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Stabilization of Duplex DNA Structure and Suppression of Transcription in Vitro by Bis(quinone diimine) Complexes of Rhodium(III) and Ruthenium(II)

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The ability of octahedral complexes possessing quinone diimine ligands to inhibit transcription by stabilization of the DNA duplex structure was investigated. Rh(III) and Ru(II) complexes possessing two quinone diimine ligands in their coordination sphere were found to significantly increase the melting temperature (ΔT_m) of a 15-mer duplex DNA. $[Rh(phi)_2phen]^{3+}$ and $[Ru(phi)_2phen]^{2+}$ (phi = 9,10-phenanthrenequinone diimine, phen = 1,10-phenanthroline) exhibit ΔT_m values of +21 and +15 °C relative to free 15-mer duplex (T_m = 55 °C) at [complex]/[DNA bases] = 0.067 (two complexes/duplex). Similarly, a shift in the melting temperature of +14 °C was measured for $[Rh(bqdi)_2phen]^{3+}$ (bqdi = 1,2-benzoquinone diimine), which possesses the nonintercalating bqdi ligand. In contrast, $[Ru(phen)_2phi]^{2+}$ and $[Rh(phen)_2L]^{3+}$ (L = phi, bqdi), which possess a single quinone diimine ligand, the parent $[Ru(phen)_3]^{2+}$ and $[Rh(phen)_3]^{3+}$ complexes, and ethidium bromide result in small shifts in the melting temperature of the duplex oligonucleotide. A distinct correlation between ΔT_m and the relative concentration of each complex required to inhibit 50% of the transcription (R_{inh}^{50}) was observed, independent of the presence of an intercalative ligand. The duplex stabilization by bis(quinone diimine) complexes results in inhibition of transcription in vitro at significantly lower complex concentrations than for the corresponding $[Ru(phen)_2phi]^{2+}$ and $[Rh(phen)_2L]^{3+}$ (L = phi, bqdi) complexes. Possible explanations for these observations are discussed.

Introduction

The replication and growth of cancerous cells can be prevented through the inhibition of transcription.[1−5] Many antitumor drugs, including actinomycin, anthracycline antibiotics, and cisplatin, utilize this mechanism as their mode of action.[6−8] Within cells, the transcription of duplex DNA results in the formation of messenger RNA (mRNA), which is then translated into proteins. Therefore, inhibition of transcription can result in the incomplete coding of mRNA and proteins and can ultimately lead to cell death.[1−8] Inhibition of transcription was recently reported for cationic mixed monolayer protected gold clusters.[9]

Rhodium(III) complexes possessing phi ligands (phi = 9,10-phenanthrenequinone diimine; Figure 1) have been shown to undergo a variety of photoinduced reactions with DNA,[10−15] including direct DNA photocleavage,[13−15] long-

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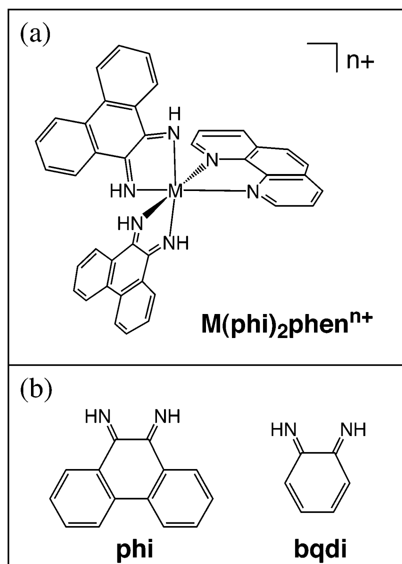

Figure 1. Molecular structures of (a) $M(phi)_2phen^{n+}$ (M = Rh, $n = 3$; M = Ru, $n = 2$) and (b) the phi and bdqi ligands.

range photooxidative damage to guanines,[10] and thymine dimer photorepair with visible light.[11] Additional interest in phi complexes of Rh(III) arises from their binding to mismatches in duplex DNA,[16] as well as in their ability to act as electron acceptors in charge-transfer reactions when bound to the duplex.[17−19] Two-dimensional ^{1}H NMR has been utilized extensively to elucidate the binding of Rh(III) complexes possessing a single phi ligand to duplex DNA. The complexes in these studies include $[Rh(NH_3)_4phi]^{3+}$,[20] $[Rh(phen)_2phi]^{3+}$ (phen = 1,10-phenanthroline),[21] Δ-α-{Rh[(*R*,*R*)-Me_2trien]phi}$^{3+}$ [(*R*,*R*)-Me_2trien = 2*R*,9*R*-diamino-4,7-diazadecane],[22] and $[Rh(en)_2phi]^{3+}$ (en = ethylenediamine).[23] Furthermore, the crystal structure of Δ-α-{Rh[(*R*,*R*)-Me_2trien]phi}$^{3+}$ bound to an eight base-pair oligonucleotide duplex[24] also shows that the binding of these mono-phi complexes to DNA takes place through the intercalation of the phi ligand between the bases of the duplex from the major groove side. Although bis-phi complexes of Rh(III), such as $[Rh(phi)_2bpy]^{3+}$ (bpy = 2,2′-bipyridine), have been utilized extensively in DNA photocleavage experiments,[10−19] no ^{1}H NMR studies or crystal structures have been reported to date on their DNA binding.

