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Molybdenocene–oligonucleotide binding study at physiological pH using NMR spectroscopy and cyclic voltammetry

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Abstract—The self-complementary oligonucleotide CGCATATATGCG was used as a model to establish the binding interactions of antitumor molybdenocene dichloride and DNA. The free dodecamer was first characterized using ^{1}H, NOESY, and DQF-COSY NMR experiments, which enable to pinpoint the guanines and adenines as well as the cytosines and thymines signals in the aromatic region. Molybdenocene dichloride was characterized in saline and buffer solutions as function of pH by ^{1}H NMR spectroscopy. In 10 mM NaCl/D_2O solution at pH of 6.5 and above, $Cp_2Mo(OD)(D_2O)^+$ is in equilibrium with its dimeric species, $[Cp_2Mo(\mu\text{-}OH)_2MoCp_2]^{2+}$. In 25 mM Tris/4 mM NaCl/D_2O at physiological pH, a new stable species is formed, coordinated by the buffer, Tris(hydroxymethyl)aminomethane. The interactions of molybdenocene dichloride species with CGCATATATGCG were studied at different pH. At pH 6.5, in 4 mM NaCl/D_2O solution, ^{1}H NMR spectra of CGCATATATGCG exhibit downfield shifts in the signals associated mainly to adenines and guanines, upon addition of molybdenocene dichloride. At pH 7.4, in 25 mM Tris/4 mM NaCl/D_2O, molybdenocene species causes broadening and small downfield shifts to the purines and pyrimidine signals, suggesting that molybdenocene dichloride can get engaged in binding interactions with the oligonucleotide in a weak manner. ^{31}P NMR spectra of these interactions at pH 7.4 showed no changes associated to Mo(IV)-OP coordination, indicating that molybdenocene–oligonucleotide binding interactions are centered, most likely, on the bases. Cyclic voltammetry titration showed a 4.9% of molybdenocene–oligonucleotide interaction. This implicates that possible binding interactions with DNA are weak.

1. Introduction

Molybdenocene dichloride (**1**) is a sandwich complex that belongs to a new class of organometallic antitumor agents.[1–5] These sandwich complexes (M = Ti, V, Nb, Mo) have demonstrated interesting antitumor activity profiles with less toxic effects than *cis*-platin.[1–18] Currently the biochemical aspects of these species are being investigated leading to new hypothesis about the possible mechanisms of action. Early precedents on the coordination chemistry of molybdenocene dichloride with biologically important molecules have reported the binding of nucleotides to Mo(IV) center.[19–21] However, in subsequent studies using NMR spectroscopy, Harding and co-workers claimed that molybdenocene does not bind oligonucleotides at pH above 6.0 thus, ruling out DNA as a primary target place for this metallocene. But notably, their NMR molybdenocene–oligonucleotide binding studies were carried out in saline (NaCl) unbuffered solutions.[18,22] On the other hand, Harding and co-workers have proposed glutathione as a target biomolecule and carrier of molybdenocene species.[23] In marked contrast, in the latest report by the same group, it was shown that glutathione functions as deactivating molecule and not a transport agent.[24]

In order to investigate, in more detail, the binding capabilities of molybdenocene dichloride to DNA, we have monitored the interaction of the self-complementary oligonucleotide CGCATATATGCG with molybdenocene dichloride at pH 6.5 and at physiological pH in buffer solution. Our NMR and cyclic voltammetry experiments were carried out under buffer conditions and provide new insights with regard to the possible molybdenocene–DNA binding interactions.

Abbreviations: A, adenine; C, cytosine; G, guanine; T, thymine.

Keywords: Antitumor; Molybdenocene dichloride; NMR; Oligonucleotide; DNA.

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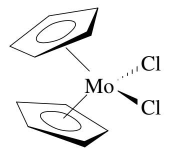

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2. Results

In order to explore the binding capability of molybdenocene dichloride to DNA we have performed a thorough characterization of molybdenocene dichloride and the oligonucleotide CGCATATATGCG (as model compound for DNA) as function of pH in saline and Tris buffer solutions. Figure 1 describes the behavior of Cp_2MoCl_2 in NaCl solution as function of pH. At pH of 3.0, two signals are observed in the Cp region. Since the initial complex is in 10 mM NaCl (D_2O) solution, the signals correspond, most likely, to the $Cp_2Mo(D_2O)_2^{2+}$ and $Cp_2Mo(D_2O)(Cl)^+$ species. Incomplete chloride hydrolysis is expected in this saline solution.[22] As we increase the pH, the aqua-chloro species starts to vanish and the bis-aqua species increases accompanied by Cp hydrolysis. At pH 6.5 a new species appeared at 6.53 ppm and must likely correspond to $Cp_2Mo(D_2O)(OD)^+$ species and the intensity of this signal increases in concentration at expense of the $Cp_2Mo(D_2O)_2^{2+}$ species (5.82 ppm), as the pH is raised to 7.4. According to Marks and co-workers, the predominant species at physiological pH is $Cp_2Mo(H_2O)(OH)^+$,[20] therefore we expect that $Cp_2Mo(D_2O)(OD)^+$ species is present in D_2O solution. $Cp_2Mo(D_2O)(OD)^+$ species should exist in equilibrium with the dimeric species $[Cp_2Mo(\mu\text{-}OD)_2MoCp_2]^{2+}$, as reported by Tyler and co-workers.[25] For simplicity, we will use $Cp_2MoCl_2(aq)$ referring to $Cp_2Mo(D_2O)(OD)^+$ and its dimeric species. Substantial amount of Cp protonolysis is observed at physiological pH as well as other transient molybdenocene species at pH of 5.5.

