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RESEARCH ARTICLE

Specific Interactions of Antitumor Metallocenes with Deoxydinucleoside Monophosphates

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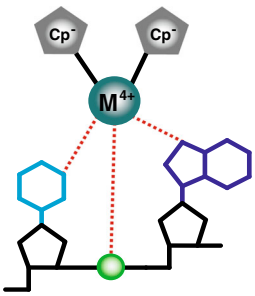

Abstract. Bent metallocenes Cp_2MCl_2 (M = Ti, V, Nb, Mo) are known to exhibit cytotoxic activity against a variety of cancer types. Though the mechanism of action is not fully understood yet, the accumulation of the metal ions in the nucleus points towards DNA as one of the primary targets. A set of eight deoxydinucleoside monophosphates was used to study the adduct yields with metallocenes and cisplatin. The binding affinities are reflected by the relative intensities of the adducts and were found to follow the order of Pt > V > Ti > Mo (no adducts were detected with Nb). High-resolution tandem mass spectrometry was applied to locate the binding patterns in the deoxydinucleoside monophosphates. Whereas cisplatin binds to the soft nitrogen atoms in the purine nucleobases, the metallocenes additionally interact with the hard phosphate oxygen, which is in good agreement with the hard and soft (Lewis) acids and bases (HSAB) concept. However, the binding specificities were found to be unique for each metallocene. The hard Lewis acids titanium and vanadium predominantly bind to the deprotonated phosphate oxygen, whereas molybdenum, an intermediate Lewis acid, preferentially interacts with the nucleobases. Nucleobases comprise alternative binding sites for titanium and vanadium, presumably oxygen atoms for the first and nitrogen atoms for the latter. In summary, the intrinsic binding behavior of the different metallodrugs is reflected by the gas-phase dissociation of the adducts. Consequently, MS/MS can provide insights into therapeutically relevant interactions between metallodrugs and their cellular targets.

Keywords: Anticancer, Metallodrugs, Metallocene, Nucleic acids, Tandem mass spectrometry, HSAB concept

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Introduction

Understanding of the interaction of antitumor metallodrugs with their biomolecular targets is of major importance for the development of novel chemotherapeutic agents. A majority of today's anticancer treatments is based on platinum compounds, such as *cis*-diamminedichloroplatinum (II) (DDP, cisplatin) and its derivatives. Cisplatin was found to be effective against various epithelial malignancies, such as lung, head, neck, ovarian, bladder, and testicular cancer [1]. However, due to severe side effects, evolving resistances of cancer cells, and the inefficiency against several types of tumors, much effort is put in the development of potent alternatives.

Clinical Studies

Bent metallocenes based on titanium, vanadium, niobium, and molybdenum were shown to exhibit cytotoxic activity against different cancer cell lines [2–5]. They comprise two negatively charged cyclopentadienyl (Cp^-) ligands and two labile chloro (Cl^-) ligands, of which the latter are readily replaced by two hydroxo ligands (OH^-) in aqueous environment, thus forming the active compound [6, 7]. Owing to their effectiveness against cisplatin resistant or sensitive cancer cell lines, bent metallocenes represent a promising class of therapeutic antitumor agents [8]. Though titanium-based drugs never made it beyond phase II clinical trials [9–13], the lack of any adverse effect on the proliferation of the bone marrow encouraged the development of improved derivatives exhibiting functionalized cyclopentadienyl ligands, which result in an improved cytotoxic activity. Examples thereof are titanocene Y [14, 15], metallocenes with salan [16, 17] or oximato ligands [18], and inclusion complexes with cucurbit[*n*]urils [19].

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Mechanism of Action

Among the transition metal-based drugs, the mechanism of action has been studied in most detail for cisplatin. It is commonly accepted that the cytotoxic activity of cisplatin is mainly based on the binding to two neighboring guanine nucleobases in the DNA double helix, thus distorting the helix and arresting replication. The electron-rich positions in purine nucleobases were identified as the preferred binding sites of cisplatin [20]. Electron energy loss spectroscopic and synchrotron-based X-ray fluorescence [21, 22] studies on metallocene dichlorides revealed a cellular distribution similar to cisplatin [23], suggesting DNA as a target. Titanium ions derived from titanocene were proposed to enter the cell with the help of the iron transporter transferrin and to be further transported into the nucleus with the assistance of ATP [24, 25]. However, the activity of the metallodrug is not only determined by the cell import but essentially depends on the binding to the target and the evoked biochemical response as well. Recent research showed that the ligands in the metal complex affect the antiproliferative activity [14–19]. Titanocene [8] and vanadocene [26] essentially interfere with proper cell division during several phases of the cell cycle, which finally initiates apoptosis. Though the mechanisms of action of metallocene-based drugs are supposed to be very diverse and not fully understood yet, the binding to nucleic acids is crucial for their antitumor activity.