We recently reported the inhibition of transcription by the bis-phi complex $Rh(phi)_2phen^{3+}$, whose structure is shown in Figure 1.[25] In addition, the competitive binding to duplex DNA between a transcription factor, yAP-1, and the related complex Λ-1-$[Rh(MGP)_2phi]^{5+}$ (MGP = 4-(guanidylmethyl)-1,10-phenanthroline) was recently reported.[26] The Λ-1-$[Rh(MGP)_2phi]^{5+}$ complex, possessing a single phi ligand and a 5+ charge, binds tightly and selectively to the 5′-CATATG-3′ sequence in the major groove of duplex DNA. In templates where the protein's recognition element was modified with the complex's binding sequence, no inhibition of the binding of yAP1 to DNA at Rh(III) complex concentrations as high as 20 μM was observed.[26] In our experiments, we found that the bis-phi complex $[Rh(phi)_2phen]^{3+}$ is able to stabilize the duplex DNA structure.[25] Such stabilization is known to result in suppression of transcription through inhibition of the DNA elongation (or duplex opening) step.[6a,27] The present work focuses on the exploration of duplex stabilization and transcription inhibition by octahedral $[Rh(L)_n(phen)_{3-n}]^{3+}$ (L = phi, bqdi (bqdi = 1,2-benzoquinone diimine); $n = 0-2$) and $[Ru(phi)_n(phen)_{3-n}]^{2+}$ ($n = 0-2$) complexes, and the complexes that possess the intercalative phi ligand were compared to those with the nonintercalative bqdi in their octahedral coordination spheres. The molecular structures of all the ligands are shown in Figure 1.

Experimental Section

Materials. Agarose, ethidium bromide, $RuCl_3$, $RhCl_3$, 1,2-phenylenediamine, 9,10-diaminophenanthrene, 1,10-phenanthroline, and hydrazine were purchased from Aldrich and used without further purification. The $[Ru(phi)_n(phen)_{3-n}]^{2+}$ and $[Rh(phi)_n(phen)_{3-n}]^{3+}$ ($n = 1, 2$) complexes were synthesized by following methods previously reported.[13,21,28] $[Ru(phen)_3]^{2+}$ was purchased from Aldrich, dissolved in acetone, and precipitated with ether followed by filtration to remove excess ligand which was present

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to up to 30% in the commercial sample. $[Rh(phen)_3]^{3+}$ was prepared by reported methods.[29] The BF_4^- salt of the precursor complex $[Rh(phen)_2(H_2O)_2]^{3+}$ was prepared by slight modification of reported methods,[28b,c] where $AgBF_4$ was utilized for the removal of chloride ions from $[Rh(phen)_2Cl_2]Cl$. The ^1H NMR, electronic absorption, and FAB mass spectra of the products were compared to those reported in the literature.

$[Rh(bqdi)_2Cl_2]Cl$. $RhCl_3$ (0.10 g) was dissolved in 6 mL of anhydrous ethanol, and 0.12 g of 1,2-phenylenediamine (PDA) was dissolved separately in 15 mL of DMF with 120 μL of hydrazine. Each solution was purged with nitrogen for 20 min. The PDA solution was slowly added to the stirring $RhCl_3$ solution under a positive N_2 pressure, resulting in the formation of a yellow precipitate. The reaction turned pale orange after it was refluxed overnight under N_2 atmosphere, and it was then allowed to stir in air at room temperature for 48 h. The reaction mixture was filtered, and the orange solid product was washed with ethanol and ether (46% yield). ^1H NMR in DMSO-d_6 (δ, ppm): 10.19 (s), 7.22 (s, br), 7.10 (s, br), 6.97 (s, br).

$[Rh(bqdi)_2phen]Cl_3$. $[Rh(bqdi)_2Cl_2]^+$ (0.03 g) was heated slightly with 1,10-phenanthroline (0.018 g) in 20 mL of DMF under N_2. A nitrogen-purged anhydrous ethanol solution (10 mL) containing 200 μL of hydrazine was slowly added to the reaction vessel. The mixture was refluxed overnight under N_2. The orange/red mixture was opened to air and filtered. After the filtrate was dried, the orange product was dissolved in water and filtered to remove water-insoluble starting materials. The solid product was collected by precipitation from an acetone solution with ether. Electronic absorption in ethanol [nm (ϵ, M^{-1} cm^{-1})]: 225 (40 900), 266 (31 190), 292 (10 200), 310 (3350), 440 (210). ^1H NMR in DMSO-d_6 (δ, ppm): 10.08 (bqdi, br), 9.18 (phen, br), 8.72 (phen, br), 8.17 (phen, s), 8.13 (bqdi, br), 8.03 (bqdi, s), 7.93 (phen, m). Electrospray MS: m/z = 563, $[Rh(bqdi)_2phen{\cdot}2Cl]^+$; m/z = 383, $[Rh(bqdi)_2Cl_2]^+$.

