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Transcription inhibition by $Rh(phi)_2(phen)^{3+}$

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$Rh(phi)_2(phen)^{3+}$ (phi = 9,10-phenanthrenequinone diimine, phen = 1,10-phenanthroline) increases the melting temperature (ΔT_m) of a 15-mer duplex DNA by 21 °C and it is able to inhibit transcription *in vitro*; the concentration ratio of $Rh(phi)_2(phen)^{3+}$ relative to DNA bases of the template required to inhibit the RNA transcribed by 50%, R_{inh}^{50}, was found to be 0.13; in contrast, $Rh(phen)_2(phi)^{3+}$, which also possesses the intercalating phi ligand, exhibits only a +7 °C shift in T_m and R_{inh}^{50} = 4.5; $Rh(phen)_3^{3+}$, $RhCl_3$, and ethidium bromide result in negligible or small ΔT_m and exhibit R_{inh}^{50} values that range from 4.8 to 12.5; these results suggest that the intercalation of the phi ligand between the DNA bases and electrostatic binding are not the only means of duplex stabilization by these complexes.

The inhibition of transcription is one of the crucial manners in which the replication of cancerous cells and viruses can be prevented.[1] This mechanism is exploited by antitumor drugs including actinomycin, anthracycline antibiotics and cisplatin, among others.[2] Inhibition of transcription leads to incomplete coding of RNA and proteins, and can ultimately lead to cell death. In the present work we compare duplex stabilization and inhibition of transcription by octahedral $Rh(phi)_n(phen)_{3-n}^{3+}$ (phi = 9,10-phenanthrenequinone diimine, phen = 1,10-phenanthroline; n = 0, 1, 2) complexes, some of which possess the intercalative phi ligand (structures shown in Fig. 1). The interactions and reactivity of phi complexes of Rh(III) with DNA have been extensively investigated.[3]

Two-dimensional ^{1}H NMR spectroscopy was previously utilized to investigate the binding of Rh(III) complexes possessing a single phi ligand to duplex DNA, including $Rh(NH_3)_4(phi)^{3+}$,[4] $Rh(phen)_2(phi)^{3+}$,[5] Δ-α-Rh[(*R*,*R*)-Me_2-trien]phi^{3+} [(*R*,*R*)-Me_2trien = 2*R*,9*R*-diamino-4,7-diazadecane],[6] and $Rh(en)_2(phi)^{3+}$ (en = ethylenediamine).[7] These NMR studies, as well as the crystal structure of Δ-α-Rh[(*R*,*R*)-Me_2trien](phi)$^{3+}$ bound to an eight base-pair oligonucleotide duplex,[8] show that the binding of these complexes to DNA takes place through the intercalation of the phi ligand between the bases of the duplex from the major groove. Although bis-phi complexes of Rh(III), such as $Rh(phi)_2(bpy)^{3+}$ (bpy = 2,2′-bipyridine), are better DNA photocleavage agents than those possessing a single phi ligand,[3] no ^{1}H NMR studies or crystal structures have been reported to date on their DNA binding.

The shifts in the melting temperatures (ΔT_m) of a 15-mer oligonucleotide duplex (see Table 1 for sequence) in the presence of $Rh(phi)_n(phen)_{3-n}^{3+}$ (n = 0, 1, 2), $RhCl_3$, and ethidium bromide are listed in Table 1.[9] The relative concentration of metal complex or ion was fixed at R = 0.067 (R = [complex]/[bases]; two metal complexes per duplex). Inspection of Table 1 reveals that the complex with two phi ligands results 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 $Rh(phi)_2(phen)^{3+}$ relative to free duplex, and a ΔT_m value of +7 °C was observed for $Rh(phen)_2(phi)^{3+}$. Ethidium bromide, a known DNA intercalator, results in ΔT_m of +5 °C. The Rh^{3+} ion, $RhCl_3$, and the non-intercalative $Rh(phen)_3^{3+}$ complex resulted in negligible changes to T_m.

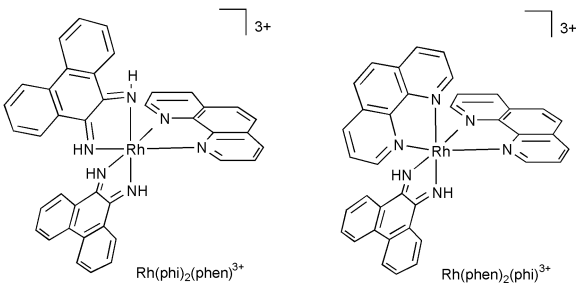

Fig. 1 Structures of $Rh(phi)_2(phen)^{3+}$ and $Rh(phen)_2(phi)^{3+}$.

