# Covalent Binding of Aquaruthenium Complexes to DNA

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Thermal denaturation studies were perfomed on a group of mononuclear mono- and diaqua polypyridyl complexes of Ru(II) covalently bound to calf thymus DNA. Adducts of monofunctional complexes show small, positive values of $\Delta T_m$ (0.8–3.5). The adducts of difunctional complexes exhibit much larger values (6–13), and the thermal denaturation is irreversible, which is consistent with formation of an interstrand diadduct. The dinuclear complex $[(bpy)_2Ru(OH_2)]_2O^{4+}$ was also studied and found to bind stereoselectively to calf thymus DNA (bpy = 2,2′-bipyridine). Circular dichroism spectroscopy showed the filtrate obtained from ultrafiltration of calf thymus DNA and $[(bpy)_2Ru(OH_2)]_2O^{4+}$ to be enriched in one enantiomer. We also report here the synthesis and electronic properties of the model complex $[(bpy)_2(EtG)RuOH_2]^{2+}$ (EtG = 9-ethylguanine). The complex is stable and possesses all of the characteristic electronic properties of the other polypyridylaquaruthenium complexes. Surprisingly, the $Ru^{IV}O$ form is accessible via electrochemical oxidation and is an effective DNA cleavage agent.

Metal complexes can bind to DNA via both covalent and non-covalent interactions.[1-3] In the former case, a labile ligand of the metal complex is replaced by a nucleophile in DNA, usually from a nitrogenous base, such as guanine. Non-covalent interactions include electrostatic, intercalative, and groove (surface) binding. Polypyridyl complexes of oxoruthenium(IV) have proven to be efficient oxidative cleavage agents,[4-7] and their aquaruthenium-(II) analogs have been shown to bind covalently to the nitrogenous bases.[8,9] The conversion of the $Ru^{II}OH_2$ complex to the reactive $Ru^{IV}O$ cleavage agent is accomplished chemically or electrochemically, according to eqs 1 and 2.

$$[Ru(tpy)(bpy)OH_2]^{2+} \rightarrow [Ru(tpy)(bpy)OH]^{2+} + H^+ + e^- \quad (1)$$

$$[Ru(tpy)(bpy)OH]^{2+} \rightarrow [Ru(tpy)(bpy)O]^{2+} + H^+ + e^- \quad (2)$$

We reported previously that the complexes $[(L)_5Ru(OH_2)]^{2+}$ and $[(L)_4Ru(OH_2)_2]^{2+}$ bind covalently to DNA at a surprisingly low ratio of bound ruthenium to DNA ($r_b$) of approximately $r_b = 0.015 \pm 0.005$.[9] The time required for half of the ruthenium bound at saturation to bind was determined by ethanol precipitation experiments to be about 30 min. The chiral selectivity of these Ru(II) aqua complexes was surprisingly high, with an enantiomeric excess for covalent binding of $[Ru(phen)_2(py)(OH_2)]^{2+}$ of 80%, favoring the Λ isomer. The degree of selectivity was much higher for bis(phen) complexes than for bis(bpy) complexes, and the degree of selectivity was a factor of 2 higher for $[Ru(L)_2(py)(OH_2)]^{2+}$ complexes compared to $[Ru(L)_2(OH_2)_2]^{2+}$ complexes (L = bpy or phen). We report here thermal denaturation studies on the covalent adducts of the same family of complexes with calf thymus DNA. Our studies show that the $\Delta T_m$ values are small and positive for covalent adducts of monoaqua complexes and are large and positive for adducts of diaqua complexes. This result is consistent with the formation of monoadducts by the monofunctional complexes and interstrand diadducts for the difunctional complexes.

The covalent interactions of ruthenium(II) with DNA were modeled previously using $Ru(NH_3)_5^{2+}$ fragments[10,11] coordinated to guanosine derivatives and more recently by reaction of $(bpy)_2RuCl_2$ with 9-ethylguanine to yield the displacement product $[Ru(bpy)_2(9\text{-}EtG)Cl]^+$.[12] The crystal structure of the latter complex confirms the binding to be to N7 of guanine. An unanswered question involves the existence of stable higher-valent ruthenium bound to guanine. For example, we have been interested in whether a $Ru^{II}OH_2$ complex bound to DNA could be oxidized to the $Ru^{IV}O$ form, allowing us to perform cleavage reactions on the DNA template.

We report here the synthesis and electronic properties of the model complex $[(bpy)_2(EtG)RuOH_2]^{2+}$. The electronic properties of the model are similar to those of the covalent adducts of DNA. The $Ru^{II}OH_2$ form is easily synthesized, and perhaps surprisingly, the $Ru^{IV}O$ form is accessible via electrochemical oxidation of dilute solutions of the $Ru^{II}OH_2$ complex.