Molybdenocene dichloride was also characterized in 25 mM Tris/4 mM NaCl solution as function of pH. Figure 2. At low pH, a mixture of species most likely corresponding to the $Cp_2Mo(D_2O)_2^{2+}$ and $Cp_2Mo(D_2O)(Cl)^+$ are evident. At pH of 6.5 and above one species predominates at 5.69 ppm while the other two signals vanished. The exact structure of this molybdenocene species is unknown, but the chemical shift does not correspond to $Cp_2Mo(D_2O)(OD)^+$, $Cp_2Mo(D_2O)_2^{2+}$ or $Cp_2Mo(D_2O)(Cl)^+$ complexes. We believe that some sort of ‘Cp_2Mo-Tris’ complex is formed (the Tris(hydroxymethyl)aminomethane chelating Mo(IV) through the oxygen and nitrogen atoms). Our proposed molybdenocene species is supported based on the fact that $Cp_2Mo(D_2O)(OD)^+$ is coordinated by amines and alcohols at pH of 7.0.[25] The existence of ‘Cp_2Mo–Tris’ complex is further corroborated by mass spectrometry analysis. Mass spectrum of ‘Cp_2Mo–Tris’ complex shows a *m*/*z* peak at 348 which corresponds to the $[Cp_2Mo\text{–}Tris+H]^+$ species. MS/MS of this species result in a major species at 229 (*m*/*z*), indicating the presence of $[Cp_2Mo-H]^+$ species. In any event, there is substantial amount of molybdenocene species at pH 7.4 and very minimum amount of Cp protonolysis under this buffer condition.

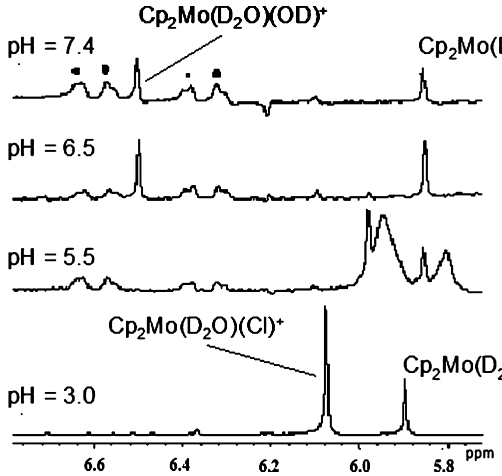

Figure 1. ^1H NMR molybdenocene dichloride (8 mM) spectra in a 10 mM NaCl/D_2O solution as a function of pH. Black dots show the cyclopendienyl hydrolysis products.

Likewise, the oligonucleotide was characterized as function of pH. We determined 26 °C, the optimum temperature where the oligonucleotide has enough resolution at pH of 6.5 and above. The ^1H spectra of the oligonucleotide at pH 5.5 in saline solution (Supplementary Material) showed poor resolution in the aromatic region, only the thymines and cytosines can be identified. At pH of 6.5 and higher, the spectrum in this region becomes resolved and changes in the δs are observed when the pH is raised from 6.5 to 7.4, Figure 3. The oligonucleotide has been further characterized using COSY DQF and NOESY experiments.

Three doublets are observed in the 7 ppm region corresponding to the H(6)’s of the cytosines which are coupled to H(5): 7.62–5.90, 7.36–5.47, and 7.29–5.47 (two H(5) coincidentally overlap). The other three singlets are the thymines, see Table 1.

The NOESY spectrum of the oligonucleotide CGCATATATGCG in 25 mM Tris-d_{11}/4 mM NaCl buffer solution in D_2O at pH 7.4 is presented in Figure 4. This spectrum allowed us to determine the adenines and guanines signals via NOE with the corresponding thymines and cytosines. NOESY spectrum in this region revealed that guanines are located at δ 7.973 (Guanine (10)), 8.003 (Guanine (2)), and 8.012 (Guanine (12)) ppm as corroborated by the corresponding cross-peaks through the anomeric protons (H1) of the sugar: Cytosine(1), H(6) 7.401 and Cytosine(3), H(6) 7.330 ppm—Guanine(2); Thymine(9), 7.287 and Cytosine(11), H(6) 7.665 ppm-Guanine(10); and Cytosine(11), H(6) 7.665 ppm-Guanine(12). The remaining resonances from 7.89 to 8.30 ppm correspond to the H(2) and H(8) of the adenines.