Analysis of Adducts

To better understand the mode of action of metallocenes and to support the tailored design of functionalized derivatives, precise knowledge on their binding to nucleic acids is essential. Different analytical techniques, such as X-ray spectroscopy [7], ^{1}H and ^{31}P NMR [27–29], cyclic voltammetry [27], and inductively coupled plasma-atomic emission spectroscopy [30, 31], as well as DFT calculations [32] have been applied for this purpose. For titanocene and molybdenocene, a bidentate chelation mode to nucleotides was suggested, involving binding to the phosphate oxygen and nucleobase nitrogen atoms [7, 29, 32–34]. It is further assumed that the binding to the phosphate linker results in the phosphodiester bond cleavage, which significantly contributes to the anticancer properties of metallocenes [35, 36]. By contrast, vanadocene exhibits only outer-sphere coordination to the phosphate group [37], and no evidence was found for the interaction of niobocene with nucleosides or nucleotides [38].

MS of Metal Adducts

The diversity of possible metallodrug-DNA adducts often renders unambiguous pinpointing of the binding sites and the elucidation of the binding preferences extremely complex. With its ability to isolate and fragment selected precursor ions, tandem mass spectrometry constitutes an excellent tool that can fill this gap and provide detailed structural information about the adducts.

Past studies have shown that the binding of alkali and alkaline earth ions (Li^+, Na^+, K^+, Cs^+, Mg^{2+}, and Ca^{2+} [39]) and organometallic complexes to nucleic acids induces a pronounced alteration of the gas-phase dissociation, as the phosphate protons are replaced by metal ions. Studies on adducts with deoxydinucleoside monophosphates (DMs) [40] further revealed that alkali metal ions preferentially target the pyrimidine nucleobases, whereas alkaline earth and transition metal ions were found to coordinate to the phosphate groups in the central region of oligodeoxynucleotides [41, 42]. Moreover, ion/ion reaction experiments revealed the selective insertion of multivalent coordination centers into gaseous oligonucleotides by substitution of their ligands [43]. The adducts with further organometallic complexes, such as cisplatin, have been studied as well. The selectivity of cisplatin for purine nucleobases is reflected by previous tandem mass spectrometric investigations of dinucleoside monophosphates [44], unmodified and modified hexanucleotides [45], and double-stranded DNA [46, 47] in the negative ion mode that revealed a pronounced site-specific backbone cleavage of the cisplatin adducts. Furthermore, infrared multiphoton dissociation (IRMPD) and ultraviolet photodissociation (UVPD) are well-suited for pinpointing the platinum binding sites in oligonucleotides as these activation techniques result in an increased number of sequence-specific fragment ions [48].

Here, we report on the binding of several antitumor metallocene dichlorides to a set of deoxydinucleoside monophosphates. Positive electrospray ionization tandem mass spectrometry (ESI-MS/MS) was applied to elucidate the binding specificities of the metallocenes and the binding patterns in the adducts. The most prevalent fragment ions and the influence of the metallodrugs on the gas-phase dissociation of the DMs are discussed.

Experimental

Oligonucleotides, Chemicals, and Solvents

Deoxydinucleoside monophosphates [d(ApT), d(TpA), d(ApG), d(GpA), d(CpG), d(GpC), d(CpT), d(TpC)] were synthesized by TriLink (San Diego, CA, USA). All lyophilized nucleic acids were used without further purification and redissolved in HPLC grade water to a final concentration of 1 nmol/μL. All solvents and *cis*-diamminedichloroplatinum (II) were purchased from Sigma Aldrich (Buchs, Switzerland). The dissolved DMs were incubated with cisplatin solution (c = 1.6 mM) in a molar ratio of 1:1.6 (DM:cisplatin) for 24 h at 37 °C. All metallocene dichlorides [*bis*(cyclopentadienyl) M(IV) dichloride, with M = Ti, V, Nb, Mo] were purchased from Sigma Aldrich (Buchs, Switzerland) and used as provided. All solutions were prepared fresh by dissolving them in HPLC grade water followed by sonication for 30 min to yield a final concentration of 1 mM. The DMs were incubated in a 1:1 ratio with the corresponding metallocene dichloride solution for 24 h at 37 °C. For mass spectrometric analysis, all samples were diluted in 80% acetonitrile in water containing 0.5% formic acid to a final DM concentration in the range of 10–20 μM.

Electrospray Ionization Mass Spectrometry (ESI-MS)

All experiments were performed on a LTQ Orbitrap XL instrument (Thermo Fisher Scientific, Bremen, Germany) equipped with a nanoESI source. All samples were analyzed in the positive ion mode with a potential of +800 V to +1300 V applied to the nanospray needle. Mass spectra were acquired in the FTMS mode from *m/z* 150 to 2000 with the mass resolution set to 100,000. The source parameters were set as follows: capillary temperature 200 °C, capillary voltage 35 V, and tube lens voltage 100 V. Ion trap CID experiments were performed with the precursor ions selected within a window of ±2–3 *m/z* and relative collision energies in the range from 20% to 35%. The activation time was 30 ms and helium was used as collision gas. The Xcalibur Software Suite including Qualbrowser ver. 2.0.7 (Thermo Fisher Scientific) was used for data processing. Peak assignment was supported by the OMA&OPA software tool [46].

