III)OH^{2+}$ and $Ru(II)OH_2^{2+}$. Freshly prepared solutions of the $Ru(IV)O^{2+}$ forms of each complex were added to buffered solutions of DNA. Special care was required to insure complete mixing. Absorbance vs time data were generated from the initial spectra at the appropriate isosbestic point, and decay curves such as those shown in Figure 3 were obtained. The data were fit using the OLIS nonlinear least squares fitting routines.

Results

Binding Studies. Binding of small molecules to DNA can be detected by a number of techniques,[15,17-25] and we report here thermal denaturation, viscometry, and absorption hypochromicity studies on the $Ru(tpy)(L)OH_2^{2+}$ complexes. In thermal denaturation experiments (Table I), the L = bpy complex shows a modest value of ΔT_m, consistent with perhaps simple electrostatic binding of the complex to the DNA.[14,23] The low binding affinity of 660 M^{-1} is also consistent with simple electrostatic binding. When L = dppz, however, the value for ΔT_m is strikingly high, *actually higher than that of the organic intercalator ethidium bromide.*[14] A higher value of ΔT_m is probably due in part to the

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Table I. Binding Parameters of Polypyridyl Complexes to Calf Thymus DNA

complex	ΔT_m (°C)[a]	slope[b]	$\Delta\lambda_{max}$ (nm) (%H)[c]	relative rate[d]	ϕ[e] (deg)	K_B (M^{-1})[f]
$Ru(tpy)(bpy)OH_2^{2+}$	4.2 ± 0.5		4.1 ± 0.7 (2.3%)	fast (1.0)		660
$Ru(tpy)(phen)OH_2^{2+}$	7.2 ± 0.4		7.3 ± 0.7 (8.0%)	fast (1.6)		3700
$Ru(tpy)(dppz)OH_2^{2+}$	14.1 ± 0.8	1.13	8.7 ± 0.5 (9.6%)	slow (0.12)	17	700000
ethidium bromide	13.0[g]	1.14	39 (26%)[h]	slow[i]	26[i]	

[a] Determined in 5.0 mM Tris-HCl buffer, 10:1 DNA/metal complex. [b] Slope of the plot of L/L° vs the metal complex/DNA ratio between metal/DNA ratios of 0–0.15. [c] $H = (A_{free} - A_{DNA})/A_{free}$ in 50 mM phosphate buffer, 350:1 DNA/metal complex. [d] Ratio of k_2 at 10:1 DNA/metal complex for each complex relative to $Ru(tpy)(bpy)O^{2+}$, see text and Table II. [e] Unwinding angle measured using topoisomerase, error ±2°. [f] Measured by emission titration at 50 mM ionic strength, error ±30%, see supplementary material. [g] Reference 14a. [h] Reference 14b. [i] Reference 20.

dicationic charge of $Ru(tpy)(dppz)OH_2^{2+}$ as compared to the ethidium monocation. The binding affinity of 7.0×10^5 M^{-1} is similar to those estimated for intercalating metal complexes, such as $Ru(bpy)_2(dppz)^{2+}$.[25] The L = dppz complex also shows an extremely high ($\Delta T_m > 33°$) affinity for poly(dA)·poly(dT), while the ΔT_m values of the bpy and phen complexes for this polymer are similar to those for calf thymus DNA. Recent NMR studies of oligonucleotides show that related complexes appear to have higher affinities for AT regions.[18,26,27] Absorption hypochromicity studies are similar, with a small red shift of the MLCT band upon binding of the bpy complex but a sizable red shift upon binding of the dppz complex to DNA. In terms of thermal denaturation, binding affinity, and absorbance hypochromicity, the phen complex gives results intermediate between those of the bpy and dppz derivatives.