The interaction of $Cp_2MoCl_2(aq)$ with the oligonucleotide was investigated by ^1H NMR spectroscopy. The ^1H NMR spectra of CGCATATATGCG at pH 6.5 (Fig. 5) upon addition of increasing amounts of molybdenocene

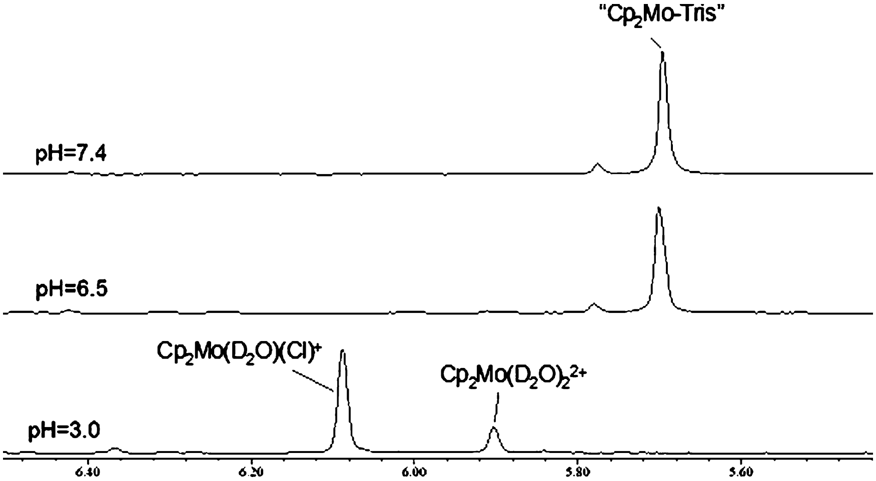

Figure 2. ^{1}H NMR spectra of molybdenocene dichloride (8 mM) dissolved in 25 mM Tris/4 mM NaCl/D_2O as a function of pH. The scale is in ppm.

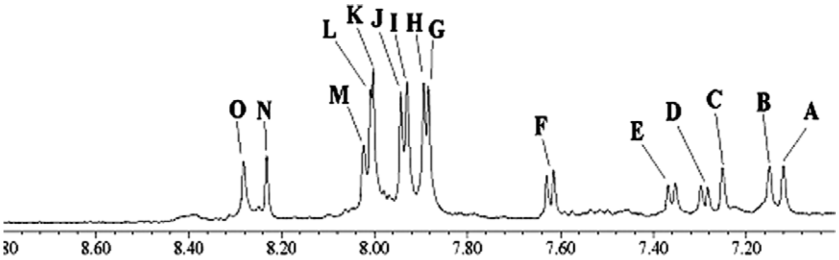

Figure 3. ^{1}H NMR aromatic region spectra of CGCATATATGCG (1 mM), 25 mM Tris/4 mM NaCl/D_2O solution, at pH 6.5 and 26 °C, assigned by letters. Table 1 shows the tabulated assignments of these signals.

Table 1. ^{1}H-NMR chemical shifts of the free oligonucleotide for the nitrogen bases at pH = 7.4, 6.5, and 5.5

Nitrogen base (tentative assignment)	Peak	pH 7.4 δ (Tris/NaCl) (ppm)	pH 6.5 δ (NaCl) (ppm)	pH 5.5 δ (NaCl) (ppm)
T_7(H6)	A	7.182	7.120	7.120
T_5(H6)	B	7.229	7.150	7.150
T_9(H6)	C	7.327	7.251	7.251
C_3(H6)	D	7.347	7.290	7.290
C_1(H6)	E	7.425	7.360	7.360
C_{11}(H6)	F	7.688	7.622	7.622
A_6(H8)	G	7.963	7.884	—[a]
G_{10}(H8)	H	7.973	7.894	—[a]
G_2(H8)	I	8.003	7.930	—[a]
G_{12}(H8)	J	8.012	7.943	—[a]
A_4(H2)	K	8.050	8.003	—[a]
A_6(H2)	L	8.059	8.008	—[a]
A_8(H2)	M	8.078	8.023	—[a]
A_4(H8)	N	8.298	8.232	—[a]
A_8(H8)	O	8.334	8.281	—[a]

[a] Signals could not be determined.

dichloride (at pH 6.5), in saline solution, showed small changes in the aromatic region corresponding to the purine bases. Signals H, I, K, and M (G_{10}, G_2, A_4, and A_6) showed downfield shifts, suggesting these bases are involved in binding to Mo(IV) center. The other signals remained in the same original positions.

Likewise, the interaction of molybdenocene species with the oligonucleotide was pursued, in saline solution, at pH of 7.4 using ^{1}H NMR spectroscopy but failed to exhibit evidence of molybdenocene–oligonucleotide binding. Similar results were obtained by Harding and co-workers using d(CGCATATGCG)$_2$ in 50 mM NaCl solution.[22]

Surprisingly, the interaction of the oligonucleotide with molybdenocene species at physiological pH in Tris *buffer* solution, Figure 6, afforded very different results. Table 2 summarizes the chemical shifts of free and bound oligonucleotide species. In contrast to the above experiment and previous reports,[18,22] the addition of