Results and Discussion

Gas-Phase Fragmentation of Deoxydinucleoside Monophosphates

Deoxydinucleoside monophosphates are the simplest model nucleic acids that bear sequence information and can reflect the influence of the nucleobase order on the adduct formation and gas-phase dissociation. For comprehensive comparison of unadducted and adducted target molecules, the fragmentation of four self-complementary [d(ApT), d(TpA), d(CpG), d(GpC)] and four homo-purine/pyrimidine [d(ApG), d(GpA), d(CpT), d(TpC)] DMs was investigated by nanoelectrospray ionization tandem mass spectrometry.

As depicted in Figure 1, the dissociation of singly protonated DMs follows two major fragmentation channels: the scission of the 3'-C–O bond resulting in the w_1^+ ion, and the loss of a nucleobase.

The dissociation of DMs is characterized by strong site-specificity attributable to the formation of hydrogen bonds between the 3'-nucleobase and the phosphate group, and to the bridging of the charging proton between the two nucleobases, resulting in preferred release of the 3'-nucleobase as an ion and the excision of the ribose without the loss of the adjunctive nucleobase from DMs with a pyrimidine base in 3'-position, respectively [40, 49].

Adduct Formation with Transition Metal Complexes

DMs were incubated with cisplatin and four bent metallocenes, comprising the transition metal ions titanium (Ti), vanadium (V), niobium (Nb), and molybdenum (Mo). Based on their ion radii and charge states, the metal centers can be classified as hard (Ti^{4+}, V^{4+}, Nb^{4+}), intermediate (Mo^{4+}), or soft (Pt^{2+}).

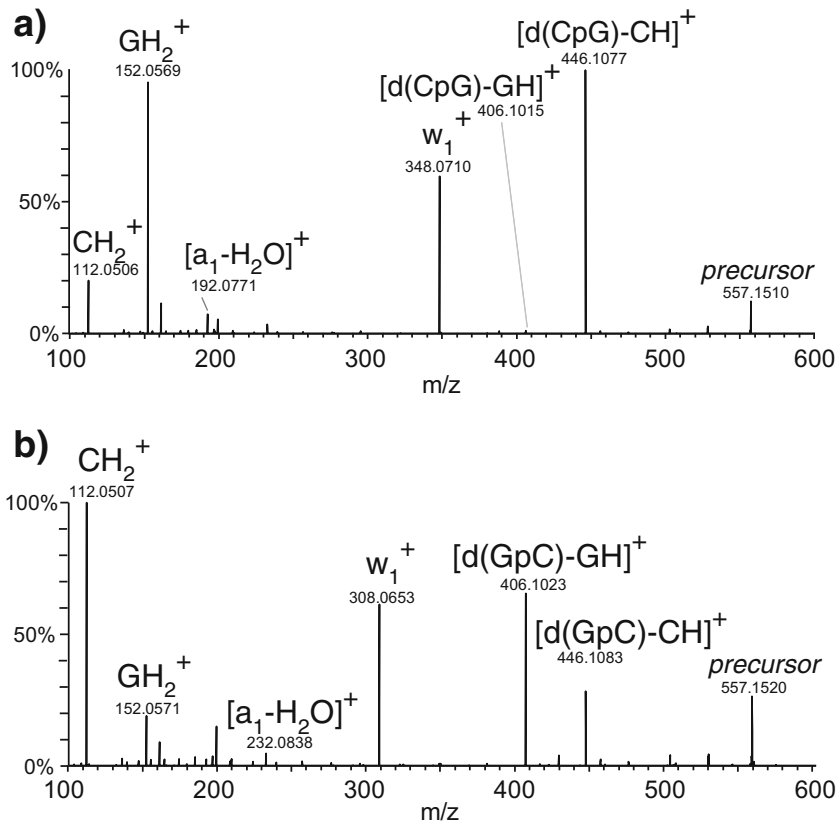


Figure 1. Product ion spectra of (**a**) d(CpG) and (**b**) d(GpC) demonstrate a sequence-dependent fragmentation pattern. Experimental and calculated *m/z* values are listed in Supplemental Table ST1

According to Pearson's hard and soft Lewis acids and bases (HSAB) concept different binding partners are expected, namely oxygen for hard and nitrogen for soft Lewis acids.

