Interactions of the Antitumor Agent Molybdocene Dichloride with Oligonucleotides

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The interactions between the antitumor-active metallocene molybdocene dichloride (Cp_2MoCl_2) and four oligonucleotides have been studied by ^1H and ^{31}P NMR spectroscopy. In 50 mM salt solutions of molybdocene dichloride, hydrolysis of the halide ligands occurs to give a solution with pD 2, containing a species in which both Cp rings remain metal bound for 24 h. At pD 7, however, partial hydrolysis of the Cp rings (∼30%) occurs after 24 h. Addition of an aqueous solution of molybdocene dichloride in 50 mM salt to the self-complementary sequence $d(CGCATATGCG)_2$, maintaining the pD at 6.0−7.0, showed no evidence for the formation of a metallocene−oligonucleotide complex, and only peaks arising from hydrolysis of molybdocene dichloride were detected. A similar result was obtained in titration experiments with the single-stranded sequence d(ATGGTA) at pD 6.5−7.0. However, at pD 3.0, new signals assigned to a molybdocene−oligonucleotide complex or complexes were detected in the ^1H NMR spectrum. No change was observed in the ^{31}P NMR spectrum. The complex or complexes formed between molybdocene dichloride and d(ATGGTA) are stable for hours at pD 3.0; at higher pD, the complex is destabilized and only peaks arising from hydrolysis of molybdocene dichloride are detected. Titration experiments at low pD with the dinucleotide dCpG showed a new set of signals in the ^1H NMR spectrum, tentatively assigned to formation of a complex arising due to coordination of molybdenum to guanine N7 and/or cytosine N3. At pD 7.0, these signals disappeared. The results obtained show that stable oligonucleotide adducts are not formed in 50 mM salt at pD 7.0, and hence it is highly unlikely that formation of molybdocene−DNA adducts in vivo is the primary action that is responsible for the antitumor properties of molybdocene dichloride.

Introduction

The metallocene dihalides Cp_2MX_2 (M = Ti, Mo, Nb, V; X = F, Cl, Br, I, NCS, N_3) are a class of small hydrophobic organometallic complexes that exhibit antitumor activity against a wide range of murine and human tumors.[1] Titanocene dichloride has recently entered clinical trials, and initial results suggest a lack of experimental cross-reactivity with cisplatin and patterns of antitumor activity different from those produced by the platinum anticancer drugs.[2] Formation of metallocene−DNA complexes is believed to be a key process related to the antitumor properties of the metallocenes, as both Cp_2TiCl_2 and Cp_2VCl_2 inhibit DNA and RNA synthesis[3] and titanium and vanadium accumulate in nucleic acid-rich regions of tumor cells.[4] However, in contrast to the well-characterized platinum-based anticancer drugs,[5] the active species responsible for antitumor activity in vivo has not been identified and the mechanism whereby irreparable DNA damage and/or structural modification occurs is poorly understood.

Studies of the hydrolysis and coordination chemistry of several metallocenes,[6−10] as well as their interaction with calf thymus DNA,[11,12] strongly suggest that each of the metallocenes operates via an independent mechanism of action. Full characterization of metallocene−DNA adducts has been hampered by their hydrolytic instability in water at physiological pH values,[6] and the basis for rational modification of the metallocene framework or the development of related complexes as potential antineoplastic agents is therefore lacking. This study reports the first detailed analysis of the interaction of molybdocene dichloride with oligonucleotides. Molybdocene dichloride was the focus of this study as this is the only metallocene in which the two cyclopentadienyl rings remain metal bound in water at pH 7.0.[8] This property has allowed characterization of both solution and solid-state adducts with a range of nucleic acid constituents,[8,9] confirming that formation of molybdocene−nucleic acid adducts in vivo is feasible. The results obtained in this study however show that, in contrast to the conclusion

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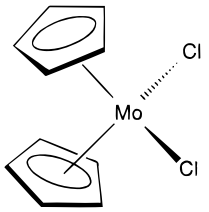
reached from studies with mononucleotides,[8,9] stable oligonucleotide adducts are not formed in the presence of salt at pD 7.0, and hence it is highly unlikely that formation of molybdocene−DNA adducts in vivo is responsible for the antitumor properties of molybdocene dichloride.