The ability of metal complexes to unwind DNA has been put forth as an important criterion for proving an intercalative binding mode and has been observed with other complexes of phen, dppz, and phi (phi = phenanthrenequinone diimine).[7,15,17,25] The enzyme topoisomerase can be used to determine if small molecules unwind DNA, according to published procedures.[15] We find, using this assay, that $Ru(tpy)(dppz)OH_2^{2+}$ unwinds DNA by 17°, which is consistent with intercalative binding. We have not observed unwinding by the bpy and phen complexes at metal/DNA phosphate ratios up to 2:1, although related phen complexes have been shown to unwind DNA at higher ratios.[28] The low solubility of $Ru(tpy)(phen)OH_2^{2+}$ limited our ability to perform the unwinding experiments confidently at high metal/DNA ratios. On the basis of previous results on $Ru(phen)_3^{2+}$,[15] we can estimate from the binding affinity of $Ru(tpy)(phen)OH_2^{2+}$ that observation of unwinding would require higher concentrations than we can access. Thus, we cannot comment on the ability of $Ru(tpy)(phen)OH_2^{2+}$ to unwind DNA. The similarity of the binding affinity, absorbance hypochromicity, and thermal denaturation results for our phen complex to those for $Ru(phen)_3^{2+}$ suggests $Ru(tpy)(phen)OH_2^{2+}$ would unwind DNA at sufficiently high concentrations.

Another convincing test of intercalation comes from viscometry studies that test the ability of a small molecule to lengthen DNA, which occurs when base pairs separate to accommodate intercalators.[14,19,29] The slope of the viscometry plot gives the amount of lengthening per metal complex. The bpy complex does not appear by viscometry to lengthen DNA; however, *$Ru(tpy)(dppz)OH_2^{2+}$ gives results in the viscometry experiment that are quantitatively identical to those for ethidium bromide.* As can be seen in Figure 2, the ratio of the length of DNA in the presence of $Ru(tpy)(dppz)OH_2^{2+}$ to the length of DNA in the absence of the complex increases linearly with the concentration of metal complex, with a slope of 1.13. Shown in Figure 2 are two separate

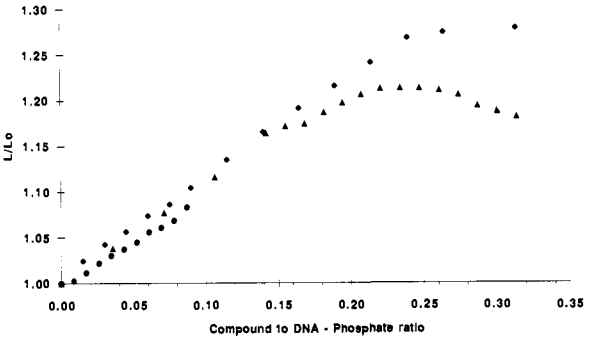

Figure 2. Viscometric titrations with sonicated calf thymus DNA with buffer conditions as described in the Experimental Section. The titration shown for ethidium bromide (◆) gives a slope of 1.14 for the range of ethidium/DNA ratios of 0–0.15. Two separate titrations (●) and (▲) are shown for $Ru(tpy)(dppz)OH_2^{2+}$: one over a narrow range and one over the full range. The slope for $Ru(tpy)(dppz)OH_2^{2+}$ is 1.13 over the range of metal/DNA ratios of 0–0.15 (▲).

titrations for $Ru(tpy)(dppz)OH_2^{2+}$ (circles and triangles), one of which is over a smaller range of metal complex/DNA ratios (circles). Both experiments clearly give the same slope within experimental error. The same behavior is observed with ethidium bromide (diamonds), which gives an identical slope of 1.14. The values of L/L° for both ethidium and $Ru(tpy)(dppz)OH_2^{2+}$ plateau at a small molecule/DNA ratio of 0.2–0.25, which is indicative of neighbor exclusion intercalation in both cases,[14,29] with saturation occurring once metal complexes are bound approximately every two base pairs. Viscometry studies of $Ru(tpy)(phen)OH_2^{2+}$ are also impaired by relatively low solubility; we have not observed significant lengthening of DNA, but only concentrations less than those required for significant unwinding by related phen complexes were accessible.