The transition metal complexes were probed for their efficiency to form adducts with the DMs and for their nucleobase selectivity (purine versus pyrimidine). The incubation of the DMs with cisplatin, titanocene, vanadocene, and molybdenocene resulted in the formation of adducts, whereas no interaction with niobocene was detected. In addition to two neutral NH_3 ligands, the active complex of cisplatin carries two negatively charged OH^- ligands that are released upon binding to the DMs. It is thus assumed that two coordinative bonds to electron-rich or deprotonated sites in the oligonucleotide are formed in solution. By contrast, the metal centers of the metallocenes feature four positive charges, two of which are compensated by the negatively charged Cp^- ligands. Titanocene and molybdenocene yielded adducts retaining both cyclopentadienyl ligands and can thus form two coordinative bonds to the target molecule, similar to cisplatin. On the other hand, adduct formation with vanadocene was found to be accompanied by ligand exchange. Vanadocene adducts experienced extensive loss of one Cp^- ligand in combination with the exchange of the second one for a hydroxo ligand ($\Delta m = -113.0749$ Da), resulting in a monohydroxovanadium adduct. These adducts were observed for all investigated DMs with a maximum deviation of 2.0 ppm. This observation contradicts previous findings that the cyclopentadienyl–metal bond is hydrolytically more stable in vanadocene than in titanocene or molybdenocene [37]. The fact that vanadocene was detected with both Cp^- ligands in the absence of the DMs further implies that exchange of the Cp^- ligands does not occur prior to, but coincides with the binding to the DMs. Furthermore, the identification of the adducts was based on the characteristic isotopic patterns of the corresponding transition metal. The comparison of the experimental and simulated isotopic patterns of the d(TpC) adducts is shown in Supplemental Figure S1.

Competition experiments performed with each of the four DMs d(GpA), d(TpC), d(CpG), and d(ApT) incubated with an equimolar mixture of all adduct-forming metallodrugs resulted in the extensive suppression of the total ion signal. Therefore, the DMs were incubated with two different metallodrugs at a time. The preference for adduct formation with purine-containing DMs was found to generally follow the order Pt > V > Ti > Mo, whereas the homo-pyrimidine DMs revealed a slightly different order with V > Pt > Ti > Mo (see Supplemental Figures S2–S5). In agreement with the HSAB concept, platinum binds preferentially to the soft purine nucleobases, whereas vanadium rather targets the hard oxygen atoms in the phosphate group and the pyrimidine nucleobases.

Cisplatin Adducts

ESI-MS data on DMs in the positive ion mode give proof for the formation of intra- and intermolecular adducts and the preferred binding of cisplatin to the electron-rich positions in the purine nucleobases, though binding to pyrimidine bases was also observed. MS/MS experiments on the intramolecular adducts with d(ApG) and d(GpA) revealed the crosslinking of the two nucleobases and demonstrated that the nucleobase

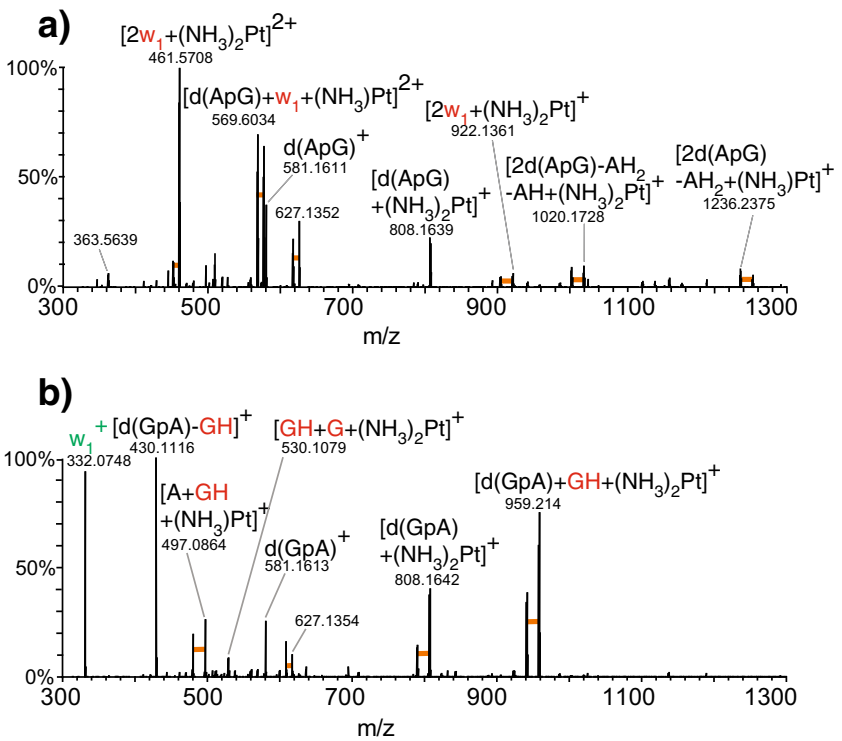


Figure 2. Product ion spectra of intermolecular adducts (**a**) [2 d(ApG) + $(NH_3)_2Pt]^{2+}$ and (**b**) [2 d(GpA) + $(NH_3)_2Pt]^{2+}$. A indicates a negatively charged and AH_2 a positively charged nucleobase, whereas AH corresponds to a neutral adenine. The orange bar indicates the loss of one ammine (NH_3) ligand. Experimental and calculated *m/z* values are listed in Supplemental Table ST2

order has no impact on the fragmentation (Supplemental Figure S6).