$[Rh(phen)_2bqdi](BF_4)_3$. $[Rh(phen)_2(H_2O)_2](BF_4)_3$ (0.041 g) and PDA (0.040 g) were dissolved and bubbled with N_2 separately in 10 and 5 mL of anhydrous ethanol, respectively. The PDA solution was slowly added to that containing the Rh(III) complex, and the reaction mixture was refluxed overnight under N_2. The red solution was then opened to air and was stirred at room temperature for 24 h. The reaction mixture was filtered, and the filtrate was dried. The solid was twice dissolved in acetone and precipitated with ether. Electronic absorption in ethanol [nm (ϵ, M^{-1} cm^{-1})]: 261 (45 970), 273 (58 800), 435 (6300), 454 (6300). ^1H NMR in DMSO-d_6 (δ, ppm): 10.00 (bqdi, d), 9.26 (phen, d), 8.87 (phen, d), 8.52 (phen, m), 8.40 (bqdi, d), 8.05 (bqdi, d), 7.74 (phen, m). Electrospray MS: m/z = 605, $[Rh(phen)_2bdqi{\cdot}Cl]^+$.

***cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.** The tetrafluoroborate salt of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (activated cisplatin) was synthesized by stirring *cis*-$[PtCl_2(NH_3)_2]$ (purchased from Aldrich) in the presence of 1.9 equiv of $AgBF_4$ for 15 h in deionized water (18 MΩ). The AgCl precipitate was removed by filtration.

Instrumentation. Absorption measurements were performed in a Hewlett-Packard diode array spectrometer (HP8453) with HP8453 Win System software equipped with a Peltier temperature-controlled sample cell and driver (HP89090A) for thermal denaturation studies. The ethidium bromide stained agarose gels (1%) were imaged using a GelDoc 2000 transilluminator (BioRad) connected to a desktop computer. The digital images were analyzed using the Quantity One software package (BioRad), which includes intensity integration. All ^1H NMR spectra were measured on a 400 MHz Bruker instrument.

Methods. The binding constants of the metal complexes to calf thymus DNA were determined by absorption titrations at room temperature utilizing 5−50 μM metal complex and increasing the calf thymus DNA concentration from 0 to 300 μM (5 mM Tris/HCl, pH 7.5). Calf thymus DNA was purchased from Sigma and was dialyzed three times against 5 mM Tris/HCl, pH = 7.5, 50 mM NaCl, over a 24 h period. The changes in the metal complex concentration due to dilution at the end of each titration were negligible. The binding constants, K_b, were determined from the reciprocal of the slopes of plots of $(\epsilon_a - \epsilon_b)/(\epsilon_b - \epsilon_f)$ vs $1/[DNA]_t$, which follow eq 1 at high DNA concentrations, such that $[DNA]_t \gg [M]_t$ and $[DNA]_t[M]_t \gg [M]_b^2$, where $[DNA]_t$ and $[M]_t$ are the total concentrations of DNA and metal complex in solution, respectively, and $[M]_b$ is the concentration of complex bound to the DNA.[30,31]

In eq 1, ϵ_a, ϵ_f, and ϵ_b are the molar extinction coefficients observed for the complex at a given DNA concentration, of free complex in solution, and for the complex bound DNA, respectively. The value of ϵ_b was determined from the plateau of the DNA titration, where addition of DNA did not result in further changes to the absorption spectrum. The linear regression fits were conducted with either Kaleidagraph or Excel commercial software.

$$(\epsilon_a - \epsilon_b)/(\epsilon_b - \epsilon_f) = (1/K_b)(1/[DNA]_t) + 1 \quad (1)$$

The pK_a values of the quinone diimine complexes were determined from photometric titrations. The 15-mer 5′-AGTGCCAAGCTTGCA-3′ and its corresponding complementary strand utilized in the thermal denaturation experiments were purchased from the Midland Reagent Co. The annealing of the 15-mers to form duplexes was performed by placing a solution containing ∼4 mM bases in 50 mM NaCl, 20 mM Tris/HCl (pH = 7.5), at 90 °C for ∼8 min, followed by slow cooling in the heat block to room temperature (∼3 h). Thermal denaturation studies of 15-mer duplex oligonucleotides were carried out by measuring the absorbance at 260 nm as a function of temperature. The melting curves were recorded in media containing 20 mM NaCl, 5 mM Tris/HCl, pH = 7.5. The melting temperature (T_m) was determined using the thermal denaturation routines in the Biochemical Analysis software package available with the Hewlett-Packard absorption instrument. The T_m values were determined with an accuracy of ±2 °C.