Oxidation Kinetics and Mechanism. We have been studying the present complexes in their oxidized forms, $Ru(tpy)(L)O^{2+}$, because these complexes are effective DNA cleavage agents.[9-12] Oxidation of calf thymus DNA by $Ru(tpy)(bpy)O^{2+}$ leads to release of all four nucleic acid bases, as shown in Figure 3. The cleavage is nonspecific, leading to release of approximately equal amounts of adenine, thymine, guanine, and cytosine. The observation of base release implicates sugar oxidation as the primary cleavage mechanism.[5,7,30]

One of the interesting features of the cleavage reactions of these complexes is that the kinetics can be followed using optical spectroscopy.[9] Kinetics studies of $Ru(tpy)(bpy)O^{2+}$ have shown that oxidations of small organic substrates occur in two stages.[31] The first stage is characterized by two-electron oxidation by $Ru(IV)O^{2+}$ (eq 1), with an isosbestic point at 368 nm.

$$Ru(IV)O^{2+} + R\text{–}H + H^+ \rightarrow Ru(II)OH_2^{2+} + R^+ \quad (1)$$

The second stage is characterized by one-electron oxidation by $Ru(III)OH^{2+}$, which is formed via comproportionation of $Ru(II)OH_2^{2+}$ and $Ru(IV)O^{2+}$ (eqs 2 and 3).

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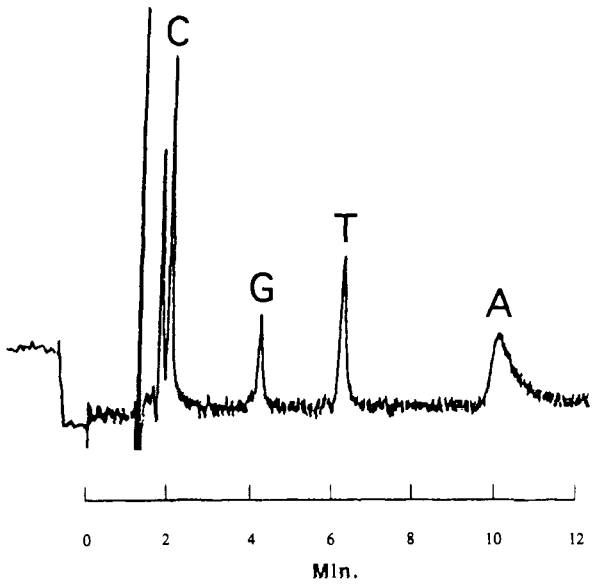

Figure 3. HPLC results obtained following oxidation of calf thymus DNA (1.0 mM) by Ru(tpy)(bpy)O^{2+} (0.05 mM). Chromatograms were run on a Rainin Microsorb-MV "Short-One" C_{18} column with 0.1 M ammonium formate buffer (pH 7) at a flow rate of 1.0 mL/min. Peaks are labeled as adenine (A), thymine (T), guanine (G), and cytosine (C).

$$Ru(IV)O^{2+} + Ru(II)OH_2^{2+} + 2Ru(III)OH^{2+} \quad (2)$$

$$Ru(III)OH^{2+} + R\text{–}H \rightarrow Ru(II)OH_2^{2+} + R^{\bullet} \quad (3)$$

The kinetics of the second stage are characterized by an isosbestic point at 406 nm, which is where $Ru(III)OH^{2+}$ and $Ru(II)OH_2^{2+}$ have the same extinction coefficient. We have reported previously that DNA oxidation occurs in these same two stages.[9] By monitoring the reaction at 406 nm, the rate of disappearance of $Ru(IV)O^{2+}$ can be followed, and with small organic substrates, first-order kinetics are observed.[31] We will report here only these kinetics of the disappearance of $Ru(IV)O^{2+}$ for the DNA oxidation reaction.

For the three complexes, the isosbestic point for the $Ru(III)OH^{2+}$ and $Ru(II)OH_2^{2+}$ complexes in DNA solutions varies somewhat from 406 nm with the metal complex and with the DNA/metal ratio (R). The change in isosbestic point as a function of R is simply a consequence of the effect of binding on the absorption spectrum of the metal complex, as discussed above. The isosbestic points at which data were collected for the three complexes are given in Table II.