The dissociation of the intermolecular adducts [2 d(ApG) + $(NH_3)_2Pt]^{2+}$ and [2 d(GpA) + $(NH_3)_2Pt]^{2+}$ (Figure 2) yielded distinct fragment ions that reflect the preference of cisplatin in binding to guanine. This interaction is not disrupted after the cleavage of the 3'-C–O bond (e.g., [2 w_1 + $(NH_3)_2Pt]^{2+}$ from the 2 d(ApG) adduct and [d(GpA) + GH + $(NH_3)_2Pt]^{2+}$ from the 2 d(GpA) adduct). The high relative intensity of the unplatinated w_1^+ ion in the product ion spectrum of [2 d(GpA) + $(NH_3)_2Pt]^{2+}$ (Figure 2b, marked in green) reflects the influence of the nucleobase order on the dissociation.

Titanocene Adducts

Compared with the soft Lewis acid cisplatin, the yield of titanocene adducts was generally lower. ESI-MS data revealed that the transition metal complex retains both Cp^- ligands and binds to one deprotonated site in the DMs to yield a singly charged ion. Competition experiments with equimolar concentrations of the DM pairs d(ApG)/d(CpT) and d(GpA)/d(TpC) incubated with the hard Lewis acid titanocene resulted in identical adduct yields. The lack of discrimination between homopurine and homopyrimidine DMs and the low pKa of the phosphate group imply that the phosphate oxygen is involved in the adduct formation.

This binding behavior is also reflected by MS/MS data. Common to all investigated titanocene-DM adducts is the formation of the $[H_2PO_4 + Cp_2Ti]^+$ fragment ion (Figure 3, labeled in red), which was detected with mass deviations of less than 2.9 ppm in all MS/MS spectra. This fragment ion identifies the phosphate group as one of the preferred binding sites, irrespective of the nucleotide composition and order. This preference is further evidenced by the $[w_1 - BH - H_2O + Cp_2Ti]^+$ fragment ion (structure marked in green in Figure 3), formed upon cleavage of the 3'-C–O and the *N*-glycosidic bonds as well as concomitant loss of a water molecule. Similar to cisplatin, titanocene targets the nucleobases and thereby induces a partial positive charge on the nucleobase, which weakens the *N*-glycosidic bond and triggers the release of the nucleobase. The additional loss of water from the 3'-terminal hydroxyl group enables the formation of an aromatic furan ring system that presents an energetically favored product ion and resembles the DNA-typical [a-B]-ion. Although its interaction with the nucleobase is responsible for the prominent base loss, titanocene is not released together with the nucleobase, which points towards a stronger bond between the phosphate oxygen and titanocene.

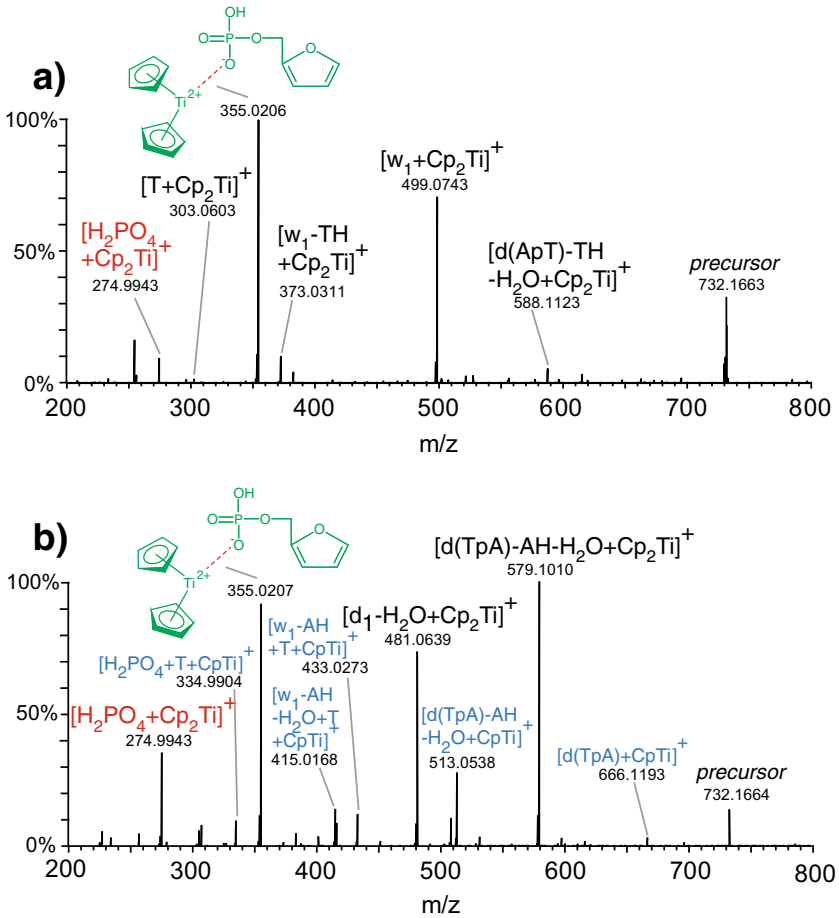


Figure 3. Product ion spectra of (**a**) d(ApT) and (**b**) d(TpA) adducted with titanocene reveal the significant influence of the sequence order on the dissociation. Experimental and calculated *m/z* values are listed in Supplemental Table ST3