## Experimental Section

**Materials.** 9-Ethylguanine and calf thymus DNA were purchased from Sigma and used as recieved. Plasmid φX174 DNA was purchased from Pharmacia and used as recieved. All other chemicals were purchased from Aldrich and used as recieved.

**Complexes. $[Ru(bpy)_2(OH_2)]_2O^{4+}$.** Synthesis of $\{[Ru(bpy)_2(OH_2)]_2O\}(ClO_4)_4$ was performed as reported previously.[13]

**$[(bpy)_2(EtG)RuOH_2]^{2+}$.** $(bpy)_2RuCl_2 \cdot 2H_2O$ (0.521 g, 1 mmol) was refluxed for 48 h in 40 mL of 3:1 $EtOH/H_2O$ with 9-ethylguanine (0.233 g, 1.3 mmol). The solution was filtered hot and the volume reduced by three-fourths by rotary evaporation. The solution was added to a 10-mL

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saturated solution of $NH_4PF_6$, and the resulting solid was collected, washed with water, and dried *in vacuo*. The solid was dissolved in acetonitrile, and the solution was chromatographed on alumina in acetonitrile and 20:1 acetonitrile/water. The second fraction was collected, and diethyl ether was added to precipitate the complex $[(bpy)_2(EtG)Ru(NCMe)](PF_6)_2$. The FAB mass spectrum (nitrobenzyl alcohol matrix) showed a series of peaks centered at $m/z$ = 779 which gave the appropriate theoretical ion distribution for M – $PF_6$. Anal. Calc for $[(bpy)_2(EtG)RuNCMe](PF_6)_2 \cdot 3H_2O$: C, 34.6; H, 3.70; N, 14.4. Found: C, 34.6; H, 3.27; N, 13.7. In pH 7 buffered solutions, electronic absorption spectroscopy and cyclic voltammetry showed the quantitative aquation of the acetonitrile ligand within 30 min to yield the complex of interest: $[(bpy)_2(EtG)RuOH_2]^{2+}$.

Syntheses of all the other metal complexes were reported previously.[9]

**Measurements.** Cyclic voltammetry and construction of Pourbaix diagrams were perfomed as described[4,6] using a PAR 273A potentiostat with a tin-doped indium oxide working electrode. Electronic absorption spectra were acquired on an HP8452 diode array spectrophotometer.

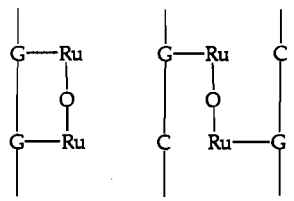
Controlled-potential electrolysis was performed in a three-compartment cell using a reticulated vitreous carbon working electrode. DNA cleavage experiments using electrogenerated $[(bpy)_2(EtG)RuO]^{2+}$ were performed as described.[7]

Thermal denaturation studies were performed as described previously.[14,15] The samples were prepared by treating DNA with an excess of metal complex for 12 h in phosphate buffer (50 mM, pH 7) and subjecting the solution to ultrafitration to remove all the non-covalently bound metal complex. The buffer was changed by ultrafiltration in 5 mM Tris–HCl, 10 μM EDTA, and 5% DMSO prior to performing thermal denaturation measurements. Solubility is not a complication in these systems; however, the DMSO-containing buffer system was used in order to compare the present results with others from our laboratories.[14,15]

Ethanol precipitation, ultrafiltration, and circular dichroism experiments were performed as described previously.[9]

## Results and Discussion

**Covalent Binding of $[Ru(bpy)_2(OH_2)_2]_2O^{4+}$.** We have been studying the covalent binding of $[Ru(bpy)_2(OH_2)]_2O^{4+}$ because the Ru···Ru separation of 3.4 Å is the same as the base pair separation in B-DNA.[16] We reasoned that a special type of diadduct may form, where each ruthenium center is bound to a guanine from adjacent base pairs. Both inter- and intrastrand adducts can be easily envisioned:




Ultrafiltration of solutions of $[Ru(bpy)_2(OH_2)]_2O^{4+}$ incubated with calf thymus DNA for 12 h yields DNA solutions that retain the absorption spectrum of the ruthenium complex even upon continued ultrafiltration, indicating covalent binding of ruthenium to DNA. These observations are entirely analogous to those we have made with monomeric complexes,[9] where the measured concentration of bound ruthenium for a wide range of complexes was the same if determined by quantitation from the spectrum of either the labeled DNA or the filtrate. Thus, the extinction coefficient is not altered appreciably by DNA binding. We have published the shifts in $\lambda_{max}$ and the hypochromicities for the mononuclear complexes elsewhere.[9] The ratio of bound ruthenium to DNA–phosphate $r_b$ = 0.020 can be calculated from the strong band at 640 nm in the absorption spectrum of the ruthenium-labeled DNA, assuming the extinction coefficient does not change upon binding to DNA. The $\lambda_{max}$ for the DNA-bound $[Ru(bpy)_2(OH_2)]_2O^{4+}$ complex is 644 nm, a red shift of only 4 nm compared to that of the free aqua complex. Thus, covalent binding does not appreciably alter the optical properties of the ruthenium complex.