The in vitro transcription experiment used the pGEM linear DNA template (Promega) and the Ribomax Large Scale RNA Production System with T7 RNA polymerase (Promega). The transcription reaction was allowed to proceed for 1 h at 37 °C (40 mM Tris/HCl, 10 mM NaCl, pH = 7.5) in nuclease-free water in the presence of 6 mM $MgCl_2$, 2 mM spermidine, and 1.0 mM each ATP, CTP, GTP, and UTP. The inhibition of mRNA production by Ru(II) and Rh(III) in vitro was determined through the measurement of mRNA produced upon addition of increasing amounts of metal complex relative to template DNA bases, R_{inh} = [complex]/[bases], to the assay. Modifications of these methods were utilized in the assays conducted to determine the role of binding of the complexes to T7 RNA polymerase and their possible displacement of Mg^{2+} ions.

Results and Discussion

DNA Binding Constants. The binding constants of each metal complex to DNA were determined from changes in the absorption spectrum of each complex upon addition of

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Table 1. Changes in Melting Temperatures (ΔT_m) of a 15-mer Duplex in the Presence of Various Metal Complexes, Relative Concentration Required to Inhibit 50% of the Transcription, $R_{Inh}{}^{50}$, pK_a of Quinone Diimine Complexes, and Binding Constants (K_b) of Each Complex to DNA

complex	ΔT_m/°C[a]	$R_{inh}{}^{50}$	pK_a	K_b/M^{-1}
$[Rh(phi)_2phen]^{3+}$	21	0.15	6.7	~10^7 [b]
$[Rh(phen)_2phi]^{3+}$	7	4.5	6.0	~10^7 [b]
$[Rh(bqdi)_2phen]^{3+}$	14	0.49	6.2	4.5×10^4
$[Rh(phen)_2bqdi]^{3+}$	6	6.1	5.8	3.1×10^4
$[Rh(phen)_3]^{3+}$	5	6.2		c
$RhCl_3$	3	12.5		
$[Ru(phi)_2phen]^{2+}$	15	0.67	6.6	2.5 10^5
$[Ru(phen)_2phi]^{2+}$	11	3.3	5.1	2.2×10^5
$[Ru(phen)_3]^{2+}$	5	7.9		6.2×10^3 [d]
$RuCl_3$	3	11.5		
ethidium bromide	5	4.8		1.9×10^5 [e]

[a] Error = ±2 °C; for duplex alone T_m = 55 °C. [b] From refs 13b, 31, and 33. [c] Spectrum overlaps with that of the DNA bases. [d] Reported value 5.5×10^3 (ref 31). [e] Reported value 1.7×10^5 M^{-1}.[34]

calf thymus DNA. Spectrophotometric titration methods have been widely utilized in the determination of binding constants for Rh(III) and Ru(II) complexes to duplex DNA.[30,31] As the calf thymus DNA concentration is increased changes in the absorption of $[Rh(bqdi)_2phen]^{3+}$ (325 nm) and $[Rh(phen)_2bqdi]^{3+}$ (458 nm) are observed, until a plateau is reached from which the molar extinction coefficient of the bound complex, ϵ_b, can be determined. The binding constants, K_b, were determined from the absorption titrations using eq 1 from plots of $(\epsilon_a - \epsilon_b)/(\epsilon_b - \epsilon_f)$ vs 1/[DNA]$_t$. The binding constants determined using this method for the phi complexes of Ru(II) and Rh(III) were similar to those previously reported and are listed in Table 1 for all the complexes. $[Rh(bqdi)_2phen]^{3+}$ and $[Rh(phen)_2bqdi]^{3+}$ were found to bind to DNA with $K_b = 4.5 \times 10^4$ M^{-1} and $K_b = 2.3 \times 10^4$ M^{-1}, respectively, which are approximately 2−3 orders of magnitude lower than those measured for $[Rh(phi)_2phen]^{3+}$ and $[Rh(phen)_2phi]^{3+}$. Such difference is not unexpected, since, unlike phi, the π-system of the bqdi ligand does not extend sufficiently to permit intercalation of the ligand between the DNA bases. However, the binding constants of the bdqi Rh(III) complexes are approximately 1 order of magnitude greater than that of $[Rh(phen)_3]^{3+}$ (Table 1),[28b] indicating that electrostatic attraction and van der Waals interactions between the phen ligands and the hydrophobic major groove are not the sole source of the DNA binding observed in $[Rh(bqdi)_2phen]^{3+}$ and $[Rh(phen)_2bqdi]^{3+}$. However, the mode of binding of $[Rh(bqdi)_2phen]^{3+}$ and $[Rh(phen)_2bqdi]^{3+}$ to DNA and their sequence specificity remain unknown and are currently under investigation.