Cp_2MoCl_2

d(CGCATATGCG)$_2$	**1**
dATGGTA	**2**
dApT	**3**
dCpG	**4**

Experimental Section

Molybdocene dichloride was obtained from Aldrich Chemical Co. Dinucleotides **3** and **4** were purchased from Sigma Aldrich Chemical Co. Oligonucleotides **1** and **2** were supplied as the triethylammonium salts by AUSPEP Pty Ltd, Melbourne, Australia. Samples were converted to the sodium salts using standard precipitation procedures,[13] and concentrations were determined by UV spectroscopy. Binding studies were carried out in D_2O with TSP (0.00 ppm) or dioxane (3.73 ppm) used as a chemical shift reference. pD values were measured using a Beckman Φ11 meter and a Mettler NMR tube pH probe and are related to the pH meter reading by the formula pD = pH(meter reading) + 0.4.[14] NMR experiments with **1** and **2** were carried out on a Bruker AMX600 spectrometer while those with **3** and **4** were carried out on a Bruker AC200 spectrometer. All experiments were carried out at 25 °C. Sonication of samples was carried out using an Elma Transsonic Digital Ultrasound water bath.

Hydrolysis Experiments. The general procedure involved dissolving 0.01−0.02 mmol of molybdocene dichloride in 500−600 μL of D_2O by sonication for 3 h, with heat, to give a deep maroon solution; complete dissolution was generally not achieved. Samples were handled in a manner identical to that of our earlier studies,[9,12] i.e., without rigorous exclusion of oxygen, because we wished to mimic the conditions used for biological testing[1−4] and because our earlier study[9] showed that the presence of oxygen did not significantly alter the types and amounts of complexes formed with nucleotides compared with studies[8] in which oxygen was rigorously excluded. The pD was adjusted to the required value with DCl (0.2−1.0 M) and NaOD (0.2−1.0 M), by addition of not more than 5 μL aliquots of either base or acid solution. Addition of 5 μL of NaOD (0.25 M) raised the pD by 3 units. For hydrolysis experiments carried out at pD 7.0, the pD dropped slowly with time and was readjusted to maintain the pD as required; a solution with initial pD 7.0−7.4 was at pD 5.5−6.0 after 24 h. Measured hydrolysis times were estimated once the pD was adjusted. Measured pD values are ±0.3 due to fluctuations in sample pD which occurred over 30 min. ^{1}H NMR spectra were recorded at time intervals with any developing precipitate ignored. The rate of Cp hydrolysis was estimated by integration of one of the two multiplets arising from free cyclopentadiene C_5H_5D (6.5 and 6.6 ppm at pD = 5.8) versus the metal-bound C_5H_5 signals taking into account the deuterium scrambling that occurs under these conditions.[6,8]

Oligonucleotide Binding Studies. NMR samples were prepared in deuterated 50 mM NaCl (pD 7.0−8.0). Molybdocene dichloride (6.0 mg) was suspended in 50 mM NaCl solution in D_2O (600 μL) and dissolved by vigorous sonication with heat for 3 h to give a saturated deep mauve solution (pD 1.0−1.5). Any precipitate was ignored, and hence mole equivalents added refer to the theoretical amount of molybdocene dichloride, assuming all the solid had dissolved. For double-stranded oligonucleotide **1**, which has a melting temperature of 45 °C, aliquots of the metallocene solution were added to the oligonucleotide (pD 7−8) as required such that the pD remained above 5.5, to maintain the duplex structure. The pD of the resultant solution was monitored and adjusted to 6.5−7.4 (0.1 M NaOD) after each addition. Typically, for single-stranded oligonucleotide **2** and the dinucleotides, the pD of the solution dropped to ∼3.0 upon addition of 1.0 equiv of molybdocene dichloride; the solution pD was then adjusted back to pD ∼7.0 using NaOD (0.25 M, ∼40 μL). Fluctuations in sample pD occurred with variations of ±0.3 pH unit occurring over 30 min and decreased by 1−2 pD units over 24 h. Any precipitate that formed during the titration experiments was ignored.