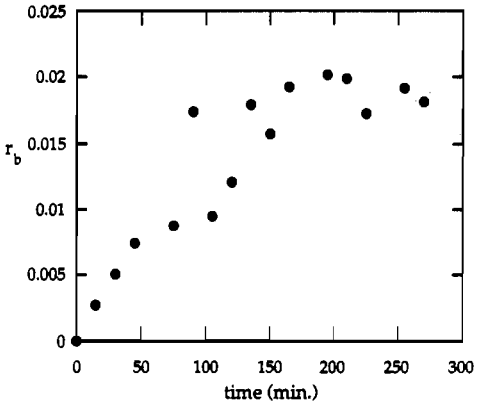



**Figure 1.** Values of $r_b$ as a function of time for the covalent binding of $[(bpy)_2Ru(OH_2)]_2O^{4+}$ to calf thymus DNA.

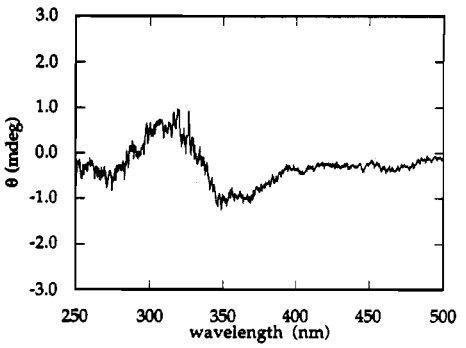



**Figure 2.** CD spectrum of the filtrate obtained after the ultrafiltration of calf thymus DNA incubated with $[(bpy)_2Ru(OH_2)]_2O^{4+}$ for 12 h.

The $r_b$ value for $[Ru(bpy)_2(OH_2)]_2O^{4+}$ could also be obtained from ethanol precipitation experiments. An advantage of the ethanol precipitation experiment is that the time-dependent characteristics of the reaction can be assessed by precipitating the DNA from aliquots of the DNA–ruthenium solution at particular time intervals. The amount of metal complex in the supernatant can then be quantitated by absorption spectroscopy. Figure 1 shows the plot of $r_b$ versus time obtained from aliquots collected every 15 min. The plot shows the maximum value for $r_b$ is about 0.02, which is similar to the values obtained with the other metal complexes we have already reported.[9] The results remain unchanged beyond the time period shown in Figure 1. The amount of time required for saturation is very similar to that for the other complexes we have studied. The value of $r_b$ for $[Ru(bpy)_2(OH_2)]_2O^{4+}$ obtained by ultrafiltration agrees with that from ethanol precipitation, and both values indicate that a relatively small amount of the complex is covalently bound to DNA. In addition, the value determined from the spectrum of the labeled DNA agrees with those determined from ultrafiltration and ethanol precipitation, strongly supporting the assumption that there is very little change in extinction coefficient upon DNA binding.

Circular dichroism (CD) of the filtrate obtained following ultrafiltration shows that the covalent binding of $[Ru(bpy)_2(OH_2)]_2O^{4+}$ is stereoselective (Figure 2). Formation of covalent adducts of the monomeric complexes we have studied previously leads to enrichment of the solution in the Δ isomer, indicating preferential covalent binding of the Λ isomer.[8,9] The dimeric complex $[Ru(bpy)_2(OH_2)]_2O^{4+}$ exists potentially as three different enantiomers. The ΔΔ and ΛΛ isomers are present in the published crystal structure,[13] and a meso ΔΛ form could also exist. These

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**Table 1.** Thermal Denaturation Results for Covalent Adducts of Calf Thymus DNA with Aquaruthenium(II) Complexes