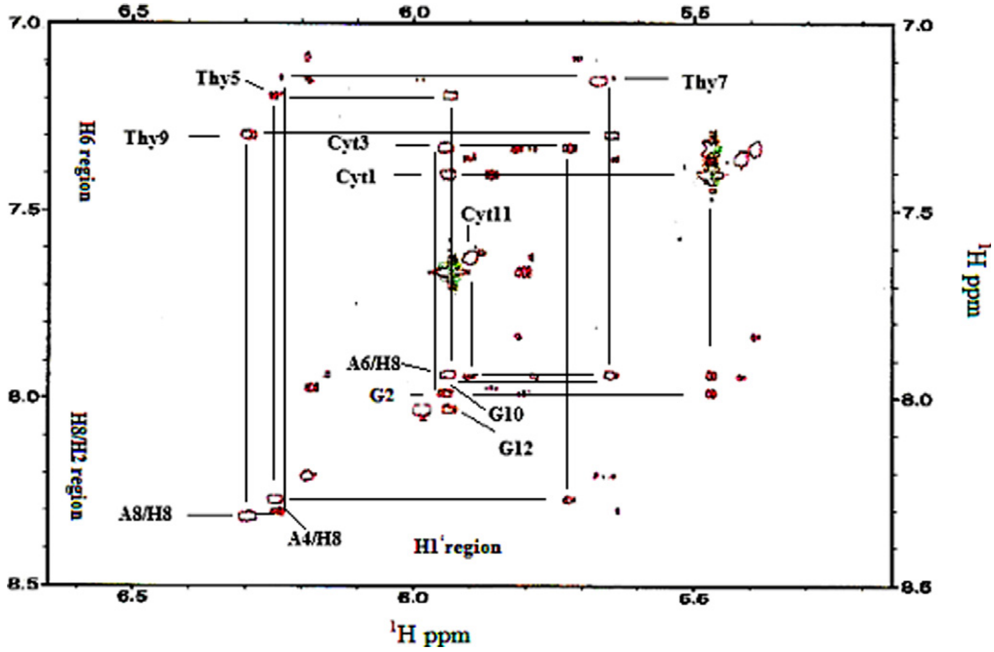

Figure 4. 750 MHz NOESY spectrum of CGCATATATGCG in 25 mM Tris-d_{11} buffer/4 mM NaCl solution in D_2O at pH 7.4, 26 °C.

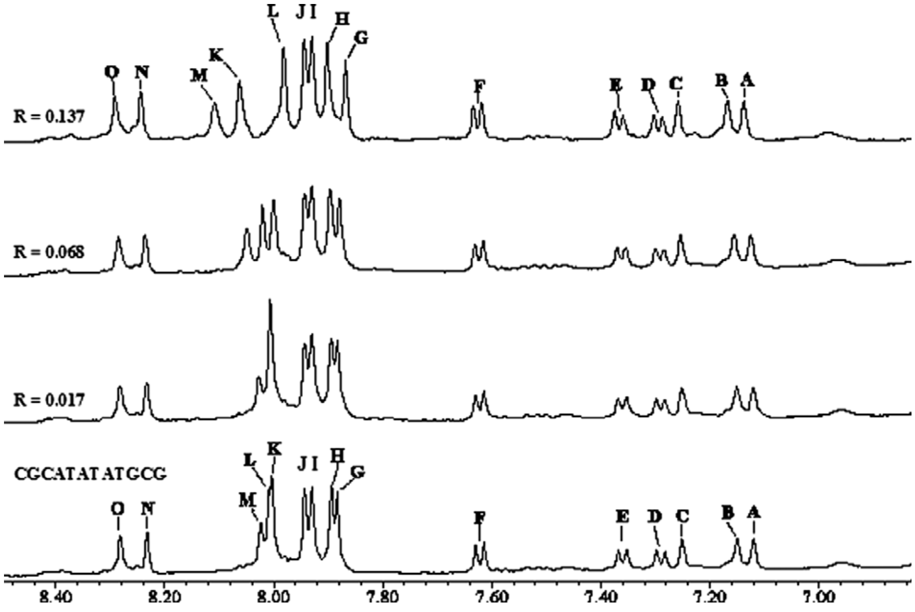

Figure 5. ^{1}H NMR aromatic region spectra of CGCATATATGCG (1 mM), 25 mM Tris/4 mM NaCl/D_2O, at pH 6.5 upon addition of aliquots of molybdenocene dichloride in 25 mM Tris/4 mM NaCl/D_2O solution.

molybdenocene aliquots (in buffer solution) to CGCATATATGCG produced broadening and small downfield shifts on G, K, and N signals (purine bases) but in addition, new resonances attributed to the H(8) of the guanines/adenines are evident. The J, I, N, and O signals decrease in intensity suggesting that these are being transformed into new species. Furthermore, the cytosine and thymine H(6)s broaden first and eventually minor signals in the cytosine/thymine H(6)s region increase in intensity, at high molybdenocene ratio. However, these changes in the ^{1}H NMR spectra are subtle which suggest weak molybdenocene–oligonucleotide binding. The coexistence of the original signals with the new emerging signals suggests that two species are present in solution. The two species are in a ratio of about 12:1. The emerging signals could arise from molybdenum coordination to the purine bases or a phosphoester bond cleavage promoted by molybdenocene dichloride.[26]

To rule out the phosphoester bond cleavage, the interaction was also monitored by ^{31}P NMR spectroscopy. The ^{31}P NMR spectra of the addition of molybdenocene dichloride to CGCATATATGCG, Figure 7, showed minor changes in the original signals proceeding from the oligonucleotide which suggests that, only weak coor-