Thermal Denaturation of Duplex Oligonucleotides. The shifts in the melting temperatures (ΔT_m) of a 15-mer oligonucleotide duplex in the absence and in the presence of $[Rh(L)_n(phen)_{3-n}]^{3+}$ (L = phi, bqdi; n = 0, 1, 2), $[Ru(phi)_n(phen)_{3-n}]^{2+}$ (n = 0, 1, 2), $RhCl_3$, $RuCl_3$, and ethidium bromide are listed in Table 1. The ratio of the concentration of metal complex or ion to that of nucleotide bases was fixed at [complex]/[bases] = 0.067 (two metal complexes/duplex). Inspection of Table 1 reveals that complexes of Rh(III) and Ru(II) with two quinone diimine ligands result in the largest increase in the melting temperature of the duplex. An increase in T_m of 21 °C was measured in the presence of $[Rh(phi)_2phen]^{3+}$ relative to free duplex, and ΔT_m values of +15 and +14 °C were observed for $[Ru(phi)_2phen]^{2+}$ and $[Rh(bqdi)_2phen]^{3+}$, respectively. Complexes possessing a single phi ligand in their coordination sphere, such as $[Rh(phen)_2phi]^{3+}$ and $[Ru(phen)_2phi]^{2+}$, raised the melting temperature of the duplex by 7 and 11 °C, respectively. In contrast, ethidium bromide, a known DNA intercalator, only results in ΔT_m of +5 °C. Similarly, $RuCl_3$, $RhCl_3$, $[Ru(phen)_3]^{2+}$, $[Rh(phen)_3]^{3+}$, and $[Rh(phen)_2bqdi]^{3+}$ resulted in negligible changes to T_m.

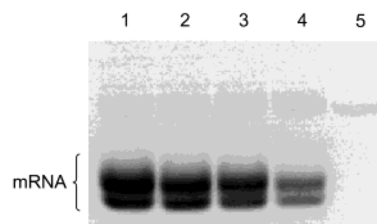

Figure 2. Ethidium bromide stained agarose gel (1%) of transcribed mRNA in the presence of $Rh(bdqi)_2phen^{3+}$ at various [complex]/[template DNA base] ratios, R. Lane, R: 1, 0.0; 2, 0.5; 3, 1.0; 4, 2.0; 5, 4.0.

During the transcription process, the formation of an "open" bubble DNA structure (elongation) is necessary for the nucleotides to function as templates to the nascent mRNA. The ability of the bis-phi and bis-bqdi complexes of Rh(III) and Ru(II) to stabilize duplex DNA led us to investigate the possible inhibition of transcription by these molecules, since they could hinder the formation of the "open" bubble DNA structure.

In Vitro Transcription Assays. A typical imaged agarose gel showing the RNA produced in vitro in the presence of various concentrations of $[Rh(bqdi)_2phen]^{3+}$ indicates that an increase in the concentration of the complex relative to that of the DNA template bases (R = [complex]/[bases]) results in a decrease in the amount of RNA produced (Figure 2). The ratio at which 50% of the RNA is transcribed, $R_{inh}{}^{50}$, was calculated for each coordination complex, $RhCl_3$, $RuCl_3$, and ethidium bromide, from the interpolation of the integrated areas of the imaged mRNA signal of each lane of the gel (Table 1). It is evident from the comparison of the $R_{inh}{}^{50}$ values listed in Table 1 that complexes with two quinone diimine ligands, $[Rh(phi)_2phen]^{3+}$, $[Ru(phi)_2phen]^{2+}$, and $[Rh(bdqi)_2phen]^{3+}$, are able to suppress transcription at lower relative concentrations than the other complexes, the metal ions, and ethidium bromide.

Experiments were also performed to rule out the binding of the complex to T7 RNA polymerase as the mechanism of transcription inhibition. $[Rh(phi)_2phen]^{3+}$ binds duplex DNA with $K_b \sim 10^7$ M^{-1};[13,33] therefore, with 15 μM $[Rh(phi)_2phen]^{3+}$ and 150 μM DNA bases (R = 0.1), it would be expected that $\sim 10^{-8}$ M rhodium complex would remain

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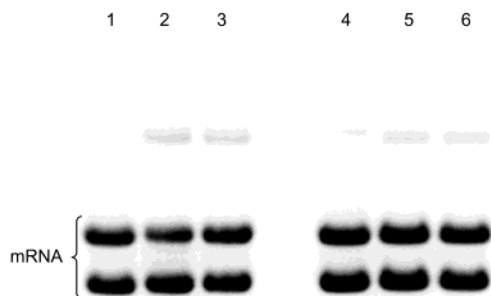