| metal complex | $\Delta T_m$ (°C) | $r_b$[a] | $\Delta T_m$ (°C)[b] |
|---|---|---|---|
| $[Ru(tpy)(bpy)(OH_2)]^{2+}$ | 0.8 ± 0.3 | 0.015 | 4.2 ● 0.5[c] |
| $[Ru(tpy)(tmen)(OH_2)]^{2+}$ | 0.8 ± 0.7 | 0.018 | 1.0 ± 0.1 |
| $[Ru(tpy)(phen)(OH_2)]^{2+}$ | 3.5 ± 0.7 | 0.019 | 7.2 ± 0.4[c] |
| $[Ru(tpy)(dppz)(OH_2)]^{2+}$ | 1.6 ± 0.2 | 0.012 | 14.1 ± 0.8[c] |
| $[Ru(bpy)_2(py)(OH_2)]^{2+}$ | 2.4 ± 0.3 | 0.012 | 2.5 ± 0.3 |
| $[Ru(phen)_2(py)(OH_2)]^{2+}$ | 2.4 ± 0.5 | 0.010 | 4.1 ± 2.7 |
| $[Ru(bpy)_2(OH_2)_2]^{2+}$ | 6.1 ± 0.5 | 0.024 | 1.6 ± 0.2 |
| $[Ru(phen)_2(OH_2)_2]^{2+}$ | 12.9 ± 0.8 | 0.015 | 4.7 ± 1.0 |
| $[Ru(bpy)_2(OH_2)]_2O^{4+}$ | 8.1 ± 0.5 | 0.020 | 6.6 ± 0.1 |

[a] $[Ru]_{bound}$/[DNA–phosphate] determined spectrophotometrically. [b] Values for non-covalently bound metal complex measured at DNA: metal complex = 10:1. [c] Reference 9.

isomers have not been resolved, so the absolute configuration of the preferentially bound isomer cannot be assigned from Figure 2, nor can the enantiomeric excess be determined. Certainly, the degree of rotation is much less than we have observed with $[Ru(phen)_2(OH_2)_2]^{2+}$,[9] suggesting that the stereoselectivity is much lower. Analogy with our earlier results would also suggest that the ΛΛ isomer is the preferred one; however, this cannot be assigned until the isomers are resolved. Nonetheless, this is to our knowledge the first CD spectrum of $[Ru(bpy)_2(OH_2)]_2O^{4+}$, which is a catalyst for a number of important reactions, such as the oxidation of water to dioxygen.[13] An understanding of the stereochemistry of this important complex may allow for its catalytic properties to be applied in a stereoselective fashion. In fact, DNA binding may provide a means for separating the stereoisomers, as with mononuclear complexes.[17]

**Thermal Denaturation Studies.** We recently reported the results of thermal denaturation studies on non-covalent adducts of DNA and $[Ru(tpy)(L)OH_2]^{2+}$ (L = bpy, phen, dppz).[14] These studies were conducted at DNA:metal complex ratios of 10:1 on solutions prepared immediately preceding the measurement, before appreciable covalent binding could occur. These studies show that the measured $\Delta T_m$ increases with increasing binding affinity, from a small value of 4.2 °C for the electrostatically bound bpy complex to a large value of 14.1 °C for the dppz complex, which has been demonstrated by viscometry, unwinding, and photophysical methods to bind intercalatively.[14,18] Values of $\Delta T_m$ for a number of other complexes non-covalently bound to DNA have been determined and are given in Table 1. These values are consistent with those we have published previously, with phen complexes showing higher values than bpy complexes. The dinuclear complex $[Ru(bpy)_2(OH_2)]_2O^{4+}$ shows a higher $\Delta T_m$ than the analogous mononuclear $[Ru(bpy)_2(OH_2)_2]^{2+}$ complex because of the increased positive charge. This finding is consistent with the largely electrostatic nature of binding of bpy complexes, which has been demonstrated.[14,19]

Thermal denaturation studies of the covalently bound ruthenium–DNA adducts were performed, and the results are given in Table 1. These samples were prepared by incubation of the complexes with DNA for several hours followed by removal of the non-covalently bound metal complex by ultrafiltration. The positive (or negative) values of $\Delta T_m$ imply that the covalent adduct is more (or less) difficult to melt than the unlabeled DNA.

The thermal denaturation results are dramatically different for the covalently and non-covalently bound complexes. Covalent adducts of the monofunctional complexes $[Ru(L)(tpy)(OH_2)]^{2+}$ and $[Ru(L)_2(py)OH_2]^{2+}$ show small, positive values of $\Delta T_m$ (1–3 °C), consistent with the formation of monoadducts. A related complex of platinum(II) that is capable of forming only monoadducts, $[Pt(dien)Cl]^+$, shows a comparable $\Delta T_m$ at $r_b$ = 0.05 of +0.5 °C.[20] The $\Delta T_m$'s for the monofunctional complexes therefore either remain essentially constant or *decrease* upon formation of covalent adducts relative to the non-covalent binding. Strikingly, the $\Delta T_m$ for $[Ru(tpy)(dppz)OH_2]^{2+}$ decreases from 14.1 to 1.6 °C upon going from non-covalent intercalative binding to covalent binding. In fact, there is no correlation of the $\Delta T_m$'s with the ancillary ligand (bpy, phen, or dppz), indicating that these ligands do not interact appreciably with the DNA upon formation of the covalent adduct and that electrostatics could account for much of the $\Delta T_m$.