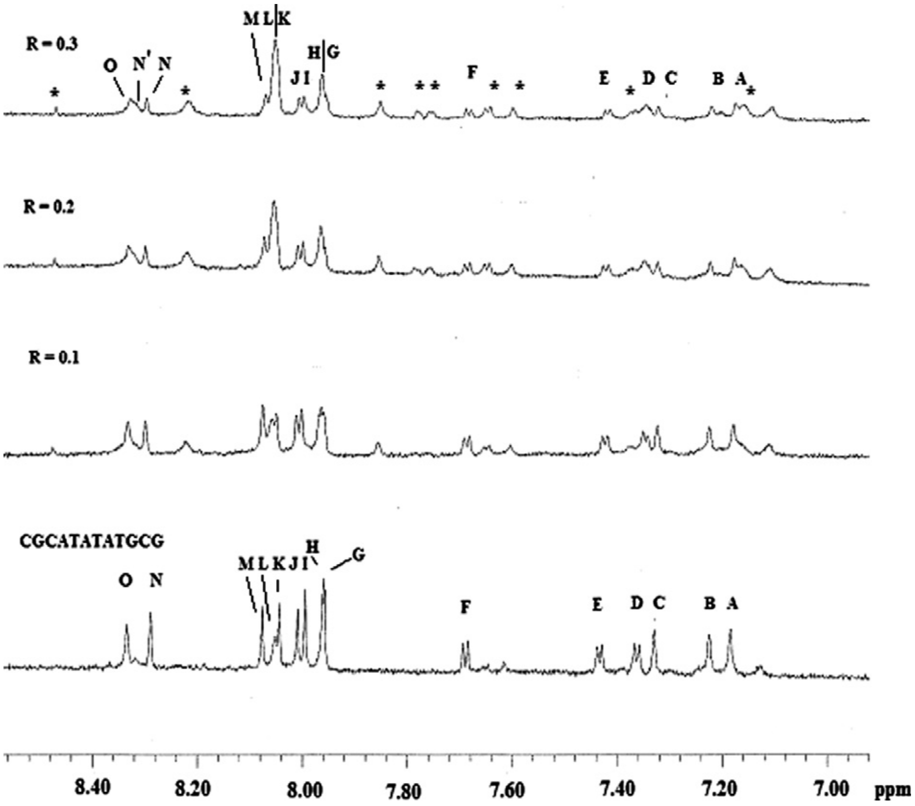

Figure 6. ^{1}H NMR aromatic region spectra of CGCATATATGCG (1 mM) at pH 7.4, in 25 mM Tris/4 mM NaCl, upon addition of aliquots of molybdenocene dichloride in 25 mM Tris/4NaCl solution, pH 7.4. Asterisk represents new signals.

Table 2. ^{1}H NMR chemical shifts of the free and 'bound' oligonucleotide for the nitrogen bases at pH 7.4

Nitrogen base (tentative assignment)	Peak	pH 7.4 Chem. Shift (ppm), free oligo	pH 7.4 Chem. Shift (ppm), oligo + Cp_2MoCl_2	Change Δδ (ppm)
Thymine			7.107	New signal
Thymine			7.167	New signal
T_7(H6)	A	7.182	7.181	−0.001
T_5(H6)	B	7.229	7.226	−0.003
T_9(H6)	C	7.327	7.328	0.001
C_3(H6)	D	7.347	7.349	0.002
C_1(H6)	E	7.425	7.422	0.003
C_{11}(H6)	F	7.688	7.688	0
Cytosine			7.754	New signal
Cytosine			7.783	New signal
Guanine			7.854	New signal
A_6(H8)	G	7.963	7.973	0.010
G_{10}(H8)	H	7.973	7.973	0
G_2(H8)	I	8.003	8.003	0
G_{12}(H8)	J	8.012	8.013	0.001
A_4(H2)	K	8.050	8.060	0.010
A_6(H2)	L	8.059	8.060	0.001
A_8(H2)	M	8.078	8.076	0.002
Guanine			8.218	New signal
A_4(H8)	N	8.298	8.303	0.005
A_4(H8)′	N′		8.319	0.021 (N′- N)
A_8(H8)	O	8.334	8.329	−0.005
Guanine			8.474	New signal

dination of molybdenocene to the bases could promote these displacements but no new signals proceeding from the phosphoester bond cleavage were observed. Table 3 presents the change in chemical shifts of the resonances upon oligonucleotide coordination, although absolute assignment of ^{31}P signals cannot be made with these data. Notably, the Δδ(ppm) are so small and Mo–OP coordination should lead to larger displacements in the