Figure 3. Ethidium bromide stained agarose gel (1%) of transcribed mRNA using 4.0 units (lanes 1−3, reaction time 30 min) and 1.3 units (lanes 4−6, reaction time 120 min) of T7 RNA polymerase in the absence of metal complex (lanes 1 and 3) and in the presence of $R = 0.25$ Rh(bdqi)$_2$phen^{3+} (lanes 2 and 5) and $R = 0.002$ activated cisplatin (lanes 3 and 6).

free in solution which could bind to the enzyme (~10^{-9} M T7 RNA polymerase was used in each assay). Similar inhibition of transcription was also observed for each complex upon variation of the enzyme concentration by a factor of 3−5 while keeping all other concentrations constant. As shown in Figure 3, at $R = 0.50$, [Rh(bqdi)$_2$phen]$^{3+}$ results in the production of 84% mRNA (lane 3) compared to the control (lane 1). A decrease in T7 RNA polymerase by a factor of 3 (Figure 3) results in 81% transcribed RNA at $R = 0.50$ (lane 6) compared to the control (lane 4). When activated cisplatin (*cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$) was utilized ($R = 0.002$), whose inhibition mechanism proceeds via DNA binding,[32] the RNA produced was 90% (lane 2) and 95% (lane 5) as the enzyme concentration was decreased by a factor of 3. Furthermore, experiments with 1 order of magnitude less complex and DNA (1.5 μM [Rh(phi)$_2$phen]$^{3+}$ and 15 μM bases) utilizing the same enzyme concentration (~10^{-9} M) resulted in a nearly identical R_{inh}^{50} value (reaction time was increased 6-fold). These results are inconsistent with an inhibition mechanism that involves the association of the complex to the T7 RNA polymerase enzyme.

To exclude the displacement of Mg^{2+} ions by the metal complexes, the transcription reaction was carried out with [Ru(phi)$_2$phen^{2+} at a concentration where transcription was partially inhibited ($R = 1.0$) in the presence of 6 mM Mg^{2+}. The use of 12 mM instead of 6 mM MgCl$_2$ under the same conditions did not result in increased transcription. It should also be noted that photocleavage of the DNA template by room light in the presence of the metal complexes can be ruled out, since the Ru(II) complexes are not photoactive.[27]

Duplex Stabilization and Inhibition of Transcription. The binding constants (K_b) of the [Ru(phi)$_n$(phen)$_{3-n}$]$^{2+}$ and [Rh(phi)$_n$(phen)$_{3-n}$]$^{3+}$ ($n = 0-2$) complexes and ethidium bromide to duplex DNA are in the 10^3 to ~10^7 M^{-1} range.[13,31] It appears that the relative concentration of each molecule required for inhibition of 50% of the transcription, R_{inh}^{50}, is related to ΔT_m rather than K_b. For example, the K_b value for [Rh(phen)$_2$phi]$^{3+}$ is ~10^7 M^{-1},[13,33] whereas for [Rh(phen)$_3$]$^{3+}$ and [Ru(phen)$_3$]$^{2+}$ $K_b \sim 10^3$ M^{-1};[13,27,28] however, similar values of R_{inh}^{50} were measured for these three complexes (Table 1). In contrast, the measured R_{inh}^{50} values for systems with similar binding constants, such as [Ru(phen)$_2$phi]$^{2+}$ ($K_b = 2.2 \times 10^5$ M^{-1}),[27] [Rh(bqdi)$_2$phen]$^{3+}$

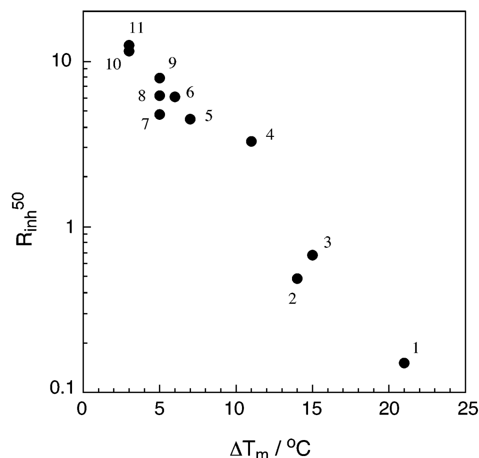

Figure 4. Semilog plot of R_{inh}^{50} vs ΔT_m for Rh(phi)$_2$phen^{3+} (1), Ru(phi)$_2$phen^{2+} (2), Rh(bdqi)$_2$phen^{3+} (3), Ru(phen)$_2$phi^{3+} (4), Rh(phen)$_2$phi^{3+} (5), Rh(phen)$_2$bdqi^{3+} (6), ethidium bromide (7), Rh(phen)$_3$$^{3+}$ (8), Ru(phen)$_3$$^{2+}$ (9), RuCl$_3$ (10), and RhCl$_3$ (11).