The thermal denaturation studies suggest that the monofunctional complexes form monoadducts. This is certainly not surprising in the case of the $[Ru(tpy)(L)OH_2]^{2+}$ complexes, because a nucleophile from the DNA is not likely to replace an arm of a bidentate or tridentate ligand. Similarly, the $[Ru(L)_2(py)OH_2]^{2+}$ complexes are also coordinatively inert and the pyridine ligand is known to be stable to substitution.[21] The similarity in the thermal denaturation results for both the Ru(tpy)(L) and $Ru(L)_2(py)$ complexes indicates the formation of similar types of adducts.

Covalent adducts of the difunctional complexes $[Ru(L)_2(OH_2)_2]^{2+}$ and $[Ru(bpy)_2(OH_2)]_2O^{4+}$ exhibit much larger values of $\Delta T_m$, which are also positive. The $\Delta T_m$'s for the non-covalent adducts of the bifunctional complexes are similar to those of the monofunctional analogues, indicating similar modes of non-covalent interaction. The $[Ru(phen)_2(OH_2)_2]^{2+}$ complex shows a striking *increase* of $\Delta T_m$ from 4.7 to 12.9 °C upon going from a non-covalent adduct to a covalent adduct. Thermal denaturation studies of adducts of $Pt(NH_3)_2Cl_2$ show that *trans*-$Pt(NH_3)_2Cl_2$ has a large, positive $\Delta T_m$ (4.5 °C, at $r_b$ = 0.05) because the platinum center forms an interstrand diadduct, thereby covalently cross-linking the two DNA strands and making them more difficult to separate.[3,20] The *cis*-$Pt(NH_3)_2Cl_2$ adduct shows a large, negative $\Delta T_m$ (–5.5 °C at $r_b$ = 0.05), because the platinum center forms primarily an intrastrand diadduct, which destabilizes the double helix and makes the strands easier to separate. The diplatinum complex of Farrell et al., $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$, has been shown to induce interstrand cross-linking and exhibits a $\Delta T_m$ of 9.3 °C under loading and buffer conditions similar to those described here.[22]

The bifunctional complexes appear to form interstrand diadducts, because of the very large $\Delta T_m$ values exhibited. In particular, the $[Ru(phen)_2(OH_2)_2]^{2+}$ adduct shows a value of +12.9 °C. This is particularly striking in light of the low level ($r_b$ = 0.015) of loading of the ruthenium complex; the analogous platinum complex *trans*-$Pt(NH_3)_2Cl_2$ exhibits a $\Delta T_m$ of only 1.5 °C at the same loading level.[20] The adduct of $[Ru(bpy)_2(OH_2)]_2O^{4+}$ exhibits a $\Delta T_m$ that is also quite high at 8.1 °C. If an interstrand adduct is formed, the thermal denaturation should be irreversible, because initial strand separation will involve breaking the covalent bonds between the metal center and the DNA. Upon cooling of the sample, these bonds are no longer present, and the re-formation of the double helix should be dramatically different. Melting of poly(dA)·poly(dT) in the absence of metal complex occurs at 43.4 °C, and reannealing simply retraces the initial melting curve. Similar experiments on the covalent adduct of $[Ru(phen)_2(OH_2)_2]^{2+}$ and poly(dA)·poly(dT) show that, upon cooling of the sample back to room temperature, the absorbance remains constant, demonstrating that re-formation of the double helix does not occur. Thus, an unusual adduct must be formed which, once disrupted, damages the DNA to the point that it can no longer re-form the double

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helix. This dramatic result is consistent with the very large $\Delta T_m$ in pointing to the formation of an interstrand diadduct.

An interesting aspect of the interstrand adduct formation is that the $[Ru(L)_2(OH_2)_2]^{2+}$ complexes are in the cis geometry. Formation of an interstrand diadduct might be more efficient with a trans geometry at the metal center, as in the case of platinum(II); however, *cis*-$Pt(NH_3)_2Cl_2$ does form both inter- and intrastrand adducts.[3] It is known that $[Ru(L)_2(OH_2)_2]^{2+}$ complexes are capable of cis–trans isomerization.[23] Adduct formation may induce a stereochemical rearrangement from the cis configuration to the trans configuration to facilitate formation of an interstrand diadduct. Alternatively, a highly strained interstrand diadduct with a cis metal center may form, which might explain the unusual thermal denaturation results. Readily detectable changes in the spectra of the metal complex are apparent upon cis–trans isomerization[23] and were not detected in any of our experiments. Thus, if an interstrand adduct is formed, it likely involves a cis complex. Oxo-bridged dimers have very strong absorptions in the 600–700-nm range,[13] and no formation of oxo-bridged dimer from the mononuclear complexes was detected in any of our reactions.