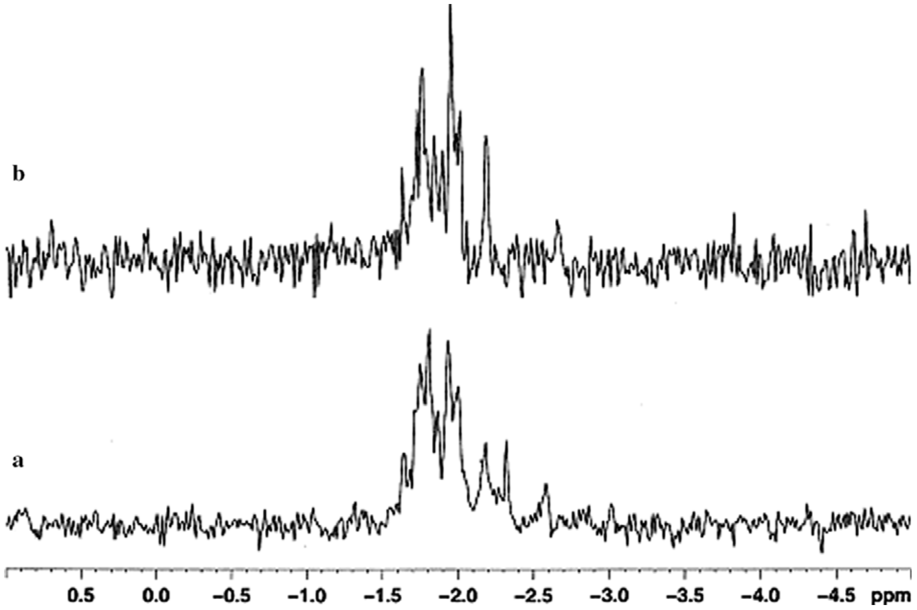

Figure 7. $^{31}P[^{1}H]$ NMR spectra of CGCATATATGCG upon addition of Cp_2MoCl_2 in 25 mM Tris/4 mM NaCl at pH 7.4.

Table 3. $^{31}P[^{1}H]$ NMR chemical shifts of the free and 'bound' oligonucleotide at pH 7.4. Labels (P) do not correspond to any particular assignment

Peak	Chem. Shift (ppm), free oligo	Chem. Shift (ppm), oligo + Cp_2MoCl_2	Change $\Delta\delta$ (ppm)
P_1	−1.633	−1.633	0
P_2	−1.681	−1.694	−0.013
P_3	−1.718	−1.730	−0.012
P_4	−1.742	−1.767	−0.025
P_5	−1.803	−1.791	0.012
P_6	−1.864	−1.840	0.024
P_7	−1.925	−1.900	0.025
P_8	−1.998	−1.949	0.049
P_9	−2.180	−1.986	0.194
P_{10}	−2.314	−2.010	0.304
P_{11}	−2.581	−2.192	0.389

chemical shifts. Attempts to obtain a NOESY spectrum of this mixture were hampered by the large signals arising from Cp's, Tris, and residual water. Additionally, mass spectrometry analysis provided no reliable results due to the amount of salts' presence in the mixture. Nevertheless, the spectral changes observed in the ^{1}H NMR spectra remain unchanged after 72 h. Therefore, it is clear that molybdenocene–Tris species, in buffer solution at pH of 7.4, could interact with the nucleotide bases in a weak manner. It should be pointed out that, different to *cis*-platin, molybdenocene dichloride apparently has no preference for guanine over adenine bases.

To corroborate our qualitatively NMR results, we investigated the molybdenocene–oligonucleotide interaction by electrochemical methods. Recently, we developed a methodology to access quantitative the molybdenocene–DNA interaction using cyclic voltammetry (cv).[27] This methodology is simpler than ICP-AES and atomic absorption but still provides accurate results regarding Mo–nucleic acid interactions.

Table 4 below and Figure S2 (Supplementary Material) describe the electrochemical behavior of molybdenocene dichloride in presence and absence of CGCATATATGCG, in buffer solution. Although the experimental conditions of the electrochemical analysis is different to the NMR spectroscopy, it is evident that the oxidation potential and current change when the oligonucleotide is present in solution.

The interaction of $Cp_2MoCl_2(aq)$ in Tris solution (presumably Cp_2Mo–Tris) with increasing amounts of oligonucleotide showed a change in E_{pa} and I_{pa} between 13–20 mV and 0.24–0.57 μA, respectively, for the first two-thirds of the titration. After the addition of 1000 μL of oligo, a net change in E_{pa} of 32 mV and I_{pa} of 0.97 μA was observed. Since the potential is more sensitive to coordination environment than current, we can calculate the % of interaction using the potentials for the free $Cp_2MoCl_2(aq)$ and $Cp_2MoCl_2(aq)$-oligonucleotide as previously reported, Eq. 1.[27]

$Cp_2MoCl_2(aq)$ in Tris buffer can attain only 4.9% of interaction to CGCATATATGCG under these experimental conditions. Thus, molybdenocene species, under physiological conditions, can bind CGCATATA TGCG weakly, as suggested using NMR spectroscopy results.

3. Discussion

In the past, we determined the degree of DNA–molybdenocene binding using electrochemistry[27] and ICP-AES.[28] Under dialysis conditions, at buffer solution and physiological pH, both analytical techniques showed that molybdenocene dichloride binds to calf-thymus DNA in 4–10% depending on the molybdenocene ancillary ligand. Although the nature of the interaction was basically unknown in buffer solution,

Table 4. Change in potential (a) and current (b) for the electrochemical titration of Cp_2MoCl_2 (5.00×10^{-4} M in 4 mM NaCl/100 Tris buffer, pH 7.4) with buffer (for dilution corrections) and oligo (2.43×10^{-4} M in 4 mM NaCl/100 Tris buffer, pH 7.4) with aliquots of 250 μL

(a) Potential	E_{pa} (mV)/aliquots μL		ΔE_{pa} (mV)	% int.
Sample:	Cp_2MoCl_2 + buffer	Cp_2MoCl_2 + oligo		
	705(4)/0 μL			
	685(6)/250 μL	698(5)/250 μL	13	1.5
	680(4)/500 μL	694(4)/500 μL	14	2.1
	670(5)/750 μL	690(2)/750 μL	20	3.0
	655(2)/1000 μL	687(3)/1000 μL	32	4.9

