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Binding Interaction of $[Re(H_2O)_3(CO)_3]^+$ with the DNA Fragment d(CpGpG)

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Insights into the interaction of the $[Re(H_2O)_3(CO)_3]^+$ 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 $[Re(H_2O)d(CpGpG)(CO)_3]^-$ 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 NMR spectrum. The presence of the 5′-bridging phosphodiester appears to stabilize the HH1 L conformer, as was 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 co-workers have demonstrated that the octahedral *trans*-$RuCl_2(DMSO)_4$ 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*-$[Re(CO)_3]^+$ core is capable of engaging two guanine bases in *cis* binding, yielding reasonably stable complexes.[16] We have also shown by structural elucidation that the guanine ligands can assume both a HH and a head-to-tail (HT) conformation around the metal center and that the two bases can freely rotate about the Re−N7 bond.[17] Furthermore, the *fac*-$[Re(CO)_3]^+$ moiety displays a principally similar reactivity pattern with plasmid

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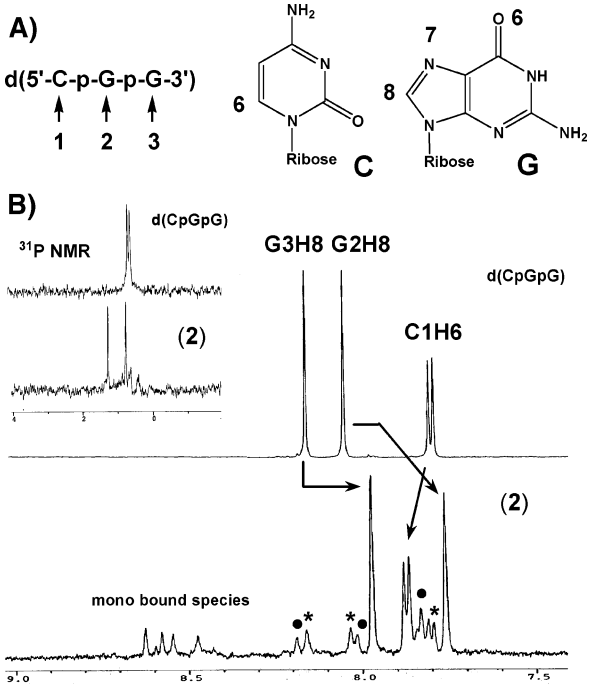

Figure 1. (A) Numbering scheme of d(CpGpG) together with the chemical formulas of the two nucleobases. (B) Aromatic region of the ^{1}H NMR spectrum (D_2O, 37 °C, 7.0−8.0 ppm) of d(CpGpG) (top) and the same sample after 1 h of incubation with 1 equiv of **1** (bottom). Residual d(CpGpG) resonances are indicated by asterisks and different HT and HH conformers are tentatively assigned by solid circles. The inset shows changes in the ^{31}P NMR spectrum.

DNA such as, e.g., cisplatin, implying a possible interaction with adjacent guanines in DNA as well.[18]

These results suggest the possibility of employing $^{99(m)}$Tc- and Re-based complexes not only as radiotherapeutic agents 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 $[Re(H_2O)_3(CO)_3]^+$ (**1**) with the d(CpGpG) sequence.

The reaction of **1** with 1 equiv of d(CpGpG) in D_2O was monitored by ^{1}H NMR spectroscopy. At 37 °C, the addition of **1** to a solution of d(CpGpG) causes the immediate appearance of four peaks in the 8.4−8.7 ppm region of the spectrum. Within 1 h, the resonances of the H8 signals of free d(CpGpG) decrease in intensity while a new set of sharp well-separated peaks of the nonequivalent H8 protons appear between 7.7 and 8.0 ppm. The peaks initially observed in the 8.4−8.7 ppm region remain relatively constant with respect to the total H8 signal intensity (Figure 1).

The peaks in the 8.4−8.7 ppm region are assigned to mono-bound Re−d(CpGpG) species. As we have previously shown, in mono-bound species of the type $[Re(Base)(H_2O)_2(CO)_3]^+$ (where Base = 9/7-MeG, G, or dG), the H8 signal is shifted downfield by about 0.55 ppm with respect to the free base[16,17] because of the electron-withdrawing effect of the metal center. Coordination of $[Re(Base)(H_2O)_2(CO)_3]^+$ to the second purine, however, results in a relative upfield shift of the same resonance.

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Table 1. ^{1}H and ^{31}P NMR Chemical Shifts (δ, ppm) for **2** and d(CpGpG) (100% D_2O, 310 K, pD = 8.45)

compound	base	H5/H6	H8	H1′	H2′/H2″ [a]	H3′	H4′	H5′/H5″ [a]	^{31}P[b]
2	C1	6.22/7.87		6.29	2.12/2.50	4.61	4.29	3.87/4.02	0.80
	G2		7.78	6.43	2.38/2.94	4.99	4.42	4.29/4.41	1.28
	G3		7.99	6.42	2.77/3.01	4.92	4.37	4.16/4.20	
d(CpGpG)	C1	6.09/7.80		6.12	2.10/2.43	4.63	4.27	3.85/4.01	0.78
	G2		8.07	6.24	2.28/2.87	4.91	4.41	4.19/4.35	0.72
	G3		8.19	6.29	2.64/2.89	4.87	4.32	4.11/4.10	

[a] Not stereospecifically assigned. [b] Shifts relative to 85% H_3PO_4 in D_2O.