We will now present results taken at the isosbestic point for $Ru(III)OH^{2+}$ and $Ru(II)OH_2^{2+}$, where the disappearance of $Ru(IV)O^{2+}$ is monitored. Thus, our analysis only applies to the first stage (eq 1) of DNA oxidation. The decay curves for the disappearance of $Ru(IV)O^{2+}$ show two distinct reaction phases (Figure 4). The first phase is rapid and occurs within the time of conventional mixing. Our mixing time is somewhat long because the viscosity of the DNA solutions makes complete mixing difficult. For Ru(tpy)(bpy)O^{2+}, the contribution of the early "burst" phase is a function of R, with the burst fraction being much larger at high R. For Ru(tpy)(phen)O^{2+}, the contribution is higher than that for Ru(tpy)(bpy)O^{2+} at the same R and also increases dramatically with increasing R. For Ru(tpy)(dppz)O^{2+}, the contribution is much larger than that for either of the other two complexes at low R (Table II).

We interpret the trend in the burst fraction in terms of the model shown in Scheme I. The results can be accounted for if the rate of binding of $Ru(IV)O^{2+}$ (k_{on}) is faster than the rate of oxidation (k_1), which is in turn faster than the dissociation rate (k_{off}). The rate-determining step in the first phase is therefore the zero-order oxidation of DNA by bound $Ru(IV)O^{2+}$. Thus, the burst fraction represents the amount of metal complex that is bound at time zero, which is higher when the binding constant of the metal complex is increased or R is increased.

The second phase of the reaction occurs over a longer time period and can be analyzed by fitting to a biexponential decay. The rates are given in Table II. Interestingly, the rates for a single complex do not vary outside of experimental error as a function of either R or the absolute concentrations of metal complex or DNA. In addition, the rates are the same within experimental error for both Ru(tpy)(bpy)O^{2+} and Ru(tpy)(phen)O^{2+}. However, the rates are an order of magnitude slower for Ru(tpy)(dppz)O^{2+} than for the other two complexes.

The results for the second phase can also be interpreted in terms of the model shown in Scheme I. Following the k_1 step, excess $Ru(IV)O^{2+}$ is present in solution, and $Ru(II)OH_2^{2+}$ is bound to DNA. For continued oxidation of DNA to occur, inactive $Ru(II)OH_2^{2+}$ must dissociate before another active $Ru(IV)O^{2+}$ complex can bind. Both oxidation states must have approximately the same binding constant, since they have the same charge and their structures differ only by two protons. We know from the analysis of the first phase that dissociation (k_{off}) is slower than oxidation (k_1) or binding (k_{on}). Thus, the rate-limiting step becomes the dissociation of the reduced ruthenium complex so that another $Ru(IV)O^{2+}$ can bind. Since dissociation is a zero-order process, we would expect the rate to be concentration-independent, as is observed.

An intercalator, such as the dppz complex, would be expected to exhibit slower dissociation kinetics than the bpy complex, which binds electrostatically. Classical intercalators, such as ethidium bromide, have been shown to exhibit exchange kinetics approximately an order of magnitude slower than those of related surface binding molecules, because structural rearrangements of the DNA occur upon binding and dissociation.[18,20] Gross structural changes in the DNA are apparently not required for binding and dissociation of the phen complex, because its kinetics are similar to those of the bpy complex.

The apparent biexponential kinetics for the k_2 phase must arise because of a variety of distinct binding sites that would be expected to exist in calf thymus DNA. The two rate constants then reflect the best fit for what is no doubt a complex ensemble of sequence- and structure-dependent binding sites, each with its own innate affinity for the metal complexes and reactivity toward the $Ru(IV)O^{2+}$ oxidant.