The ability of $Rh(phi)_2(phen)^{3+}$ to stabilize duplex DNA led us to hypothesize that it may hinder elongation during the transcription process. The imaged agarose gel showing the RNA transcribed *in vitro* in the presence of various concentrations of $Rh(phi)_2(phen)^{3+}$ is shown in Fig. 2.[10] It is clear from Fig. 2 that an increase in the concentration of $Rh(phi)_2(phen)^{3+}$ relative to DNA template, R, results in a decrease in the amount of RNA produced. The ratio at which 50% of the RNA is

Table 1 Changes in melting temperatures (ΔT_m) of a 15-mer duplex in the presence of various metal complexes and R_{inh}^{50} for each complex

Duplex = 5′-AGTGCCAAGCTTGCA-3′ / 3′-TCACGGTTCGAACGT-5′

Complex	ΔT_m[a]/°C	R_{inh}^{50}
$Rh(phi)_2(phen)^{3+}$	21	0.13
$Rh(phen)_2(phi)^{3+}$	7	4.5
$Rh(phen)_3^{3+}$	3	6.2
$RhCl_3$	3	12.5
Ethidium bromide	5	4.8

[a] Error = ±2 °C; for duplex only T_m = 55 °C; measurements performed in 5 mM Tris (pH = 7.5, 50 mM NaCl) with [complex]/[bases] = 0.067 (2 complexes per duplex) and [bases] = 20–30 μM; T_m values determined from the changes in absorbance at 260 nm using the HP Biochemical Analysis software on a diode array spectrometer (HP 8453) in a sample holder equipped with a Peltier temperature controller (1 cm path length).

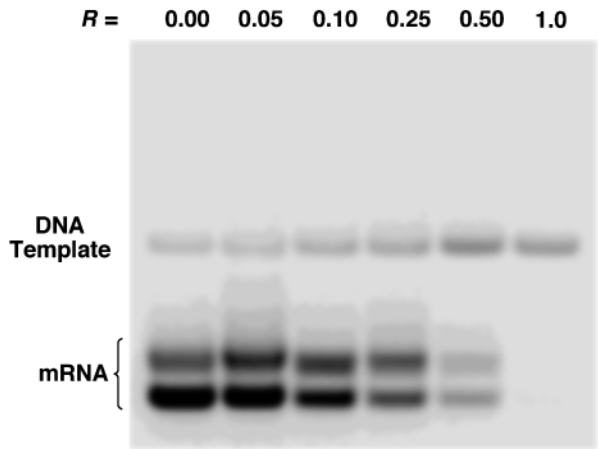

Fig. 2 Ethidium bromide stained agarose gel (1%) of transcribed mRNA in the presence of $Rh(phi)_2(phen)^{3+}$ at various [Rh]/[template DNA base] ratios, R. Both the DNA template (150 μM) and mRNA are imaged on the gel (labeled). See ref. 10 for a detailed description.

transcribed, R_{inh}^{50}, for each complex, $RhCl_3$, and ethidium bromide are listed in Table 1. The values of R_{inh}^{50} presented in Table 1 reveal that $Rh(phi)_2(phen)^{3+}$ inhibits transcription to a much greater extent than $Rh(phen)_2(phi)^{3+}$, although both complexes possess the intercalative phi ligand. In addition, large R_{inh}^{50} values were measured for $Rh(phen)_3^{3+}$, $RhCl_3$ and the intercalator ethidium bromide.

One possible explanation of the observed results is that the complexes that bind more strongly to double stranded DNA might play a greater role in duplex stabilization and transcription inhibition. However, it appears that 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 $\approx 10^6\ M^{-1}$,[11,12] for $Rh(phen)_3^{3+}$ $K_b \approx 10^3\ M^{-1}$,[12] and for ethidium bromide $K_b = 1.7\times10^5\ M^{-1}$,[13] however, the R_{inh}^{50} and ΔT_m values for all three molecules are similar (see Table 1). Although the role of the binding constant cannot be completely discounted at this time, it does not appear that the binding constant of each complex to duplex DNA plays a role in transcription inhibition or duplex stabilization.