Results

As the aim of this work was to determine the relative stability and structures of adducts formed between molybdocene dichloride and DNA in vivo, titration experiments were carried out in aqueous solutions at approximately physiological pH. In general, samples were prepared in 50 mM salt and the pD of the solutions was maintained in the range 6.0−7.5. Phosphate buffer, which is generally used for the study of drug−oligonucleotide complexes, was not used as blank experiments showed that Cp_2MoCl_2 binds significantly to this buffer.

Hydrolysis of molybdocene dichloride was initially carried out in order to characterize the predominant metallocene species present in 50 mM salt at pD 7.0. Three different oligonucleotide NMR titration experiments were carried out. The self-complementary 10-base pair sequence d(CGCATATGCG)$_2$ **1**, was used as a model of double-stranded DNA. These results were compared with the non-self-complementary sequence dATGGTA, **2**, which was used as a model for single-stranded DNA. Titration experiments with the dinucleotides dAT, **3**, and dCG, **4**, were also carried out in order to assist in the assignment of resonances obtained in spectra with the oligonucleotides **1** and **2**. To preclude the possible formation of complexes involving sterically accessible terminal phosphate groups which are not present in chromosomal DNA, oligonucleotides **1**−**4** lacked terminal phosphate groups.

Hydrolysis Chemistry of Molybdocene Dichloride. The hydrolysis of the cyclopentadienyl (Cp) and chloride ligands in molybdocene dichloride in water has been well-characterized by Kuo et al.[8] Rapid hydrolysis of the two chloride ligands occurs to give an acidic solution (pH 2.0); the predominant species is believed to be the charged complex $Cp_2Mo(OH)(OH_2)^+$.[8] Negligible hydrolysis of the Cp rings occurs in the range pH 2.0−7.0. However, the different experimental conditions used in the NMR studies with oligonucleotides required the rate of Cp hydrolysis in molybdocene dichloride at different pH values in 50 mM NaCl to be determined and to provide reference spectra for comparison with titration spectra obtained with **1**−**4**.

^{1}H NMR spectra of freshly prepared samples of Cp_2MoCl_2 in 50 mM NaCl at pD 2.0 showed the presence of two sharp Cp signals (Figure 1a). The spectrum remained unchanged after 24 h, consistent with the presence of two molybdocene species in which the Cp rings are metal bound. No significant Cp hydrolysis occurred as evidenced by the absence of multiplets corresponding to cyclopentadiene or dicyclopentadiene or the appearance of any other signals in the ^{1}H NMR spectrum.

In contrast, at higher pD values in 50 mM NaCl, molybdocene dichloride undergoes some Cp hydrolysis. The pD levels of freshly prepared samples (pD 1.0−1.5) were raised to either pD 7−7.5 or pD 9−9.5, and NMR spectra were recorded over a 24 h period. The relative intensities of the signals in these spectra varied with each sample, but a typical example is shown in Figure 1. At pD ∼7, in addition to two sharp signals around 6 ppm (Figure 1a), assigned to metal-bound Cp rings, signals assigned as arising from cyclopentadiene (∼6.6 ppm) and minor

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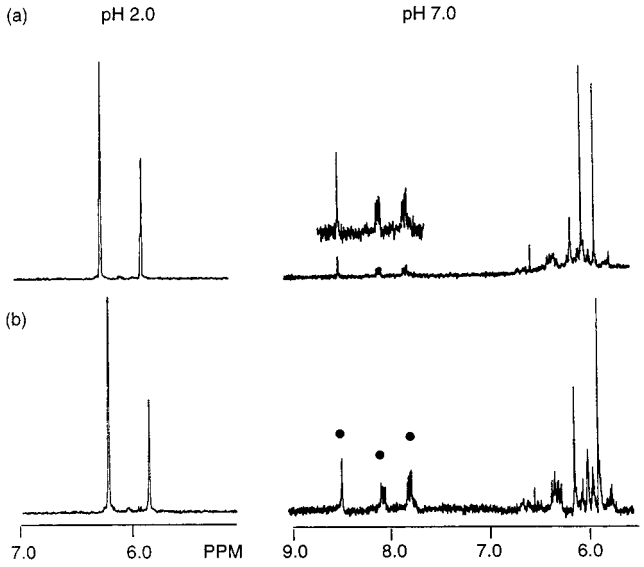