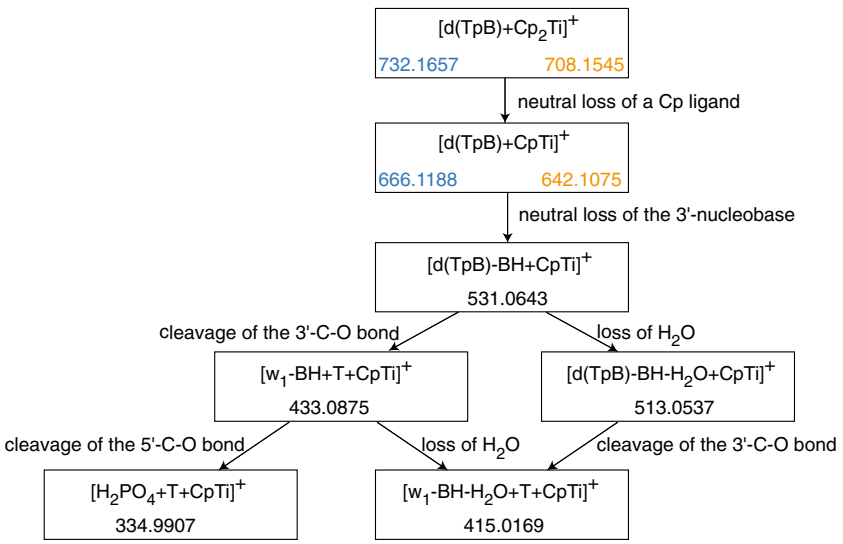
The interaction of titanocene with thymine gives rise to fragment ions derived from site-specific dissociation. The dissociation of adducts with thymine in the 3'-position [i.e., d(ApT) and d(CpT)] is largely identical and produces the abundant $[w_1 + Cp_2Ti]^+$ fragment ion (Figure 3a). On the other hand, the main backbone dissociation product of adducts with thymine in the 5'-position is the loss of the 3'-nucleobase. The subsequent cleavage of the 5'-C–O bond and the additional loss of a water moiety results in the abundant $[d_1 - H_2O + Cp_2Ti]^+$ ion, instead of $[w_1 + Cp_2Ti]^+$ (Figure 3b).

A separate series of singly charged fragment ions containing one cyclopentadienyl ligand only was observed for the adducts with a thymine in 5'-position. CID of the $[d(TpA) + Cp_2Ti]^+$ adduct resulted in the $[d(TpA) + CpTi]^+$ ion. The mass difference of 66.0471 Da and the retention of the charge state imply that ligand loss occurred in combination with the deprotonation of a nucleobase, which in turn led to a Coulombic interaction between the metal ion and the base. The appearance of the $[H_2PO_4 + T + CpTi]^+$ ion indicates preferred binding to thymine. The ion is the product of a dissociation cascade starting from $[d(TpA) + CpTi]^+$ and comprising the fragment ions $[d(TpA) - AH + CpTi]^+$, $[d(TpA) - AH - H_2O + CpTi]^+$, $[w_1 - AH + T + CpTi]^+$, and $[w_1 - AH - H_2O + T + CpTi]^+$ (Scheme 1). This fragment ion series demonstrates how the loss of a ligand in the gas phase alters the binding characteristics of the transition metal–DM complexes. Thus, distinguishing between fragment ions derived from precursors formed in solution and species generated by gas-phase processes is crucial for the elucidation of the binding patterns.

For adducts without 5'-thymine the loss of a cyclopentadienyl ligand was not observed. They dissociate mainly by the ejection of one nucleobase and the scission of the 3'-C–O bond. A signal attributed to the $[G + Cp_2Ti]^+$ ion observed in the product ion spectra of the d(ApG) and d(GpA) adducts gives evidence for the interaction of titanocene with the guanine nucleobase, but not with adenine. Because titanocene was found to only interact with the nucleobases thymine, cytosine, and guanine, which all comprise at least one oxygen atom, the binding sites of titanocene within the nucleobases are proposed to be O2/O4 (thymine), O2 (cytosine), and O6 (guanine). Though this finding is in contradiction to previous localization of the binding sites [7, 32, 34], it does agree with the HSAB concept. In conclusion, we found evidence that titanocene preferentially binds to the phosphate linker by substitution of the proton. Sequence-specific dissociation of the adducts indicates that titanocene must also interact with oxygen-containing nucleobases in the DMs.

