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Binding Interaction of [Re(H₂O)₃(CO)₃⁺ with the DNA Fragment d(CpGpG)

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Insights into the interaction of the [Re(H₂O)₃(CO)₃]+ complex (1) with the DNA fragment d(CpGpG) have been obtained by one- (1D) and two-dimensional (2D) NMR spectroscopy. The H8 resonances of the single major [Re(H₂O)d(CpGpG)(CO)₃]⁻ adduct (2) exhibit pH-independent chemical shift changes attributable to metal N7 binding. The structure of this adduct has been characterized by molecular modeling studies based on 1D and 2D NMR data. In solution, 2 shows the presence of two N7-coordinated guanine moieties in a head-to-head (HH) orientation as evidenced by G2H8/G3H8 cross peaks in the [¹H-¹H]-NOESY spectrum. The presence of the 5'-bridging phosphodiester appears to stabilize the HH1 L conformer as previously described for related Pt and Rh complexes.

The anticancer drug cisplatin and the more recent compounds carboplatin and oxaliplatin remain the most effective inorganic compounds for treatment of a variety of tumors. There is consensus in the community that binding of the drug to DNA is critical for its antitumor activity. A large body of evidence indicates a preference of the drug for DNA sequences containing two or more adjacent guanosine nucleosides.¹-⁴ Bifunctional binding to purines leads to DNA modifications which contribute to a cascade of events ultimately resulting in cell death.¹-⁴

Insights into the nature of the GpG platinum adducts emerged from the X-ray structure determination of (NH₃)₂Pt{d(pGpG)} followed by those of longer oligonucleotides.⁵-¹¹ To the best of our knowledge the (NH₃)₂Pt{d(pGpG)} structure remains to date the only structure of a metal fragment bound to GG. For other transition metals of interest in cancer therapy, among which Ru and Rh play a predominant role, the structure of the d(GpG) adducts has been determined solely in solution via NMR studies.¹²-¹⁵

Cauci and coworkers have demonstrated that the octahedral trans-RuCl₂(DMSO)₃ complex forms a stable compound with d(GpG) characterized by covalent bifunctional binding to N7 with guanine bases in a head-to-head (HH) orientation.¹² More recently, Chifotides et al. presented a binding study of the d(GpG) and d(pGpG) fragments with several antitumor tetrakis(µ-carboxylato)dirhodium(II,II) compounds.¹³-¹⁵ They have shown that the interaction of the dirhodium units with d(xGpG) yields an adduct in which both rhodium centers are involved in cis binding to GG. In their model the guanine residues are found in a left-handed HH arrangement.

We have recently demonstrated that the fac-[Re(CO)₅]⁺ core is capable of engaging two guanine bases in cis
The H8 signal is shifted downfield by about 0.55 ppm with respect to the free base due to the electron withdrawing effect of the metal center. Coordination of [Re(H2O)d(CpGpG)(CO)3]− species (2). While the H8 signals of 2 resonate upfield with respect to free d(CpGpG), the H6 cytosine resonance at 7.75 ppm (Figure 1B, top) suffers a downfield shift (Table 1).

pD dependent [1H]-NMR titration experiments revealed changes in chemical shifts of all aromatic protons only between pD values of 4.5 and 7.5. The absence of chemical shift changes below a pD of 3.5, at which guanine N7 becomes protonated and the fact the cytosine H6 exhibits the largest change (Supporting Information), proves Re-coordination at the N7 positions of the guanine nucleobases. The pKₐ values of C1H6 is thereby considerably higher than the one of H2(CMP) ± (pKₐ = 4.33 ± 0.04) describing the deprotonation of the N3 site.

Binding of 1 to d(CpGpG) also causes a downfield shift of the 31P resonances in comparison to the free ligand (Figure 1B). Such a downfield shift of the 31P signal is common for Pt²⁺ and Rh³⁺ N7-N7 macrochelates of such a DNA fragment and usually indicates an increase in the unwinding angle characterized by changes in the R-O-P-O-R' torsion angles. Since this DNA fragment binds to Re via the guanine residues one might expect that the greater change in the R-O-P-O-R' torsion angles concerns the -GpG phosphate while the CpG-phosphate would be relatively less affected. The most downfield 31P signal is therefore assigned to the -GpG phosphate. The other signal, closer to free d(CpGpG) resonances, is assigned to the CpG-phosphate.