Discussion

The binding modes of polypyridyl complexes, especially those of phen, to DNA have been discussed at length.[17-19,21,24,26,27] For our purposes, this subject is important only because it is vital to understanding the kinetics of oxidation of DNA by $Ru(IV)O^{2+}$. Assignment of binding modes for the bpy and dppz complexes now seems straightforward on the basis of results reported here and elsewhere. It seems widely accepted that a complex such as Ru(tpy)(bpy)OH_2^{2+} binds to DNA almost solely because of electrostatics,[1,15,17] which is consistent with the results reported here. The binding affinity is quite modest, as are ΔT_m and the extent of hypochromicity. In contrast, the complex Ru(tpy)(dppz)OH_2^{2+} could be expected to intercalate on the basis of previous studies of dppz complexes[25,32-34] and has been shown here to give results in favor of intercalation from helical unwinding, absorbance hypochromism, and thermal denaturation. Figure 2 also shows for the first time that an octahedral metal complex is capable of lengthening DNA, as determined by viscometry, in a manner similar to that of ethidium bromide. We have also observed that the excited state of Ru(tpy)(dppz)OH_2^{2+} is not

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Table II. Kinetics Results for Oxidation of DNA by Ru(tpy)(L)O^{2+}

L	[Ru(IV)][a]	[DNA][b]	R[c]	λ_{isos}[d]	$k_2 \times 10^3$ [e]	$k_2' \times 10^3$ [e]	burst fraction[f]
bpy	0.12	1.2	10	407.0	11 ± 6	3.2 ± 1.5	0.10 ± 0.03
	0.40	4.0	10	407.8	13 ± 6	5.2 ± 2.5	0.29 ± 0.03
	0.12	5.3	45	408.9	16 ± 7	5.5 ± 2.5	0.53 ± 0.05
phen	0.11	1.1	10	389.3	20 ± 3	5.2 ± 0.9	0.15 ± 0.04
	0.37	3.8	10	392.0	19 ± 7	5.3 ± 0.9	0.15 ± 0.09
	0.11	4.9	45	394.5	20 ± 10	6.5 ± 10	0.76 ± 0.03
dppz	0.12	1.3	11	404.5	2.4 ± 0.9	0.38 ± 0.12	0.47 ± 0.07

[a] Concentration in mM. [b] Concentration in nucleotide phosphate, mM. [c] [DNA phosphate]/[Ru(IV)]. [d] Isosbestic point for Ru(III) and Ru(II) under the particular set of concentration conditions, see text. [e] Rate constants in s^{-1}. [f] Contribution of the "burst" (k_1) to the overall decay.

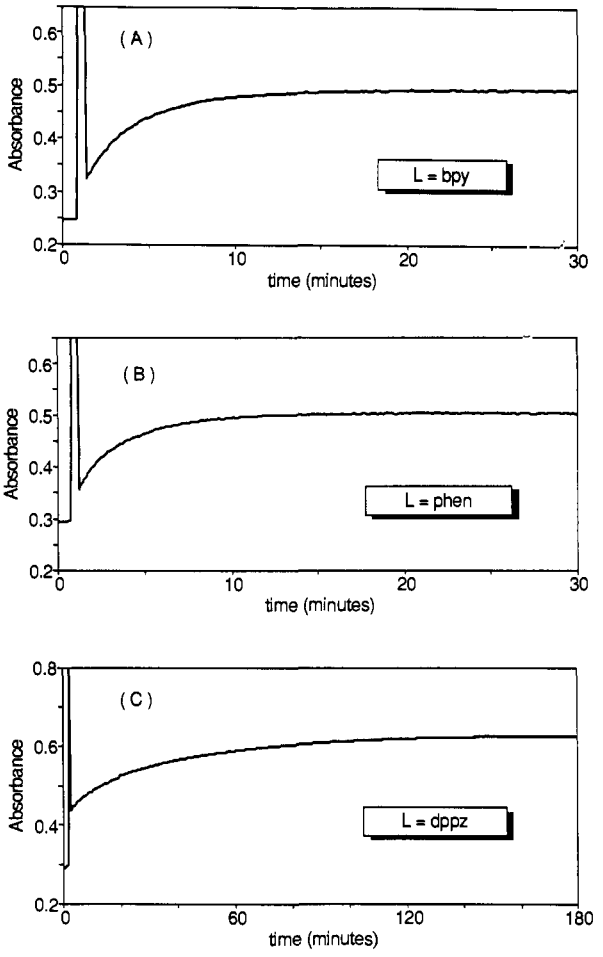

Figure 4. Absorbance vs time curves for oxidation of DNA by Ru(IV)O^{2+} species in 50 mM phosphate buffer, pH 7, containing (A) 1.2 mM DNA and 0.12 mM Ru(tpy)(bpy)O^{2+}, (B) 1.1 mM DNA and 0.11 mM Ru(tpy)(phen)O^{2+}, and (C) 1.3 mM DNA and 0.12 mM Ru(tpy)(dppz)O^{2+}.