The unusual properties of the adducts (small $r_b$'s, high stereoselectivities, high $\Delta T_m$'s for diadducts) suggest that special interactions may exist in the metal–nucleic acid complexes. The recent crystal structure of $[Ru(bpy)_2(EtG)Cl]^+$ determined by Reedijk et al.[12] shows that guanine binds with the keto group wedged between the two bpy ligands, creating a large barrier to rotation about the Ru–N(guanine) bond. Binding of a second guanine to the metal center can be envisioned to occur in a similar configuration, giving a head-to-head arrangement of the guanine ligands, which is unusual but has been noted for Ru(II).[24] This second guanine ligand would also be highly constrained, yielding a very specific structure that may be required to achieve the unusual properties of the diadducts described here.

**Model Complex.** The synthesis of the model complex $[(bpy)_2(EtG)RuOH_2]^{2+}$ is based on the lability of the chloro and subsequent aqua ligands of $(bpy)_2RuCl_2$ in refluxing ethanol–water solution. Substitution by a single purine ligand appears to proceed by analogy to the role of pyridine in the synthesis of $[(bpy)_2(py)Ru(OH_2)]^{2+}$.[21] The remaining aqua ligand is displaced by acetonitrile during purification to yield the stable solvento complex $[(bpy)_2(EtG)Ru(NCMe)]^{2+}$. The acetonitrile ligand is useful in preventing the slow oxidation to $Ru^{III}$ that occurs in solid perchlorate salts of the aqua complex. In buffered solution, quantitative aquation of the acetonitrile ligand occurs within 1 h to yield $[(bpy)_2(EtG)Ru(OH_2)]^{2+}$.

The optical properties of the complex are perturbed somewhat by coordination of guanine to the metal center. The starting $[Ru(bpy)_2(OH_2)_2]^{2+}$ complex exhibits a $\lambda_{max}$ of 484 nm, which is shifted to 476 nm upon the coordination of guanine. In addition, the extinction coefficient changes from $\epsilon = 10\,800\ M^{-1}\ cm^{-1}$ for the starting complex to $\epsilon = 7600\ M^{-1}\ cm^{-1}$ for the guanine complex.

Chemical oxidations of $[(bpy)_2(EtG)RuOH_2]^{2+}$ to the corresponding $Ru^{III}OH$ and $Ru^{IV}O$ species were not successful. Excess $Cl_2$ and $Br_2$ as well as excess and stoichiometric amounts of $Ce^{4+}$ all led to irreversible oxidation of the parent complex. This may occur because the guanine ligand is easily oxidized ($\sim$1.0 V vs SSCE),[25] and these oxidants are all thermodynamically capable of oxidizing both the ruthenium center and the coordinated guanine ligand. Oxidation of the guanine ligand may provide a pathway for metal oxidation and the formation of oxo-bridged dimers, which were observed by mass spectrometry upon standing in air of reaction solutions.

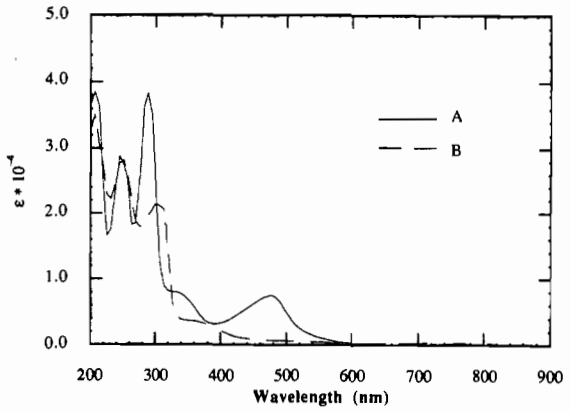


**Figure 3.** Electronic spectra of (A) $[(bpy)_2(EtG)RuOH_2]^{2+}$ and (B) $[(bpy)_2(EtG)RuO]^{2+}$.

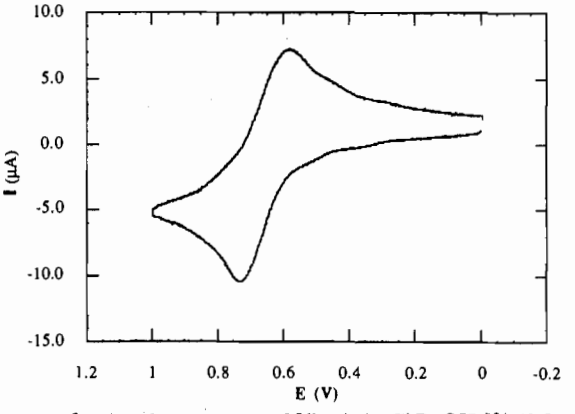


**Figure 4.** Cyclic voltammogram of $[(bpy)_2(EtG)RuOH_2]^{2+}$ (0.5 mM), pH 4. Conditions: tin-doped indium oxide working electrode, Pt wire auxiliary, Ag/AgCl reference, 100 mV/s scan rate, 50 mM phosphate buffer.

