Anaerobic Photoinduced N7-Binding of *cis*-Dichlorobis(1,10-phenanthroline)rhodium(III) Chloride to 2′-Deoxyguanosine: A One-Electron-Transfer Chain Process

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In recent papers we reported our studies of the photochemically induced covalent binding of the title complex (*cis*-Rh(phen)$_2$Cl$_2^+$) and related complexes to calf thymus DNA, nucleotides, and nucleosides.[1] These studies were prompted by our interest in the potential development of "photo *cis*-platin" derivatives as photochemotherapeutic agents. We observed that photolysis of the metal complex with deoxyguanosine (dG) or DNA under aerobic conditions gave one product corresponding to binding of the metal to dG at N1 and a second adduct involving either the oxygen at C6 or amino group at C2. The reactions are inefficient (Φ_{DNA} ca. 10^{-3}),[1b] and the binding to dG is apparently initiated by electron transfer from the base to the excited metal complex.[1c] We now report that analogous photolyses conducted *in the absence of oxygen* lead to very efficient covalent binding of the metal to dG, with the site of binding at N7 and with good evidence for the involvement of a one-electron-transfer chain reaction.

Photolysis of *cis*-Rh(phen)$_2$Cl$_2^+$ (3.8 mM) and dG (16.5 mM) in 0.1 M Tris buffer (pH 7.0) with uranium yellow filtered light (λ > 330 nm) in an argon atmosphere gives rise to two major photoproducts (adducts **I** and **II**; 38:62 after 90 s) observable by HPLC with yields of 28 and 45%, respectively. Also formed are the chloro aquo (26%) and diaquo (<1%) metal complexes. Results from experiments we conducted indicate that these aquation products do not react with dG in the dark or under irradiation in the presence of dG. The adducts were purified by HPLC and characterized by NMR spectroscopy and FAB mass spectrometry. The FAB spectra of both adducts, in an NBA matrix, show the highest mass ions at m/z = 764. This mass corresponds to a mono dG adduct minus two hydrogens (i.e. [M − 2H]$^+$). The loss of hydrogen from FAB molecular ions is well precedented for metal complexes[2] and for nucleic acids.[3] There is an isotope peak corresponding to the presence of one chlorine atom, and fragmentation ions are observed which correspond to the loss of the halogen.

For both adducts one observes the following ^{1}H NMR features: (1) resonances for 16 phenanthroline protons and 10 dG protons—evidence for *cis*-bis(phenanthroline) mono dG adducts; (2) a downfield doublet for the H2′ proton at δ 10.00, indicative of the presence of the chlorine ligand on the metal (in the absence of chlorine, these protons typically appear at δ 9.0); (3) a singlet for the dG H8 protons appearing at δ 8.42, rather than at δ 7.8 as in unmodified dG, an indication of binding of the metal to N7 of the base;[4] (4) the N1 resonance at δ 11.2 in a spectrum taken of a mixture of the adducts in deuterated DMSO (exchangeable when D_2O is added); (5) for the same mixture, the resonance for the two protons for the exocyclic amine at δ 7.4.

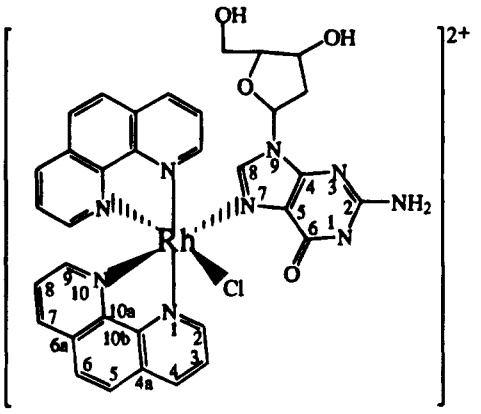

Figure 1. Structure of the photoproduct formed upon irradiation of dG with the Λ isomer of *cis*-[Rh^{III}(phen)$_2$Cl$_2$]$^+$ under anaerobic conditions.

Confirmation of N7 as the site for metal binding was provided by acidifying each adduct with DCl and noting that the chemical shift for the H8 resonance remained unchanged. It is known that protonation at N7 shifts the H8 resonance downfield[5] (in our hands, δ 0.8 for dG itself) and the absence of such an effect indicates that there has been alkylation or metalation at N7. Additional confirmation of N7 as the site for metalation was provided by the ^{13}C NMR spectra of the adducts. Both exhibit a δ 4 downfield shift for the C8 resonance and a δ 2 upfield shift for the C5 resonance, as expected for reaction at N7.[6]

On the basis of the data outlined above, we assign the formula for the adducts as [*cis*-Rh(phen)$_2$(dG)Cl]$^{2+}$, with the metal covalently bound at N7 of dG. The spectral identity of the two adducts leads us to assign them as diastereoisomers involving a reaction of the racemic *cis*-Rh(phen)$_2$Cl$_2^+$ with dG. The product corresponding to reaction of the Λ isomer of the complex is shown in Figure 1.

Quantum efficiencies for binding of the metal (7.8 mM) to dG (16.7 mM) under anaerobic conditions were determined at 308 (phenanthroline $\pi \rightarrow \pi^*$ transition) and 355 (metal d–d transition) nm using excimer and Nd:YAG lasers, respectively.[7] The data are given in Table 1. Included in the table is the efficiency for total product formation from the metal complex, i.e. adduct formation plus mono- and diaquation. Though there is a modest difference between the data obtained at the two wavelengths, in both cases the values for the overall product efficiencies exceed unity! The most reasonable explanation of

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(7) An OPHIR 3A-P-CAL-S power head was used as an actinometer to measure the amount of photons absorbed during the irradiation.