Scheme I

$$\mathrm{Ru(IV)O^{2+} + DNA} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{Ru(IV)O^{2+}\cdot DNA} \quad (K_B)$$

$$\mathrm{Ru(IV)O^{2+}\cdot DNA} \xrightarrow{k_1} \mathrm{Ru(II)OH_2^{2+}\cdot DNA}$$

$$\mathrm{Ru(II)OH_2^{2+}\cdot DNA} \underset{k_{on}}{\overset{k_2\ (k_{off})}{\rightleftharpoons}} \mathrm{Ru(II)OH_2^{2+} + DNA} \quad (K_B)$$

emissive in aqueous solution but does emit in the presence of double-stranded DNA.[35] The complex therefore exhibits the "light-switch" effect noted for $Ru(bpy)_2(dppz)^{2+}$.[25] In addition, we have reported that the dppz ligands of Ru(tpy)(dppz)OH_2^{2+} are stacked in the solid state in a manner seen for many classical intercalators.[12]

In terms of its binding affinity, thermal denaturation, and absorbance hypochromicity, Ru(tpy)(phen)OH_2^{2+} gives results intermediate between those of Ru(tpy)(bpy)OH_2^{2+} and Ru(tpy)(dppz)OH_2^{2+}. We have not observed that the Ru(tpy)(phen)OH_2^{2+} complex lengthens DNA in the viscometry assay; however, two important caveats must be applied. The first is that, if only some fraction of the metal complex is intercalated, as has been suggested for $Ru(phen)_3^{2+}$,[26,27] then the viscometry assay might not be sensitive enough to detect it. In addition, the relatively low solubility and binding affinity of Ru(tpy)(phen)OH_2^{2+} prohibited testing the complex for both unwinding and viscometry at concentrations that might be required for enough metal complex to be bound to DNA to give a positive result.[15] Thus, our unwinding and viscometry results cannot be used as evidence for or against intercalation of Ru(tpy)(phen)OH_2^{2+}.

An understanding of the binding modes of the metal complexes is tantamount to analysis of the kinetic studies. The trend in the contribution of the k_1 phase (Scheme I) to the overall decay is consistent with the trend in binding affinities reflected in the thermal denaturation, absorbance hypochromicity, and binding constants given in Table I for the three Ru(tpy)(L)OH_2^{2+} complexes. The burst fraction only reflects the concentration of bound Ru(IV)O^{2+} upon initial mixing. Thus, we have shown that the cleavage process is the rate-determining step in the initial phase, not binding.

The kinetics of the k_2 phase are strikingly concentration-independent. However, this result is consistent with our model shown in Scheme I. If the kinetics are controlled by dissociation of a bound, reduced Ru(II)OH_2^{2+} complex, then the kinetics would be expected to be independent of the initial DNA and ruthenium concentrations. This interpretation is also consistent with the fact that the contribution of the k_2 phase to the overall decay is smaller when there is less Ru(IV)O^{2+} in solution (i.e. more Ru(IV)O^{2+} bound to DNA) at time zero. Finally, the fact that the k_2 kinetics are an order of magnitude slower for Ru(tpy)(dppz)OH_2^{2+}, which is a classical intercalator, than they are for Ru(tpy)(bpy)OH_2^{2+}, which does not lengthen DNA, again points to the model given in Scheme I.