Molybdenocene Adducts

The intermediate Lewis acid molybdenum combines the binding characteristics of hard and soft Lewis acids resulting in the interaction with both the nitrogen-containing nucleobases and the phosphate oxygen. Molybdenocene shows a strong tendency to bind to the electron-rich sites of the nucleobases, as evidenced by a signal for $[B + Cp_2Mo]^+$ (with B = negatively charged nucleobase), which represents the main dissociation product of molybdenocene adducts (Figure 4). Molybdenum targets purine over pyrimidine nucleobases and thus exhibits a nucleobase preference similar to cisplatin, but not to titanocene. The preference for nucleobase binding was determined as G > A ~ C >> T, which correlates with the order of the proton affinities of the nucleobases in the gas phase [50]. This preference is not affected by the nucleobase order. Evidence for the specific interaction with a nucleobase can arise from the comparison of the fragmentation patterns from precursors with reversed base order. In addition to the binding of molybdenocene to the nucleobases, signals indicating the interaction with the phosphate oxygen, analogous to titanocene (Figure 4, structure marked in green), were detected as well. The $[w_1 + Cp_2Mo]^+$ ion gives further proof for the simultaneous interaction with the deprotonated phosphate group and a lone pair of the 3'-nucleobase. In conclusion, CID



Scheme 1. Dissociation cascade of titanocene adducts with d(TpA) and d(TpC) after the loss of the cyclopentadienyl ligand. B indicates the second nucleobase, adenine or cytosine. The calculated *m/z* values are indicated in blue for d(TpA), in orange for d(TpC), and in black when identical for both DMs

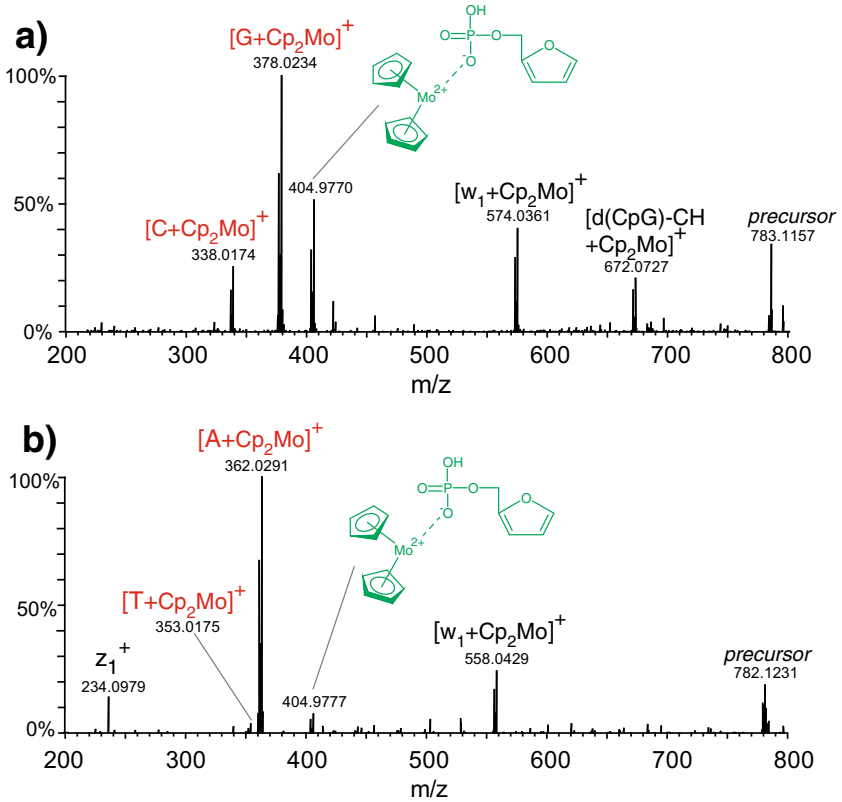


Figure 4. Product ion spectra of the molybdenocene adducts with (**a**) d(CpG) and (**b**) d(TpA). Experimental and calculated *m/z* values are listed in Supplemental Table ST4