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Table 1. Quantum Efficiencies for Photolysis of *cis*-$Rh(phen)_2Cl_2^+$ with dG

	Φ(adduct I)	Φ(adduct II)	Φ(pdt)[a]	Φ(−SM)[b]
308 nm	0.69	1.13	3.06	3.26
355 nm	0.70	1.17	2.99	3.36

[a] $\Phi_{(pdt)}$ = quantum efficiency for the total formation of products. [b] $\Phi_{(-SM)}$ = quantum efficiency for the disappearance of the starting material *cis*-$Rh(phen)_2Cl_2$.

this result is that excitation of *cis*-$Rh(phen)_2Cl_2^+$ in the presence of dG creates products through the involvement of a chain process.

Considering our previous observations, which support electron transfer from dG to the metal complex excited state,[1c] it is reasonable to assume that the chemistry described herein involves a photoinitiated electron-transfer chain process. In fact, such chain processes involving metal complexes are well precedented, with reports of both photochemical[8] and electrochemical[9] initiation. However, we are unaware of any other example of the covalent binding of a transition metal to nucleosides involving a photoinduced chain process. The details of our proposal are given in Scheme 1.

As noted above, the evidence for steps 1 and 2 has been presented earlier.[1c] Of course, step 2 can be reversed by back electron transfer, and this energy wastage step is noted in the scheme as step 8. The possibility for extensive, but indeterminate, back electron transfer makes it difficult to judge how long a chain is actually involved in the substitution chemistry. The chain begins with step 3 in which the Rh^{II} complex loses Cl^-. A subsequent reaction with dG at the nucleoside's most nucleophilic (e.g. N7) site[10] and electron transfer between the resulting Rh^{II} adduct and the starting dichloro complex complete the propagation sequence. It is possible that aquation would accompany loss of Cl^- with dG substitution following. This option is shown as steps 4a and 4b. Step 5 is directly analogous to the key propagation step invoked to explain the chain process involved in the photosubstitution of $[(CH_3CN)Re(CO)_3phen]^+$ by triphenylphosphine.[8a] We show step 5 as an equilibrium because we lack the appropriate oxidation/reduction potentials needed to judge the thermodynamics of this electron transfer process. It seems reasonable that the interaction will be approximately thermoneutral; even a modestly endothermic transfer from the Rh^{II} to the Rh^{III} species would be driven forward by the subsequent loss of chloride in step 3.[11] Oxygen interrupts the chain by oxidizing the $[Rh^{II}(phen)_2Cl]^+$ species

Scheme 1. Proposed Chain Mechanism for the Photoinduced Binding of *cis*-$[Rh^{III}(phen)_2Cl_2]^+$ to dG under Anaerobic Conditions

Initiation

$$cis[Rh^{III}(phen)_2Cl_2]^+ \xrightarrow{h\upsilon} cis[Rh^{III}(phen)_2Cl_2]^{+*} \quad (1)$$

$$cis[Rh^{III}(phen)_2Cl_2]^{+*} + dG \longrightarrow cis[Rh^{II}(phen)_2Cl_2] + dG^{+\cdot} \quad (2)$$

Propagation

$$cis[Rh^{II}(phen)_2Cl_2] \longrightarrow [Rh^{II}(phen)_2Cl]^+ + Cl^- \quad (3)$$

$$[Rh^{II}(phen)_2Cl]^+ + dG \longrightarrow cis[Rh^{II}(phen)_2(N7\text{-}dG)Cl]^+ \quad (4)$$

$$[Rh^{II}(phen)_2Cl]^+ + H_2O \longrightarrow cis[Rh^{II}(phen)_2(OH_2)Cl]^+ \quad (4a)$$

$$cis[Rh^{II}(phen)_2(OH_2)Cl]^+ + dG \longrightarrow cis[Rh^{II}(phen)_2(N7\text{-}dG)Cl]^+ \quad (4b)$$

$$cis[Rh^{II}(phen)_2(N7\text{-}dG)Cl]^+ + cis[Rh^{III}(phen)_2Cl_2]^+ \rightleftharpoons cis[Rh^{III}(phen)_2(N7\text{-}dG)Cl]2+ + cis[Rh^{II}(phen)_2Cl_2] \quad (5)$$

Termination

$$[Rh^{II}(phen)_2Cl]^+ + O_2 \xrightarrow{H_2O} cis[Rh^{III}(phen)_2Cl(OH_2)]2+ + O_2^{\cdot-} \quad (6)$$

$$cis[Rh^{II}(phen)_2(N7\text{-}dG)Cl]^+ + dG^{+\cdot} \longrightarrow cis[Rh^{III}(phen)_2(N7\text{-}dG)Cl]2+ + dG \quad (7)$$

Energy Wastage

$$cis[Rh^{II}(phen)_2Cl_2] + dG^{+\cdot} \longrightarrow cis[Rh^{III}(phen)_2Cl_2]^+ + dG \quad (8)$$

(step 6); the result is a very inefficient sequence in air which allows for the deprotonation and rearrangement of the dG radical cation to give an N1-centered radical.[1c] Adventitious oxygen may play a role in termination even with the degassed conditions being used in these studies. Another termination reaction is shown in step 7, wherein the N7-substituted Rh^{II} intermediate is oxidized by $dG^{+\cdot}$, again in good analogy with that proposed for the Re chemistry.[8a]

We are now studying the significance of these findings with regard to the efficiency and binding sites for the photolytic binding of *cis*-$Rh^{III}(phen)_2Cl_2^+$ to DNA under anaerobic conditions.

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(11) Step 3 would also be reversible at higher concentrations of chloride ion; tests of the effect of chloride concentration on the quantum efficiency are in progress.