Although chemical oxidations were unsuccessful, $[(bpy)_2(EtG)RuO]^{2+}$ was obtained electrochemically at concentrations sufficient for characterization and reactivity studies. Continuous electrolysis at +0.8 V vs SSCE at pH 7 converts $[(bpy)_2(EtG)RuOH_2]^{2+}$ stoichiometrically to the $Ru^{IV}O$ form. At this potential, the ruthenium center is oxidized without oxidation of the guanine ligand. Figure 3 shows that this conversion can be followed by monitoring the disappearance of the intense band at 476 nm due to MLCT in the $Ru^{II}OH_2$ form. Clean electrochemical conversion to the $Ru^{IV}O$ form occurs only at concentrations less than approximately 50 $\mu$M, and bands characteristic of Ru(III) and Ru(IV) are too weak to be observed at these concentrations. Addition of a small amount of solid $SnCl_2$ to the nearly colorless $Ru^{IV}O$ solutions gives complete recovery of the corresponding $Ru^{II}OH_2$ complex, as monitored by MLCT intensity.

Cyclic voltammetry shows that the redox chemistry of $[(bpy)_2(EtG)RuOH_2]^{2+}$ is similar to that of related monoaquapolypyridylruthenium complexes,[21] with $E_{1/2}(IV/II) = 0.430$ V (Figure 4). Related aqua polypyridyl complexes such as $[Ru(tpy)(bpy)OH_2]^{2+}$ exhibit two distinct waves separated by $\sim$100 mV corresponding to eqs 1 and 2. Coulometry at 0.8 V vs SSCE shows the passage of 1.9 electrons during oxidation of $[(bpy)_2(EtG)RuOH_2]^{2+}$, consistent with oxidation to Ru(IV). Resolution of the cyclic voltammogram into the two one-electron waves was also not possible at indium–tin oxide and glassy carbon working electrodes at scan rates between 20 and 500 mV $s^{-1}$. The pH dependence typical of proton-coupled oxidations is shown in Figure 5. The plot of $E_{1/2}$ vs pH is linear over the range pH 0.35–13.0 with a slope of $50 \pm 2$ mV/pH unit, consistent with a two-electron, two-proton oxidation.

Addition of $[(bpy)_2(EtG)RuO]^{2+}$ to DNA results in oxidation of the nucleic acid with corresponding recovery of the $Ru^{II}OH_2$

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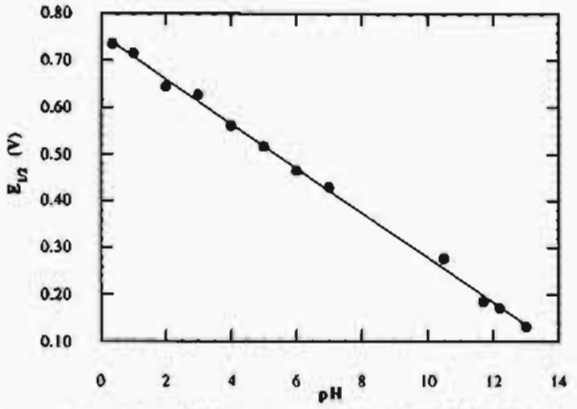


**Figure 5.** Plot of $E_{1/2}$(IV/II) vs pH for $[(bpy)_2(EtG)RuOH_2]^{2+}$.

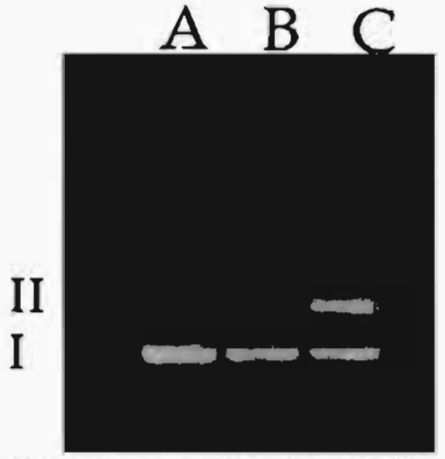


**Figure 6.** 1% agarose gel showing the results of electrophoresis of (A) 60 $\mu$M $\phi$X174 DNA and the same complex in the presence of (B) 0.1 mM $[(bpy)_2(EtG)RuOH_2]^{2+}$ and (C) 0.1 mM $[(bpy)_2(EtG)RuO]^{2+}$.