Figure 1. 200 MHz ^{1}H NMR spectra of Cp_2MoCl_2 dissolved in 50 mM D_2O at pD 2.0 and pD 7.0 at times (a) 10 min and (b) 24 h after dissolution.

peaks arising from the corresponding dimer (2.5−3.5 ppm) were observed. In addition, an unusual set of resonances, not previously observed in related hydrolysis experiments, appeared around 8 and 8.5 ppm. After 24 h (Figure 1b), the relative intensities of the metal-bound Cp signals changed to give two major singlets (5.9 and 6.1 ppm) with the singlet at 6.0 ppm reduced in intensity compared to that in the initial spectrum. In addition, the relative amounts of the downfield signals increased with time. By integration of all signals (Figure 1b), it was estimated that ∼70% of the species in solution contained metal-bound Cp rings. Increasing the pD to ∼9.0−9.5 gave results very similar to those observed at pD 7.

At high pD in 50 mM NaCl, the exact nature of the species present is difficult to determine as the chloride ion concentration is expected to have a significant influence on the initial rates of hydrolysis of the two chloride ligands. The sharp singlets at ∼6.0 ppm most probably arise from increased amounts of species containing a bound chloride ligand, e.g., $Cp_2Mo(D_2O)Cl^+$ and $Cp_2Mo(OD)Cl$. The signals at 8.0−8.5 ppm have not been assigned, but the sharp downfield singlet (8.5 ppm) is consistent with a metal-bound Cp ring and most probably arises from an intermediate hydrolysis product in which only one Cp ring is bound to the metal center. Similar signals have not been observed in any of the previous studies of molybdocene dichloride (or other metallocenes) with nucleic acid constituents,[8,9,10] but these experiments were all studied at lower pH values and in the absence of salt. As all samples were handled in air, it is possible that these peaks arise due to oxidation of molydocene dichloride at higher pH. While the oxidation chemistry of Cp_2MoCl_2 has not been fully characterized, a number of Cp_2Mo−molybdate species have been reported[15] which could arise from oxidation and/or hydrolysis of Cp_2MoCl_2. The precipitate that formed could also arise from oxidation in addition to hydrolysis of the halide and Cp rings of the metallocene. Due to the uncertainty in defining the halide or pseudohalide ligands bound at different pD values and the presence of minor unidentified hydrolysis and/or oxidation products at pD 7.0 (including possible deuterium scrambling in products), the notation Cp_2MoCl_2(aq) is used in this article to refer to an aqueous solution of molybdocene dichloride in 50 mM salt.

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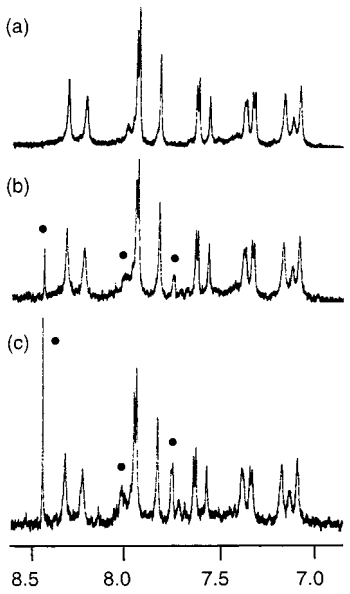

Figure 2. 600 MHz ^{1}H NMR spectra (50 mM NaCl, D_2O, pD 6.5−7.0) of d(CGCATATGCG)$_2$, **1**, on titration of Cp_2MoCl_2(aq): (a) 0.0 equiv; (b) 1.0 equiv; (c) 2.0 equiv; • = peaks assigned to hydrolysis product.