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 $[Re(H_2O)d(CpGpG)(CO)_3]^-$ 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 ^{1}H NMR titration experiments revealed changes in the 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 that cytosine H6 exhibits by far the largest change (see the Supporting Information) prove Re coordination at the N7 positions of the guanine nucleobases.[1−4,12−15] The $pK_{a,H2O} = 5.27 \pm 0.14$ calculated[19] from the titration data of C1H6 is thereby considerably higher than the one of $H_2(CMP)^{\pm}$ ($pK_a = 4.33 \pm 0.04$)[20] describing the deprotonation of the N3 site.

The binding of **1** to d(CpGpG) also causes a downfield shift of the ^{31}P NMR resonances in comparison to the free ligand (Figure 1B). Such a downfield shift of the ^{31}P NMR signal is common for Pt^{2+} and Rh^{3+} 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.[13−15,21−23]

Because 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 ^{31}P NMR 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 NMR 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.

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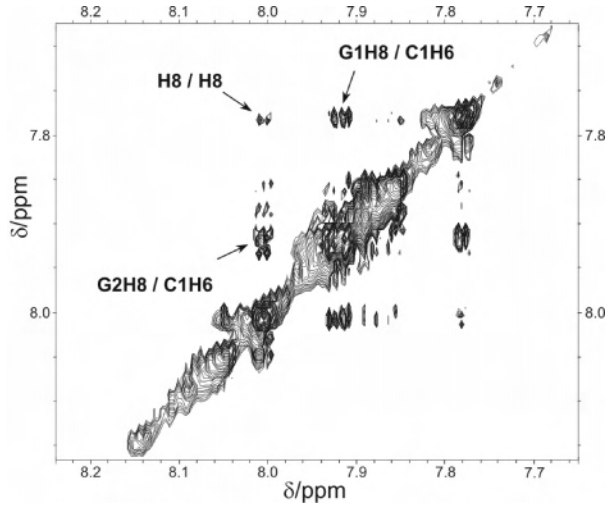

Figure 2. Aromatic region of the ^{1}H−^{1}H NOESY NMR spectrum (D_2O, 37 °C, 7.5−8.4 ppm) of d(CpGpG) after 1 h of incubation with 1 equiv of **1**.

The detection of sequential contacts between base and deoxyribose H1′, H2′, and H2″ resonances yielded the correct assignement 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 the 2D NOESY NMR spectrum of **2**, the relatively weak G2H8/G3H8 cross-peak (Figure 2) provides evidence that the adduct is in a HH base orientation, as is usually observed for Pt^{2+} [21,24−29] and Rh^{3+} 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.

Based on the NMR data, a model of **2** was designed and a preliminary structural optimization performed.[30] The model was chosen such that the carbonyl groups of G2 and G3 are oriented toward the coordinated H_2O molecule in order to optimize hydrogen-bonding interactions (Figure 3). As we have previously shown, this configuration is energetically favored.[17] Interestingly, the presence of strong G2H8− and G3H8−C1H6 cross-peaks in the NOESY NMR spectrum

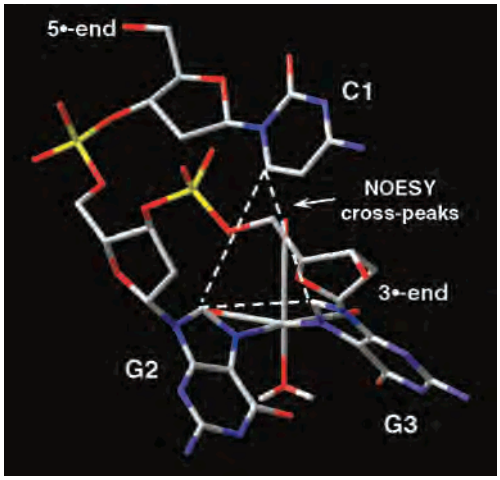

Figure 3. Preliminary optimized model of **2** based on the NMR data.

suggests 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 was 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 *fac*-$[Re^I(CO)_3]^+$ carbonyl vibrations at 2024 and 1898 cm^{-1} together with three distinguishable organic C═O vibrations (1687−1600 cm^{-1}; see the 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 **1** with the DNA fragment d(CpGpG) yields a single major 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 HH orientation. These results further support the possibility of employing $^{99(m)}$Tc- and Re-based complexes not only as radiotherapeutic agents but also as chemotherapeutic agents.

Supporting Information Available: Materials and methods, mass spectrometry, IR, and pH dependence of the ^{1}H NMR resonances of adduct **2**, calculation of pK_a values, and details of theoretical calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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