2D NOESY spectra were acquired to assign the H8 and sugar resonances of 2 (Table 1). Each sugar spin system was connected to its nucleobase via the cytosine H6 or guanine H8 NOE to the respective H2' and H3' resonances. The detection of sequential contacts between base and deoxyribose H1', H2' and H2'' resonances yielded the correct assignment of all resonances. The sugar spin system connected to the most downfield H8 peak (7.99 ppm, Figure 1) is assigned to G3. In the aromatic region of

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**Table 1.** [1H] and [31P]-NMR Chemical Shifts (δ, ppm) for 2 and d(CpGpG) (100% D2O, 310 K, pD = 8.45)

<table>
<thead>
<tr>
<th>compound</th>
<th>Base</th>
<th>H5 / H6</th>
<th>H8</th>
<th>H1'</th>
<th>H2' / H2&quot;</th>
<th>H3'</th>
<th>H4'</th>
<th>H5' / H5&quot;</th>
<th>31P</th>
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<tr>
<td>2</td>
<td>C1</td>
<td>6.22 / 7.87</td>
<td></td>
<td>6.29</td>
<td>2.12 / 2.50</td>
<td>4.61</td>
<td>4.29</td>
<td>3.87 / 4.02</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>7.78</td>
<td></td>
<td>6.43</td>
<td>2.38 / 2.94</td>
<td>4.99</td>
<td>4.42</td>
<td>4.29 / 4.41</td>
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<tr>
<td></td>
<td>G3</td>
<td>7.99</td>
<td></td>
<td>6.42</td>
<td>2.77 / 3.01</td>
<td>4.92</td>
<td>4.37</td>
<td>4.16 / 4.20</td>
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</tr>
<tr>
<td></td>
<td>C1</td>
<td>6.09 / 7.80</td>
<td></td>
<td>6.12</td>
<td>2.10 / 2.43</td>
<td>4.63</td>
<td>4.27</td>
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<tr>
<td></td>
<td>G2</td>
<td>8.07</td>
<td></td>
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<tr>
<td></td>
<td>G3</td>
<td>8.19</td>
<td></td>
<td>6.29</td>
<td>2.64 / 2.89</td>
<td>4.87</td>
<td>4.32</td>
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*a* Not stereospecifically assigned. *b* Shifts relative to 85% H3PO4 in D2O.

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**Figure 2.** Aromatic region of [1H-1H]- NOESY spectrum (D2O, 37 ºC, 7.5-8.4 ppm) of d(CpGpG) after 1h incubation with 1eq of I.
the 2D NOESY spectrum of 2 the relatively weak G2H8/G3H8 cross-peak (Figure 3) provides evidence that the adduct is in a HH base orientation as is usually observed for Pt(II) (refs. 21,24-29) and Rh(III) d(GpG)23-15 adducts. The two H8 resonances are well separated and upfield shifted (Table 1) from those of the unbound d(CpGpG). In addition, relatively strong G2H8- and G3H8-C1H6 cross-peaks are visible in the aromatic region.

Figure 3. Preliminary optimized model of [Re(H2O)d(CpGpG)(CO)3]– (2) based on the NMR data.

Based on the NMR data a model of 2 was designed and a preliminary structure optimization performed.30 The model was chosen such that the carbonyl groups of G2 and G3 are orientated towards the coordinated H2O molecule in order to optimize H-bonding interactions (Figure 4). As we have previously shown, this configuration is energetically favoured.17 Interestingly the presence of strong G2H8- and G3H8-C1H6 cross-peaks in the NOESY spectrum suggest that the cytosine residue folds on top of the bound guanines. These adopt a HH1 left-canted orientation which appears to be stabilized by the presence of a terminal 5'-phosphate group as previously described for related Pt and Rh complexes.31-35, 21, 31, 32

Species 2 is only slightly soluble in water at room temperature. During the course of the NMR experiments the complex started to precipitate already at 37°C as a white microcrystalline solid. The IR spectrum of the microcrystalline solid shows typical $\nu_{ac}$-[Re(II)(CO)3]$^+$ carbonyl vibrations at 2024 and 1898 cm$^{-1}$ together with three distinguishable organic C=O vibrations (1687-1600 cm$^{-1}$, Supporting Information) assigned to the oligonucleotide ligand. Attempts to grow single crystals from the isolated solid suitable for x-ray crystallography were so far unsuccessful.

In conclusion we have shown that the interaction of [Re(H2O)d(CO)3]$^+$ (1) with the DNA fragment d(CpGpG) yields a single major [Re(H2O)d(CpGpG)(CO)3]$^-$ adduct (2) exhibiting pH-independent titration curves attributable to metal N7 binding. Molecular modeling studies based on 1D and 2D NMR data show that the two N7-coordinated guanine moieties are in a head-to-head (HH) orientation. These results further support the possibility of employing $^{99m}$Tc and Re based complexes not only as radio- but also as chemotherapeutic agents.

Supporting Information: Materials and Methods, IR and pH dependence of the $[^1H]$-NMR resonances of adduct 2, calculation of $pK_a$ value, and details of theoretical calculations.

REFERENCES

30. The preliminary structure simulation was performed with a Spartan '06 program version 1.1.0. Details are given in Supporting Information.