Oligonucleotide Titrations. Titration experiments were carried out by adding 0.5, 1.0, and 2.0 mol equiv of a salt solution of Cp_2MoCl_2(aq) to each of **1**−**4**.[13] The pD was adjusted to ∼7 after each addition, and one-dimensional ^{1}H and ^{31}P NMR spectra were recorded. As complete dissolution of molybdocene dichloride did not generally occur (see Experimental Section), the quoted equivalents refer to the theoretical equivalents present in a completely homogeneous solution.

(a) Duplex DNA. Figure 2 shows the spectra obtained on titration of 1.0 and 2.0 equiv of Cp_2MoCl_2(aq) into the duplex 10-mer **1**. The spectra showed the appearance of several new sharp resonances (indicated by •). The relative intensities of these resonances, which correspond to the peaks observed in the hydrolysis experiments of Cp_2MoCl_2 under identical experimental conditions (Figure 1), increased on addition of each 0.5 equiv of Cp_2MoCl_2(aq).

Two sets of symmetrical pairs of multiplets centered at 6.0 ppm were also detected. These signals are characteristic of deuteriocyclopentadiene and were assigned to cyclopentadiene and dicyclopentadiene, arising from some hydrolysis of the cyclopentadienyl rings and assigned previously in related experiments.[8,9] Two sharp singlets at 5.8 and 6.5 ppm were assigned to the Cp protons of the uncomplexed molybdocene Cp_2MoCl_2(aq). ^{31}P NMR spectra recorded after each addition, showed no evidence of formation of a phosphate-*O*-centered complex. In particular, a ^{31}P signal approximately 35−40 ppm downfield of the backbone phosphorus resonances, which is characteristic of Mo phosphate-*O* coordination,[8] was not observed. Addition of further equivalents of Cp_2MoCl_2(aq) increased the amount of Cp hydrolysis and resulted in formation of dark precipitate. Attempts to characterize this precipitate, which had very poor solubility, failed using NMR and mass spectrometry.

(b) Single-Stranded DNA. Figure 3 shows the spectra obtained on titration of 1.0 and 2.0 equiv of Cp_2MoCl_2(aq) with the 6-mer single-stranded oligonucleotide **2**, under conditions

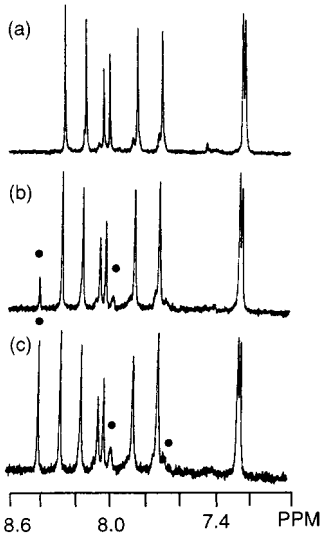

Figure 3. 600 MHz ^{1}H NMR spectra (50 mM NaCl, D_2O, pD 6.8−7.2) of d(ATGGTA), **2**, on titration of Cp_2MoCl_2(aq): (a) 0.0 equiv; (b) 1.0 equiv; (c) 2.0 equiv; • = peaks assigned to hydrolysis product.