(b) Current	I (μA)/aliquots μL		ΔI (μA)
Sample:	Cp_2MoCl_2 + buffer	Cp_2MoCl_2 + oligo	
	−4.89(7)/0 μL		
	−4.7(1)/250 μL	−4.4(1)/250 μL	0.3
	−4.1(1)/500 μL	−3.86(8)/500 μL	0.24
	−4.05(2)/750 μL	−3.48(9)/750 μL	0.57
	−4.24(6)/1000 μL	−3.27(9)/1000 μL	0.97

we know from our ICP and electrochemical studies that molybdenocene binds DNA but in a weak manner. Now, our NMR results suggest that indeed molybdenocene species can interact with oligonucleotides at pH 7.4 under buffer solution but the interaction is weak in nature. In addition, the binding interaction is centered on the bases and not on the phosphoesters.

To corroborate the NMR results, cyclic voltammetry titrations of 'Cp_2Mo–Tris' with oligonucleotide were performed, leading to 4.9% of interaction. Therefore, our NMR results at physiological pH, in buffer solution, corroborate, in a qualitatively manner, what we have been determined quantitatively by other analytical methods, weak molybdenocene-oligo binding interactions.

Recent report on the coordination chemistry of molybdenocene dichloride with biologically important molecules has suggested that DNA is not the primary target for its antitumor activity.[29] Competition studies with dAMP, ribose monophosphate, glutathione, and histidine demonstrated favorable coordination to the cysteine thiol group over the phosphate (O) or heterocyclic (N) groups. [29] While molybdenocene dichloride has preference for the thiol groups, it has been shown that glutathione (GS) reacts with Cp_2MoCl_2(aq), forming $Cp_2Mo(GS)_2$, which has no cytotoxic properties.[24] Therefore, glutathione deactivates Cp_2MoCl_2(aq) and should not be the transport agent in the blood plasma. In the plasma there are many biomolecules that could have potential to coordinate and stabilize/transport Cp_2MoCl_2(aq). Additionally, recent studies on intracellular distribution of metallocene antitumor agents (Cp_2MCl_2, M = Ti, V, Nb, Mo) have shown that molybdenum is localized in the nuclear region.[30] It is not clear what species is inside the cell and what type of interaction molybdenocene is engaged inside the nucleus.

Notably, while molydenocene dichloride in saline solution at pH of 7.4 showed no evidence of binding to the oligonucleotide, molybdenocene species in Tris buffer can indeed show some degree of binding interactions, although very weak. While 'Cp_2Mo–Tris' represents a model compound and is not the species present in the blood plasma, our results suggest that molybdenocene dichloride could be stabilized by a coordinating biomolecule present in the blood, thus reaching the nucleic acid region. While extrapolation of these results from oligonucleotide to a larger macromolecule such as DNA must be taken with caution, we believe that DNA cannot be completely ruled out as a possible target place and these results point to a more complex scenario.

4. Experimental

4.1. Materials

Molybdenocene dichloride, DCl, NaOD, and Tris-d_{11} were obtained from Aldrich and used as received. The HPLC purified and lyophilized self-complementary dodecamer CGCATATATGCG with OH on the ending 5′ and 3′ positions was purchased by BioSynthesis Corp. This oligonucleotide has no *free phosphate* on the terminal positions.

4.2. Nuclear magnetic resonance (NMR) analysis—interaction between molybdenocene and GCGATAT ATCGC

Stock solutions of 25 mM Tris-d_{11}/4 mM NaCl (pH 7.4) and 10 mM NaCl (pH 6.5) in D_2O were prepared for the oligonucleotide dissolution. Solutions of 0.5% DCl and 1% NaOD were used to control the pH of the interactions. About 1 mM solution of CGCATATATGCG was prepared by dissolving the pre-weighed amount of the oligonucleotide in the above stock solutions at 5 °C.

Molybdenocene dichloride solutions were prepared using the saline and buffer solutions to obtain a concentration of 1×10^{-2} M. This was performed by 30 min sonication, ice was used to maintain a low temperature during sonication and 1% NaOD solution was used to adjust the pH.

For the interaction studies, aliquots of molybdenocene solution were added to the NMR tube containing oligonucleotide and analyzed by nuclear magnetic resonance. Experiments were made at 26 °C and pH of 6.5 and 7.4. The interaction study at pH 7.4 was performed on a Bruker 750 MHz spectrometer while the studies at 6.5 were performed on a Bruker 500 MHz instrument. The pH was measured before and after the addition of molybdenocene aliquots. To control temperature, the variable temperature unit (VT) was used. TSP was used as external reference. NOESY experiment for the oligonucleotide (in Tris-d_{11}/NaCl) was obtained on a Bruker 750 MHz spectrometer at 26 °C using the following parameters: 2048 complex t_2 points, 256 t_1 increments and linear predicted to 512, 64 transients, relaxation delay 1 s, and mixing time of 250 ms. Square sinebell function was used to process the data in both dimensions. Watergate was used to suppress solvent signal. ^{31}P NMR spectra were performed on a Bruker 500 MHz spectrometer using 4096 complex t_2 points, delay 1 s, and 256 transients, under proton decoupling conditions.