($K_b = 4.5 \times 10^4$ M^{-1}), and ethidium bromide ($K_b = 1.7 \times 10^5$ M^{-1}),[34] exhibit R_{inh}^{50} values that differ by 2 orders of magnitude. A plot of R_{inh}^{50} vs ΔT_m is shown in Figure 4 for all the coordination complexes, RhCl$_3$, RuCl$_3$, and ethidium bromide. Figure 4 shows a distinct trend between the increase in the duplex's melting temperature by a given complex or ion and its ability to inhibit transcription.

The largest shifts in the duplex melting temperatures, ΔT_m, and the lowest concentration of complex required to observe 50% transcription inhibition, R_{inh}^{50}, were measured for [Rh(phi)$_2$phen]$^{3+}$, [Ru(phi)$_2$phen]$^{2+}$, and [Rh(bqdi)$_2$phen]$^{3+}$. The intercalation of the phi ligand between the DNA bases results in a large binding constant for the phi-containing complexes to DNA; however, it appears that intercalation alone is not enough to explain the large ΔT_m and low R_{inh}^{50} values. For example, [Rh(phen)$_2$phi]$^{3+}$ and [Ru(phen)$_2$phi]$^{2+}$ also possess a phi ligand that is able to intercalate between the DNA bases and, therefore, exhibit strong binding to DNA, but their ΔT_m and R_{inh}^{50} values are similar to those measured for [Rh(phen)$_3$]$^{3+}$ (see Table 1). In addition, although the bqdi ligand is not expected to intercalate between the DNA bases, [Rh(bqdi)$_2$phen]$^{3+}$ exhibits ΔT_m and R_{inh}^{50} similar to those measured for the bis-phi complex [Ru(phi)$_2$phen]$^{2+}$. The binding constant of the nonintercalative [Rh(bqdi)$_2$phen]$^{3+}$ complex to DNA is approximately 1 order of magnitude lower than that measured for [Ru(phi)$_2$phen]$^{2+}$. Although the magnitude of the binding constant appears to be dictated by the ability of the complex to intercalate between the DNA bases, there must be some other interaction that is more important in the stabilization of the DNA duplex structure than intercalation.

As discussed above, it appears that intercalation of the quinone diimine ligand is not necessary for duplex stabilization and transcription inhibition in vitro. Although the mechanism for duplex stabilization by bis(quinone diimine) complexes remains under investigation, a possible explanation of the observed results is that specific hydrogen-bonding

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interactions are present between the two diimine quinone ligands of the bis-phi and bis-bdqi complexes with the bases or phosphate backbone of DNA that result in increased duplex stabilization. Another possibility is that deformations in the double helix structure that take place upon binding of the complexes result in additional interstrand interactions. Structural reports on the binding of phi complexes to duplex DNA that support these possibilities are discussed below.

Structures of phi Complexes Bound to DNA. The ^{1}H solution NMR structures of several mono-phi complexes of Rh(III) bound to duplex DNA exhibit hydrogen-bonding interactions between ligands below and above the plane of the phi ligand with the DNA bases.[14b,22] Photocleavage assays by the complexes provide additional evidence for the role of hydrogen bonding from the ancillary ligands to nucleic acids, resulting in their sequence selectivity.[35] For example, the phi ligand in Δ-$[Rh(en)_2phi]^{3+}$ intercalates selectively at 5′-GC-3′ steps owing to hydrogen bonding by the amino protons from the ehthylenediamine ligands located above and below the plane of the phi ligand to the O6 position of guanine residues above and below the intercalation site, respectively.[34] Similar selectivity was also observed for $[Rh(NH_3)_4phi]^{3+}$ and $[Rh(cyclen)phi]^{3+}$ (cyclen = 1,4,7,10-tetraazacyclododecane), both of which possess amino protons above and below the plane of the phi ligand for potential hydrogen bonding.[35] In contrast, when $[Rh(S_4\text{-cyclen})phi]^{3+}$ (S_4-cyclen = 1,4,7,10-tetrathiacclododecane) was utilized, where the modified cyclen ligand lacks hydrogen-bonding protons, no sequence selectivity was observed.[35] In addition to these results, modification of the nucleic acids surrounding the binding site also led to the conclusion that hydrogen bonding from the amino protons above and below the intercalation plane to the bases was necessary in the sequence recognition by the complexes.[35] This conclusion is also supported by ^{1}H NMR studies undertaken with $[Rh(NH_3)_4phi]^{3+}$, $[Rh(en)_2phi]^{3+}$, and $[Rh(phen)_2phi]^{3+}$ bound to various duplex oligonucleotides.[20,21,23]