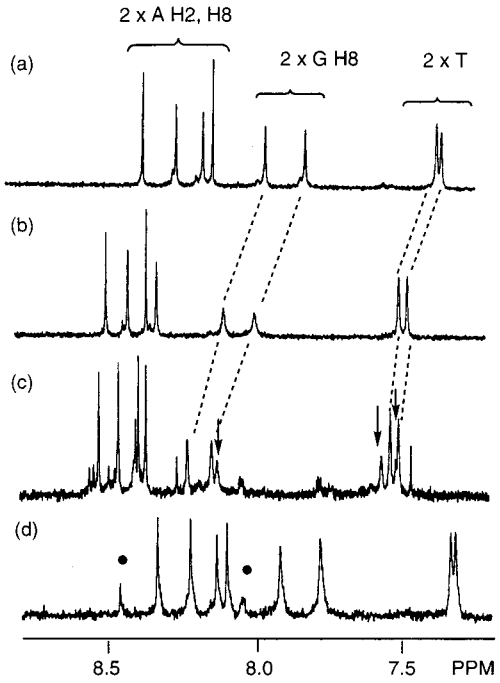

Figure 4. 600 MHz ^{1}H NMR spectra (50 mM NaCl, D_2O) of d(ATGGTA), **2**, at (a) pD 7.1 and (b) pD 3.0 and following the addition of Cp_2MoCl_2(aq): (c) 1.0 equiv, pD 3.0; (d) 2.0 equiv, pD 7.2; • = peak assigned to hydrolysis product; arrows indicate signals assigned to a metallocene−oligonucleotide complex.

identical to those used in the titration experiment carried out with the duplex **1**. The spectra obtained with **2** (Figure 3) showed trends similar to those observed for **1**, notably the appearance of a downfield singlet at 8.5 ppm. Signals due to cylopentadiene were also observed (6.4−6.6, 2.8−3.1 ppm). As in the case of **1**, there was no change in the ^{31}P NMR spectra, indicating no interaction with the phosphate backbone.

To assess the effect of pD on the interaction of Cp_2MoCl_2 with oligonucleotides, a second experiment was carried out in which the solution pD was not adjusted to pD 7.0 after each titration (Figure 4). Addition of 1.0 equiv of Cp_2MoCl_2(aq) to a solution of **2** resulted in a solution of pD 3.0. For comparison, a reference spectrum of **2** was also recorded at the same pD values by addition of a solution of DCl.

Parts a and b of Figure 4 show the 600 MHz ^{1}H NMR spectra of **2** at pD 7.0 and pD 3.0, respectively. In addition to some changes in chemical shift that occur on lowering the solution pD, some broadening of the two guanine H8 protons was observed. Upon addition of 1.0 equiv of Cp_2MoCl_2, several new resonances were observed (Figure 4c). The relative intensities of these new peaks increased upon addition of a second equivalent of Cp_2MoCl_2(aq) (data not shown). These new resonances were tentatively assigned as arising from an oligonucleotide−metallocene complex. In particular, the new thymine and guanine signals (indicated by arrows, Figure 4c) were assigned on the basis of their chemical shifts compared to the reference spectrum (Figure 4b) and studies with mononucleotides which showed similar small changes in chemical shift of the nucleic base protons on coordination of the metallocene. The new resonances in the adenine region of the spectrum are also consistent with formation of a complex. The possibility that all the new resonances arise from some other hydrolysis/oxidation pathway is highly unlikely, as new signals at these chemical shifts were not detected in reference spectra lacking the oligonucleotide or in titrations with the dinucleotides at low pH (see below). When the solution pD was raised to 7.0, the new peaks disappeared and only signals due to the oligonucleotide **2** and hydrolysis/oxidation products were observed (Figure 4d).

(c) Dinucleotides. To assist in the peak assignments made with oligonucleotide **2**, a similar set of titration experiments were carried out with the dinucleotides dCpG, **3**, and dApT, **4**. Titration of molybdocene dichloride into either dApT or dCpG and maintaining the pD at ∼7.0 showed no evidence for formation of any complexes by either ^{31}P or ^{1}H NMR spectroscopy, and the only new signals were assigned to hydrolysis products. However, in the case of dCpG at low pD values, new signals were observed consistent with the formation of a metallocene−dinucleotide complex (Figure 5). In particular, a new downfield doublet, assigned to H5 of cytosine in the complex, appeared and persisted over 24 h. This complex was only stable at low pD values, as increasing the pD to 7.0 resulted in disappearance of these new signals and formation of minor amounts (<5%) of products arising from hydrolysis (• in Figure 5c; compare Figure 1) and some cyclopentadiene. Lowering the pH again resulted in reappearance of the signals assigned to the complex and confirmed the labile and pH-dependent nature of the molybdocene−dCpG complex. In contrast, no new signals were observed in titration experiments formed with dApT under identical conditions.