A Bruker Avance 500 MHz equipped with a QXI probe was used for the 1D and 2D (COSY) experiments. To resolve some resonance overlapping, a Bruker 750 MHz equipped with a triple resonance probe was used from the NMRFAM at the University of Wisconsin-Madison.

4.3. Cyclic voltammetry—interaction between molybdenocene and GCGATATATCGC

The water used throughout the experiments was double-distilled and deionized. The instrument used for the analysis was a voltammetric analyzer model BAS 50 W of Bioanalytical Systems, Inc. with a three-stand electrode cell. The three electrodes used were glassy carbon as the working electrode, an Ag/AgCl as a reference electrode, and a Pt wire as an auxiliary electrode. The working electrode was polished with 0.05 μm alumina slurry for 1–2 min and then rinsed with double-distilled and deionized water. This cleaning process is done before each cv experiment and a sweep between 0 and 1000 mV is performed on the buffer to detect any possible deposition of molybdenum or nucleobase on the electrode surface. Blank cv experiments were carried out in fresh buffer solutions and the demonstrated to be electrochemically inactive between 0 and 1000 mV.

All the cyclic voltammetry samples were prepared using a buffer solution of 100 mM Tris and 4 mM NaCl (as supporting electrolyte) at pH 7.4 (adjusted with 1 M NaOH) and measurements were performed in triplicate.

Calibration of the voltammetric analyzer was performed using a 3 mM solution of $FeSO_4$ in 1 M HCl, using platinum as a working electrode and Ag/AgCl in 3 M NaCl as reference. The $E°$ determined in this cell, in a cv experiment, was 493 mV with respect to Ag/AgCl reference. Since Ag/AgCl reference (in 3 M NaCl) is at 209 mV with respect to NHE, the potential of the $FeSO_4$ in 1 M HCl is 702 mV vs NHE. The literature potential of Fe^{2+}/Fe^{3+} in 1 M HCl is 700 mV. The experimental uncertainty for the potential measurements is ±2.0 mV. Cp_2MoCl_2(aq) solutions were prepared at a 5.00×10^{-4} M concentration (in 100 mM Tris/4 mM NaCl, pH 7.4) for the oligonucleotide interaction study. Before analyzing the interaction between molybdenocene complexes and the oligo, we must considered the dilution factor. For this 3 mL of the molybdenocene complex dissolved in Tris buffer was added to the electrochemical cell and four aliquots of 250 μL of the buffer solution were added stepwise to the cell (to a total of 1000 μL) giving a final concentration of 3.75×10^{-4} M of the metallocene (the dilution factor is also consider for the Metal–Oligo interaction samples). The titration (interaction) between Cp_2MoCl_2(aq) and the oligo was made by adding four aliquots of 250 μL of CGCATATATGCG to 3 mL of the molybdenocene complex in the cell giving a final concentration of 3.75×10^{-5} M on molybdenocene complex and approximate 6.1×10^{-5} M on the oligo. The potentials of the molybdenocene complex solutions containing 250 μL aliquots of the oligo and buffer (oligo free) are compared to determine the % of interaction, equation given below, in this manner correcting for dilution factors.

$$\%\,\text{interaction} = \frac{E(\text{Cp}_2\text{MoCl}_2 + \text{oligo}) - E(\text{Cp}_2\text{MoCl}_2 + \text{buffer}) \times 100}{E(\text{Cp}_2\text{MoCl}_2 + \text{buffer})} \qquad (1)$$

CGCATATATGCG solution was prepared by dissolving the solid in a buffer solution of 100 mM Tris and 4 mM NaCl at pH 7.4 (adjusted with 1 M NaOH) at 5 °C, leading to a concentration of 2.43×10^{-4} M.

The cv analyses were performed using the following parameters: the initial potential was 0 mV and the maximum potential was 1000 mV, the sweeping speed was of 100 mV/s and, each sample was purged with nitrogen gas ultrapure (UHP 99.999%) for 120 s to remove oxygen from the solution.

4.4. Mass spectrometry analysis

Mass spectral data were obtained on a Bruker Daltonics Esquire 6000. The Cp_2MoCl_2 complex was dissolved in 25 mM Tris buffer solution at pH of 7.4 and added methanol and 0.2% of formic acid for mass spectral analysis. The following conditions were used: electrospray ionization (ion source type), in a direct infusion mode and using ion-trap method to analyze in the positive ion mode. The molecular ion spectrum of *m/z* 348 $[M+H]^+$ was observed for the Cp_2Mo-Tris in buffer/methanol solution. MS/MS experiment was performed on the isolated ionic species (molecular ion peak, *m/z* 348) resulting in the product ions *m/z*: 230(8), 229(100), 228(43), 227(75), 226(24). Mass spectrum of $[Cp_2Mo\text{-Tris}+H]^+$, *m/z* (%): 350(44), 348(100), 347(46), 346(73), 345(63), 344(32), 342(57). The Molecular Weight Calculator Program available on http://jjorg-chem.unc.edu/personal/monroe was used to determine the theoretical isotopic distribution pattern for the $[M+H]^+$ *m/z* peaks, which are consistent with our results.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.08.015.

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