Prediction of hydrogen-bonding contacts between the amino protons of the ancillary ligands of the complex and nearby nucleotides, as well as favorable van der Waals interactions, led Barton and co-workers to design an intercalative mono-phi Rh(III) complex, Δ-α-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$, for the selective recognition of the 5′-TGCA-3′ sequence.[14b] Indeed, Δ-Δ-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$ binds tightly and selectively to 5′-TGCA-3′ sites in duplex DNA.[14b,22] The ^{1}H NMR structure of the complex bound to the $d(GAGTGCACTC)_2$ oligonucleotide duplex shows hydrogen bonding from the axial amine to the N7 and O6 of the guanine at the intercalation site.[22] In addition, it appears that the positive slide and propeller twisting of the duplex that occurs upon intercalation of the complex results in additional interstrand hydrogen bonding.[22]

The crystal structure of Δ-α-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$ bound to a palindromic 8-base duplex oligonucleotide sequence, 5′-G-dIU-TGCAAC-3′ (dIU = 5-iododeoxyuridine) was recently reported.[24] This structure is consistent with that determined from solution NMR and shows that in DNA-bound Δ-Δ-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$ the phi ligand is intercalated between the DNA bases and that the amino protons of the (R,R)-Me_2trien ligand located above and below the phi plane are involved in hydrogen bonding with two nearby guanines, both directly and through ordered water molecules.[24] Another important feature apparent in the crystal structure was that the two imine protons of the phi ligand are hydrogen bonded to ordered water molecules in the structure.[24]

Although bis-phi complexes of Rh(III) appear to be better DNA photocleavage agents than the systems with a single phi ligand under certain conditions,[10−19] no ^{1}H NMR studies or crystal structures have been reported to date on their DNA binding. The structural characterization of bis-phi complexes is complicated by the presence of two phi ligands; however, owing to steric constraints, in each complex only one of the phi ligands can intercalate between the DNA bases. Although the DNA binding of bis-phi complexes is far less specific than that of the mono-phi complexes described above, the binding constants of the mono- and bis-phi Rh(III) complexes to duplex DNA are similar (Table 1).

The bis(quinone diimine) complexes of Rh(III) and Ru(II) reported here possess four imine hydrogens in the octahedral coordination sphere of their respective metal centers (Figure 1). In the DNA-bound complexes, hydrogen bonding by these protons is possible, similar to that discussed above for the ancillary amine ligands of mono-phi Rh(III) complexes. Hydrogen bonding is expected to be stronger by the imino protons of the metal-bound quinone diimine ligands than from amino protons of coordinated amines. Spectroscopic and potentiometric titration data indicate that the imine quinone protons of $[Rh(phi)_2phen]^{3+}$, $[Rh(phen)_2phi]^{3+}$, and related phi complexes are protonated when bound to duplex DNA.[13a] The pK_a values measured for all the quinone diimine complexes are listed in Table 1 and range from 5.1 to 6.7. The pK_a values of the bqdi complexes are similar to those measured for phi complexes of Rh(III) and Ru(II). In addition, it was recently reported that in Ru(II) complexes possessing appended quinolines ($pK_a = 7.1$), the quinoline moiety is protonated when bound to DNA at neutral pH.[36] Similar effects were also reported for the binding of other metal complexes and organic drugs to duplex DNA.[37,38] The more acidic environment around duplex DNA is believed to arise from electrostatic effects from the polyanionic backbone, which result in a higher local proton concentration.[39]

It is therefore possible that hydrogen bonding take places from the imine quinone protons to nearby DNA bases directly or through ordered water molecules, as was shown in the crystal structure of Δ-α-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$ bound to duplex DNA. Such hydrogen bonding may give rise to

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the increased stability of the duplex structure observed upon binding of bis-phi and bis-bqdi complexes of Rh(III) and $Ru(phi)_2phen^{2+}$, which would then result in the inhibition of transcription at lower complex concentrations. Alternatively, a distortion of the duplex upon binding the complexes may result in additional interstrand hydrogen bonding, such as that observed for Δ-α-$\{Rh[(R,R)\text{-}Me_2trien]phi\}^{3+}$. Although no direct evidence for hydrogen bonding from the bis(quinone diimine) complexes to DNA is presented in the current work, further studies are currently underway to determine the possible role of hydrogen bonding by these complexes in duplex stabilization.

Conclusions

The inhibition of transcription by the stabilization of the DNA duplex structure in the presence of octahedral metal complexes possessing quinone diimine ligands was investigated. Rh(III) and Ru(II) complexes possessing two quinone diimine ligands in their coordination sphere were found to significantly increase the melting temperature of a 15-mer duplex DNA. In contrast, complexes that possess a single quinone diimine ligand, the parent $[Ru(phen)_3]^{2+}$ and $[Rh(phen)_3]^{3+}$ complexes, and ethidium bromide result in small shifts in the melting temperature of the duplex oligonucleotide. A distinct correlation between the shift in the melting temperatures of the duplexes, ΔT_m, in the presence of each metal complex and its relative concentration required to inhibit transcription in vitro was observed. Our findings show a difference in behavior between complexes possessing one and two quinone diimine ligands, independent of the complex's ability to intercalate between the DNA bases.

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