Discussion

While the bulk of current evidence suggests that DNA is the principal cellular target of the metallocene dihalides, no molecular level picture of the nature of metallocene−DNA interactions has emerged. Chemical and coordination studies, however, strongly indicate that each of the metallocene dihalides acts via an independent, and probably unrelated, mechanism. From an experimental point of view, molybdocene dichloride offers the advantage of appreciable stability to Cp hydrolysis under physiological conditions and hence is the most promising candidate for characterizing the nature of molybdocene−DNA interactions at the molecular level using ^{1}H and ^{31}P NMR spectroscopy.

Analysis of the ^{1}H and ^{31}P NMR spectra obtained from titration of Cp_2MoCl_2(aq) into $d(CGCATATGCG)_2$, **1**, and d(ATGGTA), **2**, showed no evidence for the formation of metallocene−oligonucleotide adducts in 50 mM salt at pD 7.0

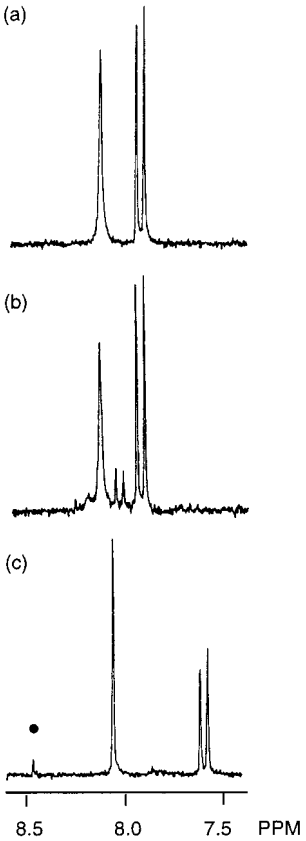

Figure 5. 200 MHz ^{1}H NMR spectra (50 mM NaCl, D_2O) of dCpG on titration of Cp_2MoCl_2(aq): (a) 0.0 equiv, pD 3.0; (b) 1.0 equiv, pD 3.0; (c) 1.0 equiv, pD 7.0; • = peak assigned to hydrolysis product.

(Figures 2 and 3). However, at low pD values, new signals were observed in the ^{1}H NMR spectra, consistent with the formation of a complex between Cp_2MoCl_2(aq) and **2**. Full characterization of this complex was not possible, but formation of molybdocene−phosphate-*O* adducts, which give a characteristic downfield shift in the ^{31}P NMR spectra,[8,9] was ruled out as no new signals were observed in ^{31}P NMR spectra. While binding at low pH is observed, coordination is less favored at high pD due to competition from the hydrolysis pathway that increases at higher pH (Figure 1). In addition, at higher pH a number of the nucleic base binding sites are no longer available, due to hydrogen bonding and steric effects.

The results obtained with **1**−**4** are quite different from those obtained with the mononucleotides dAMP, dGMP, dCMP, and dUMP.[8,9] In these systems, immediate complexation to the nucleobase-*N* and phosphate-*O* occurs in a nonlabile manner (Figure 6) that effects major conformational changes.[8] There are two major differences between **1**−**4** and nucleotides in previous studies. First, the oligonucleotides studied in this work lack terminal phosphate groups which stabilize and promote formation of the chelates shown in Figure 6. Indeed, recent preliminary studies of the interaction of Cp_2MoCl_2(aq) with the self-complementary 6-mer d(ApGpGpCpCpT) and the 5′-phosphorylated analogue d(pApGpGpCpCpT) in D_2O in the absence of salt were reported.[16] As expected, significant perturbations of resonances at the 5′-end of the phosphorylated analogue were detected, consistent with simultaneous Cp_2MoCl_2-(aq) coordination at the adenine N7 and phosphate-*O* of the 5′-terminal adenosine, but only minor changes were observed

