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

Zobi, F; Blacque, O; Sigel, Roland K O; Alberto, R

Abstract: 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.

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Insights into the interaction of the $[\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^+$ 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 $[\text{Re}(\text{H}_2\text{O})\text{d(CpGpG})(\text{CO})_3]^{-}$ 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 $[^1\text{H}-^1\text{H}]$-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 (NH$_3$)$_2$Pt{d(pGpG)} followed by those of longer oligonucleotides.$^{5-11}$ To the best of our knowledge the (NH$_3$)$_2$Pt{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-RuCl$_2$(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)dirhomodium(II,II) compounds.$^{13-15}$ They have shown that the interaction of the dirhomodium 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)$_3$]$^+$ core is capable of engaging two guanine bases in cis...
binding yielding reasonably stable complexes.\textsuperscript{16} We have also shown by structural elucidation that the guanine ligands can assume both a HH and a HT conformation around the metal center and that the two bases can freely rotate about the Re-N7 bond.\textsuperscript{17} Furthermore, the \textit{fac-} [Re(Base)(H\textsubscript{2}O\textsubscript{2})(CO\textsubscript{3})\textsuperscript{+} moiety displays a principally similar reactivity pattern with plasmid DNA as e.g. cisplatin implying a possible interaction with adjacent guanines in DNA as well.\textsuperscript{18} 

These results suggest the possibility of employing \textsuperscript{99(m)}Tc and Re based complexes not only as radio- but also as chemotherapeutic agents. However, nothing is known about the interaction of these metal ions with DNA fragments. It is this lack of knowledge that prompted us to study the interaction of [Re(H\textsubscript{2}O\textsubscript{3})(CO\textsubscript{3})\textsuperscript{2-} (2) with d(CpGpG), the H8 signal is shifted downfield by about 0.55 ppm with respect to the free base\textsuperscript{16, 17} due to the electron withdrawing effect of the metal center. Coordination of [Re(Base)(H\textsubscript{2}O\textsubscript{2})(CO\textsubscript{3})\textsuperscript{2-}] to the second purine, however, results in a relative upfield shift of the same resonance. 

This latter effect is most likely due to, first, the ring current effect of the second nucleobases shielding to some extent the proton from the external magnetic field of the probe and, second, the distribution of the electron withdrawing effect on two bases. The two peaks of the single major product between 7.7 and 8.0 ppm are consequently assigned to H8 resonances of a bis-bound [Re(H\textsubscript{2}O\textsubscript{2})(CO\textsubscript{3})(d(CpGpG))\textsuperscript{2-}] species. While the H8 signals of 2 resonate with respect to free d(CpGpG), the H8 cytosine resonance at 7.75 ppm (Figure 1B, top) suffers a downfield shift (Table 1).

Figure 2. Aromatic region of [\textsuperscript{1}H-\textsuperscript{1}H]- NOESY spectrum (D\textsubscript{2}O, 37 °C, 7.5-8.4 ppm) of d(CpGpG) after 1h incubation with 1eq of I.

Table 1. [\textsuperscript{1}H] and [\textsuperscript{31}P]-NMR Chemical Shifts (\textit{\textdelta}, ppm) for 2 and d(CpGpG) (100% D\textsubscript{2}O, 310 K, pD = 8.45)

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<tr>
<th>Compound</th>
<th>Base</th>
<th>H5 / H6</th>
<th>H8</th>
<th>H1'</th>
<th>H2' / H2''</th>
<th>H3'</th>
<th>H4'</th>
<th>H5' / H5''</th>
<th>\textsuperscript{1}P'</th>
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<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.22 / 7.87</td>
<td>6.29</td>
<td>2.12 / 2.50</td>
<td>4.61</td>
<td>4.29</td>
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<tr>
<td>G2</td>
<td></td>
<td>7.78</td>
<td></td>
<td></td>
<td>6.43</td>
<td>2.38 / 2.94</td>
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<tr>
<td>G3</td>
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<td></td>
<td>6.42</td>
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<td>6.09 / 7.80</td>
<td>6.12</td>
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<td>4.91</td>
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<tr>
<td>G3</td>
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<td>8.19</td>
<td></td>
<td></td>
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<td>4.87</td>
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\textsuperscript{a} Not stereospecifically assigned. \textsuperscript{b} Shifts relative to 85% H\textsubscript{3}PO\textsubscript{4} in D\textsubscript{2}O.
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 Pt2+ (refs. 21-24-29) and Rh3+ d(GpG)13-15 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. The model was chosen such that the carbonyl groups of G2 and G3 are oriented towards the coordinated H2O molecule in order to optimize H-bonding interactions (Figure 4). As we have previously shown, this configuration is energetically favoured. 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.13-15, 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 µfac- [Re6(CO)14]+ 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 99mTc 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 pKₐ 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.