complex. The ethidium bromide gel in Figure 6 shows the conversion of supercoiled plasmid (form I) to nicked circular (form II) upon incubation with $[(bpy)_2(EtG)RuO]^{2+}$. In control experiments, incubation with the $Ru^{II}OH_2$ complex (lane A) and continued electrolysis of the plasmid at 0.8 V vs SSCE (lane B) caused no conversion to the nicked form. Some aspects of the DNA cleavage chemistry of this complex have been published previously.[26]

As with $[Ru(tpy)(bpy)O]^{2+}$, oxidation of DNA by $[(bpy)_2(EtG)RuO]^{2+}$ is initially the dominant process and is characterized by an isosbestic point at 340 nm (Figure 7A). The second phase of the reaction is slower by an order of magnitude and is characterized by a isosbestic points at 322, 368, and 406 nm (Figure 7B) with a return of the $[(bpy)_2(EtG)RuOH_2]^{2+}$ absorption at 476 nm. This two-phase behavior is seen in oxidations by other monooxoruthenium(IV) complexes,[21,27] and the second phase must therefore correspond to oxidation by the $Ru^{III}OH$ complex. Thus, the $[(bpy)_2(EtG)RuOH]^{2+}$ form is stable, and the failure to observe two distinct waves in Figure 4 is due to slow electrode kinetics or proximity in potential of the

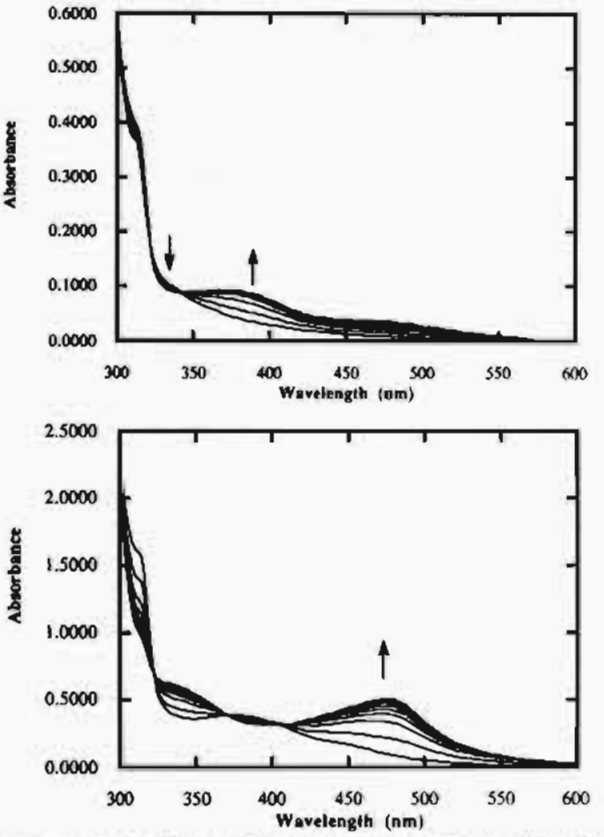


**Figure 7.** (A) Top: Electronic spectra taken at 10-s intervals during the oxidation of calf thymus DNA (0.5 mM nucleotide phosphate) by $[(bpy)_2(EtG)RuO]^{2+}$ (0.025 mM). (B) Bottom: Electronic spectra taken at 2-min intervals during the oxidation of calf thymus DNA (1.0 mM nucleotide phosphate) by $[(bpy)_2(EtG)RuO]^{2+}$ (0.1 mM).

(IV/III) and (III/II) couples, not to $[(bpy)_2(EtG)RuO]^{2+}$ being a two-electron oxidant. Interaction of the guanine ligand with the electrode surface may provide an origin for the inability to resolve the two waves.

## Conclusions

Thermal denaturation studies are consistent with binding of $[(L)_5Ru(OH_2)]^{2+}$ complexes to DNA via formation of a monoadduct of the ruthenium and the DNA and binding of $[(L)_4Ru(OH_2)_2]^{2+}$ via formation of a diadduct. The $\Delta T_m$'s for the diadducts are quite high at relatively low ($r_b \sim 0.02$) levels of covalent binding. The binding of $[(bpy)_2Ru(OH_2)]_2O^{4+}$ is stereoselective although, at present, the absolute configuration of the preferred isomer cannot be assigned, and the enantiomeric excess cannot be determined.

Synthesis of model complexes shows that $[Ru(bpy)_2(EtG)OH_2]^{2+}$ is a stable complex and posesses all of the same electronic properties as the other polypyridylaquaruthenium(II) complexes. The complex can be oxidized to the $Ru^{IV}O$ form at a surprisingly low potential, and this oxidized complex is an effective DNA cleavage agent. This presents the attractive possibility of activating the cleavage chemistry of covalently bound monoaqua complexes.

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