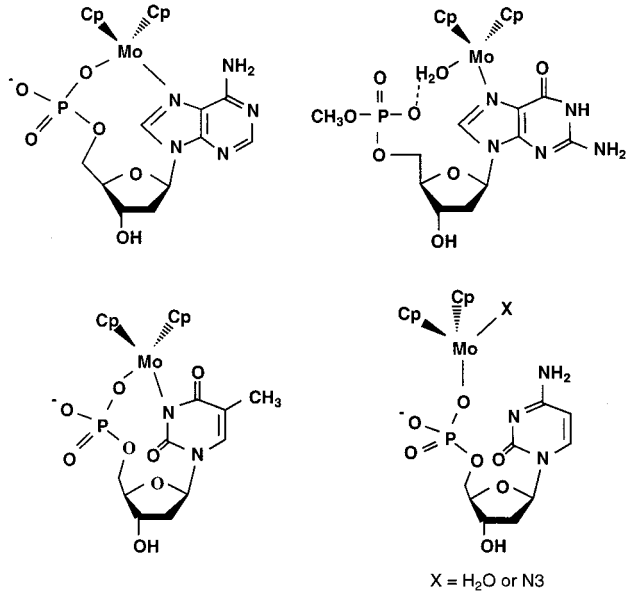

Figure 6. Complexes formed in water between Cp_2MoCl_2 and nucleotides (adapted from ref 8b).

in the nonphosphorylated sequences. It appears that phosphate binding is favored only when formation of a stabilized chelate with a neighboring heteroatom is possible. The presence of 50 mM salt, which stabilizes the duplex, also competes with the molybdocene for interaction with the negatively charged phosphate groups.

Second, different experimental conditions were used in this study; the pD of the solution was kept above 6.0 throughout the titrations, by careful addition of Cp_2MoCl_2(aq) and adjustment of the pD as required. This condition has a major effect on the formation of complexes, since at pD <4.0, a complex or complexes with **2** were formed which were stable in solution over 24 h.

At pD 7.0 in salt solution, the only new products formed between oligonucleotides and molybdocene dichloride were products previously assigned as arising from hydrolysis/oxidation of the Cp rings of Cp_2MoCl_2(aq) (• in Figure 1). While the exact species that gives rise to these new peaks was not assigned, the sharp downfield singlet (8.45 ppm) is consistent with a metal-bound Cp ring and most probably arises from an intermediate hydrolysis or oxidation product in which only one Cp ring is bound to the metal center. The relative amounts of the hydrolysis signals (• in Figures 1−5) and their rates of formation were variable in different experiments, but they were consistently detected. However, after 6−8 h, the predominant molybdocene species in each of the titration experiments contained metal-bound Cp rings which gave rise to sharp singlets at 6.5−7.0 ppm (spectra not shown).

Kuo et al.[17] recently reported that molybdocene dichloride promotes the phosphodiester bond cleavage of activated phosphodiesters and phosphomonoesters in aqueous solution. A similar reaction was considered with the oligonucleotides as a mechanism whereby DNA damage could occur. However, no new signals arising from fragmentation of the oligonucleotides with time were assigned. This is not unexpected, as the phosphates present in the oligonucleotides are unactivated to this type of hydrolysis.

The results reported here have important implications for the mechanism of antitumor action of molybdocene dichloride. In

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particular, while our results suggest that stable adducts may be formed at pH <4.0, the formation of stable metallocene−DNA adducts in vivo at pH >6.0 is unlikely. However, the possibility that blood constituents could stabilize complexes with proteins and/or DNA cannot be ruled out. Further work on the effect of molybdocene dichloride on DNA and RNA synthesis as well as polymerases and topoisomerase enzymes is required in order to clarify the mechanism of action of the drug. This interpretation is supported by recent preliminary results[16] that have identified protein kinase C and toposiomerase II as potential targets of molybdocene dichloride in vivo. This study also highlights the importance of contrasting results with nucleic acid constituents with larger fragments of DNA, where steric effects can be significant and where the same binding sites are not necessarily available.

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