In terms of the k_1 phase of the reaction, Ru(tpy)(phen)OH_2^{2+} gives kinetics results intermediate between those of the bpy and dppz complexes. This is because the contribution of the k_1 phase to the overall decay only depends on the amount of Ru(IV)O^{2+} bound upon mixing, which depends directly on the binding affinity. In the k_2 phase of the reaction, however, the results for phen are identical to those for bpy and not at all like those for dppz. Thus, the dissociation rate of Ru(tpy)(phen)OH_2^{2+} is identical to that of the bpy complex. If Ru(tpy)(phen)OH_2^{2+} were an intercalator, it should exhibit exchange kinetics in the same range as the dppz complex.[18,20] However, the same caveat concerning the failure of our viscometry measurement to detect a fraction of intercalated complexes must also apply to the oxidation kinetics. If some small fraction (<10%) of the bound Ru(tpy)(phen)OH_2^{2+} complexes were undergoing slow exchange, the kinetic analysis might not be sensitive enough to detect it. The rapid exchange

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kinetics of $Ru(phen)_3^{2+}$ have already been reported in NMR studies;[2,19,20] however, photophysical studies show that $Ru(phen)_3^{2+}$ has a significantly longer residence time on DNA than $Ru(bpy)_3^{2+}$.[5]

The measurement of the relative dissociation rates reported here offers one of the first examples of a series such as L = bpy, phen, dppz where the results for phen are not intermediate between those for bpy and dppz or other intercalating ligands.[15,17-19,22,23] In the cases of binding affinity, absorbance hypochromism, thermal denaturation, emission enhancement, and emission polarization, the results for phen are always intermediate between those for bpy and dppz. However, we show here that the significant fraction of $Ru(tpy)(phen)OH_2^{2+}$ complexes bound to DNA dissociates at a rate identical to that for $Ru(tpy)(bpy)OH_2^{2+}$. This rate is an order of magnitude slower than that for $Ru(tpy)(dppz)OH_2^{2+}$, which is consistent with the lengthening of DNA shown by the dppz complex in Figure 2. Unfortunately, viscometry studies of $Ru(tpy)(phen)OH_2^{2+}$ are complicated by low solubility and low binding affinity, prohibiting us from drawing a link between the fast dissociation rate and the viscometry experiment. Nonetheless, the results in Table II are readily interpretable for the cases of bpy and dppz and provide a new way of studying the binding of $Ru(tpy)(phen)OH_2^{2+}$ that may ultimately aid in further defining the interaction of phen complexes with DNA.

In the case of $Ru(IV)O^{2+}$, cleavage (k_1) is rate-limiting in the first phase because oxidation is slower than binding and faster than dissociation. Two other scenarios could be envisioned wherein oxidation is either faster or slower than both binding and dissociation. Different kinetics would be expected in each of these cases. Knowledge of these models is important in mechanistic studies of other systems, particularly for interpreting isotope effects on cleavage reactions of bleomycin and other agents.[8,36] In the present $Ru(IV)O^{2+}$ system, cleavage always occurs before dissociation in the first phase of the reaction, because the $Ru(IV)O^{2+}$ is an efficient oxidant. If the metal complex were a less efficient oxidant, dissociation would compete with oxidation, leading to a significantly different kinetic model. Likewise, if the metal complex were a significantly more efficient oxidant, oxidation would compete with binding, also altering the kinetic model.

The results in Table II make an unusual point concerning the interplay of binding thermodynamics and oxidation kinetics. In the first phase, the amount of metal complex bound to DNA controls the efficiency of oxidation and, therefore, complexes with higher affinities are more efficient cleavage agents. In the second phase, however, when cleavage must occur starting with solution-bound $Ru(IV)O^{2+}$, the high-affinity intercalating complex is actually a *less efficient* oxidant than the low affinity bpy and phen complexes, because dissociation of reduced complex is rate-limiting. This points out effectively that an understanding of the microscopic details of the cleavage mechanism is vital to evaluating the efficiency of individual cleavage agents.

Acknowledgment. We gratefully acknowledge the support of the North Carolina Biotechnology Center and the Burroughs-Wellcome Co. H.H.T. thanks the National Science Foundation for a Presidential Young Investigator award, the Camille and Henry Dreyfus Foundation for a New Faculty Award, and the David and Lucile Packard Foundation for a Fellowship in Science and Engineering.

Supplementary Material Available: Emission titration curves for measurement of the binding affinities of the three complexes with accompanying discussion (5 pages). Ordering information is given on any current masthead page.

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