Experiments were also performed to ensure that binding of the complexes to T7-RNA polymerase was not the mechanism of transcription inhibition. $Rh(phi)_2(phen)^{2+}$ binds duplex DNA with $K_b \approx 10^7\ M^{-1}$,[11] therefore with 15 μM $Rh(phi)_2(phen)^{2+}$ and 150 μM DNA bases ($R = 0.10$) it would be expected that $\approx 10^{-8}$ M rhodium complex would remain free in solution which could bind to the enzyme ($\approx 10^{-9}$ M). Experiments with an order of magnitude less complex and DNA [1.5 μM $Rh(phi)_2(phen)^{2+}$, 15 μM bases] utilizing the same enzyme concentration ($\approx 10^{-9}$ M) resulted in negligible change to the inhibition of transcription relative to that measured with 15 μM complex and 150 μM DNA bases.[10] In addition, no change in the R_{inh}^{50} was observed with a 10-fold increase in T7-RNA polymerase with 15 μM $Rh(phi)_2(phen)^{2+}$ and 150 μM bases (15 min reaction time).[10] These results are inconsistent with an inhibition mechanism that involves the association of the complex to the enzyme. To exclude displacement of Mg^{2+} ions by the metal complexes, aggregation of oligonucleotides, and non-specific ionic binding of the complexes to the duplex, the transcription reaction was carried out with $Rh(phi)_2(phen)^{3+}$ at $R = 0.10$ and either 6 mM or 12 mM Mg^{2+}. The use of 12 mM instead of 6 mM $MgCl_2$, under the same conditions, did not result in increased transcription.

The largest shifts in the duplex melting temperatures, ΔT_m, and the lowest concentrations of complex required to observe the transcription inhibition, R_{inh}^{50}, were measured for $Rh(phi)_2(phen)^{3+}$, which possesses two quinone diimine phi ligands in the octahedral coordination sphere of the Rh(III) metal center. Since $Rh(phen)_2(phi)^{3+}$ behaves similarly to $Rh(phen)_3^{3+}$ intercalation of the phi ligand or charge on the complex are not the sole reasons for the increased duplex stabilization or transcription inhibition observed for $Rh(phi)_2(phen)^{3+}$. One explanation of our observations is that the four imine protons of the two phi ligands of $Rh(phi)_2(phen)^{3+}$, which are not present in the other complexes, makes hydrogen bonding possible between $Rh(phi)_2(phen)^{3+}$ and the DNA nucleotides and backbone. The recent crystal structure of Δ-α-Rh[(*R*,*R*)-Me_2trien](phi)$^{3+}$ bound to duplex DNA shows that two binding modes are present. In both binding modes the phi ligand is intercalated between the DNA bases. 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 in one binding mode and with ordered water molecules hydrogen-bonded to the guanines in the other.[8] Furthermore, the two imine protons of the phi ligand are hydrogen bonded to ordered water molecules in the structure.[8]

$Rh(phi)_2(phen)^{3+}$ stabilizes the duplex DNA structure and inhibits the transcription process *in vitro* at 35-fold lower concentration than $Rh(phen)_2(phi)^{3+}$. This finding suggests that intercalation of the phi ligand and the 3+ charge on the complex are not the only factors involved in duplex stabilization. One possible explanation for the observed results is the deformation of the double helix through the binding of the $Rh(phi)_2(phen)^{3+}$ complex at various sites on the DNA template. Another possibility is that hydrogen bonding, either directly to the DNA or through ordered water molecules, may play a role in the binding of $Rh(phi)_2(phen)^{3+}$ to DNA and in the stabilization of the duplex structure, thus resulting in inhibition of transcription.

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9 $RhCl_3$ and ethidium bromide were purchased from Aldrich and used without further purification. $Rh(phi)_n(phen)_{3-n}^{3+}$ ($n = 0, 1, 2$) complexes were synthesized following methods previously reported (refs. 3 and 11).

10 The *in vitro* transcription experiments used the pGEM Express Positive Control DNA Template (Promega, 3995 base pairs) and the Ribomax Large Scale RNA Production System with T7 RNA polymerase (Promega). The transcription was allowed to proceed for 1 h at 37 °C (40 mM Tris, 10 mM NaCl, pH = 7.5) in nuclease-free water in the presence of 6 mM $MgCl_2$, 2 mM spermidine and 2.5 mM each ATP, CTP, GTP and UTP. The ethidium bromide stained agarose gels were imaged on a Bio-Rad GelDoc 2000 transilluminator, and quantitative data was determined using Quantiy One software (Bio-Rad).

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