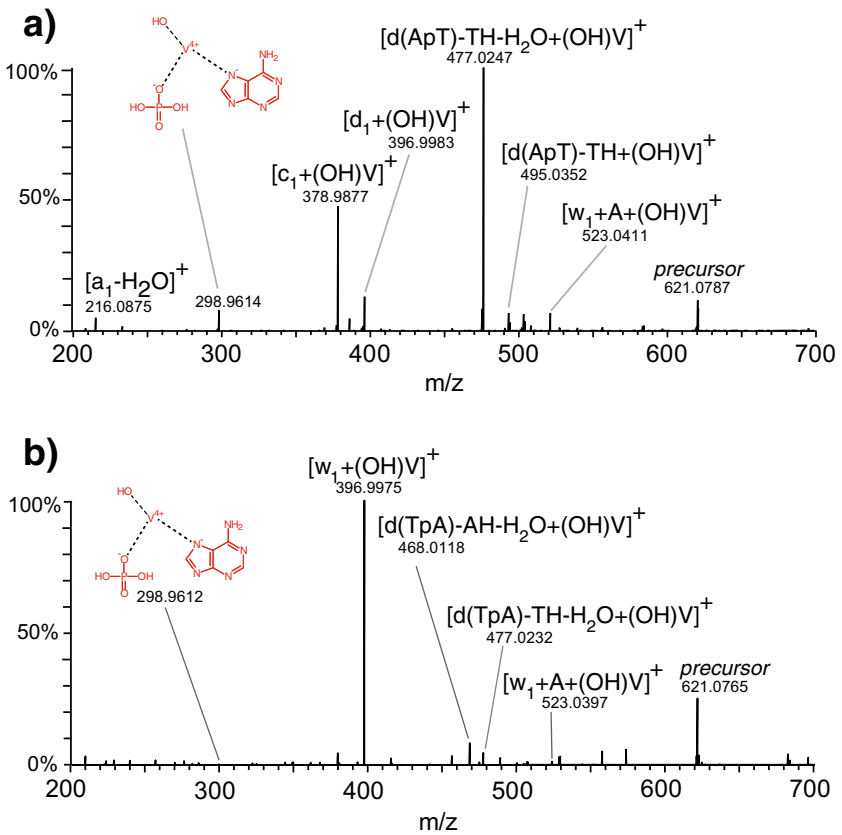


Figure 5. The dissociation of (**a**) [d(ApT) + (OH)V]$^+$ and (**b**) [d(TpA) + (OH)V]$^+$ produced specific fragment ions that demonstrate the preference of vanadium for A > T. Experimental and calculated *m/z* values are listed in Supplemental Table ST5

data identify the nucleobases and the phosphate group as preferred targets, thus reflecting the intermediate binding character of molybdenocene.

Vanadocene Adducts

In contrast to titanocene and molybdenocene, vanadocene was found to involve ligand exchange upon adduct formation in solution resulting in a monohydroxo complex. As vanadium carries four positive charges and retains one negatively charged hydroxo ligand, the singly charged precursors must exhibit two coordinative bonds between the metal and deprotonated sites in the nucleic acids. The affinity for the hydroxo ligand points towards preferred binding to oxygen. ESI-MS/MS experiments revealed that the phosphate group and one of the nucleobases, preferably a purine base, can serve as binding partners. Figure 5 shows the product ion spectra of the vanadium adducts with d(ApT) and d(TpA).The fragment ion $[A + H_2PO_4 + (OH)V]^+$ (Figure 5, structure marked in red) was detected in both product ion spectra, thus identifying the phosphate linker and adenine as targets (deviation less than 2.3 ppm), irrespective of the relative position of the nucleobases. This is further supported by the fragment ions $[c_1 + (OH)V]^+$ and $[w_1 + (OH)V]^+$ in d(ApT) and d(TpA), respectively. Additionally, the product ion spectrum of $[d(ApT) + (OH)V]^+$ (Figure 5a) revealed a remarkably high propensity for neutral thymine loss, which was not observed for the unadducted counterpart. It is, therefore, proposed that the vanadium complex also interacts with an electron-rich site of thymine, which destabilizes its N-glycosidic bond and triggers the release of the nucleobase. Due to the stronger interaction with the negatively charged phosphate oxygen and adenine, the release of thymine does not coincide with the loss of the monohydroxo–vanadium complex. In summary, the phosphate group represents the preferred binding site for the vanadium complex.

Conclusion

In this study, new insights into the binding of metallocenes to deoxydinucleoside monophosphates were gained. It is demonstrated that the Lewis acid character of the transition metal significantly determines the binding sites that are targeted in the nucleic acids. In contrast to cisplatin, which only interacts with the nucleobases, the metallocenes investigated in this study also target the phosphate oxygen. Titanocene was found to exclusively interact with oxygen as present in the phosphate linker and the nucleobases. Molybdenocene, which constitutes an intermediate Lewis acid, combines the binding properties of cisplatin and titanocene and targets the nitrogen atoms of the nucleobases as well as the phosphate oxygen. Vanadocene underwent extensive substitution of both Cp^- ligands by one hydroxo ligand (OH^-) upon binding to the DMs. Monohydroxovanadium interacts with the deprotonated phosphate group and one of the nucleobases in the DMs. In the absence of a second phosphate group, hard Lewis acid metallodrugs can bind to a nucleobase as second binding partner. Therefore, DMs constitute a useful model system to determine the nucleobase preference for the different metallocenes.

In general, adduct formation was found to be strongly influenced by the ligand composition and net charge of the activated metallocene complexes. Tandem mass spectrometric data demonstrated that the Pearson concept rationalizes the binding of the transition metal complexes to the nucleic acids. Presented results underline that MS/MS is well suited for the elucidation of the unique binding patterns of metal-based drug candidates.

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