Evidence for Binding of Dirhodium Bis-Acetate Units to Adjacent GG and AA Sites on Single-Stranded DNA

John M. Asara, Jennifer S. Hess, Elizabeth Lozada, Kim R. Dunbar,*,† and John Allison*

Contribution from the Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, and The Cancer Center, Michigan State University, East Lansing, Michigan 48824

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Abstract: Dirhodium tetraacetate is an antitumor active compound that is known to inhibit cellular DNA synthesis, but relatively little is known about its interactions with nucleic acids. A combination of matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) and enzymatic digestion experiments has established that the dirhodium bis-acetate unit forms an adduct with AA and GG sites in short DNA strands. The MALDI MS detection of the dirhodium/DNA adduct was aided by the addition of spermine, but no special sample treatment was required to obtain high quality mass spectra of the corresponding cisplatin-modified DNA strands.

Introduction

The compound cis-diamminedichloroplatinum(II), also known as cisplatin or *cis*-DDP, is a highly successful drug for the treatment of a variety of deadly tumors including bladder, cervical, ovarian, and testicular cancers. While the mechanism of activity of cisplatin is not entirely understood, there is consensus in the community that DNA binding to *cis*-DDP is critical to its antitumor activity.[1] Alteration of the DNA structure by coordination to the drug, therefore, is one possible scenario for its cytotoxic effects. Detailed footprinting studies of *cis*-DDP bound to DNA reveal a preference for sequences containing two or more adjacent guanosine nucleosides. These studies imply that 1,2 and 1,3 *intra*strand adducts of the general formulas $(NH_3)_2Pt\{d(XpX)\}$ or $(NH_3)_2Pt\{d(GpNpG)\}$, where d(XpX) is either GpG or ApG (A = adenosine, G = guanosine, and N = any nucleoside), account for ∼90% of all cisplatin/DNA binding modes, while monofunctional adducts account for the remaining 10% of bound platinum. Since the estimated number of bound molecules of *cis*-DDP is several orders of magnitude lower than the value expected for all of the potentially strong d(GpG) binding sites in the human cell, it is likely that the drug is acting on a subset of more highly susceptible GpG or ApG sites in vivo.[2]

Structural information describing *cis*-$Pt(NH_3)_2Cl_2$ adducts with oligonucleotide duplex sequences is crucial to understanding the antitumor behavior of this important drug. Recently, Lippard et al. reported an important result in this regard, namely the crystal structure of the cisplatin dodecamer duplex d(CCTCTGGTCTCC)·d(GGAGACCAGAGG), which was solved at a resolution of 2.6 Å.[1m] The Pt(II) center forms cis interactions with the N7 positions of the guanines, which themselves are almost perpendicular. This interrupts the base-stacking and base-pairing patterns of the DNA helix, which is in accord with the observation that platinated duplexes exhibit lower melting points (T_m) than unplatinated duplexes. The overall structure of the duplex is a surprising combination of A and B type helices. Apart from this general distortion, a major feature of this Pt/DNA adduct is the significant bend of the duplex toward the major groove at the site of the GPtG cross-link. This bend and the widened minor groove are reminiscent of the structural elements found in high-mobility group (HMG) domain proteins and transcription binding protein (TBP)/DNA complexes.[3]

In our laboratories, we are focusing on a class of antitumor-active transition metal compounds, the structures of which are often referred to as "lantern-type" arrangements. These are dinuclear metal−metal bonded compounds of Re, Ru, and Rh that contain at least two bridging carboxylate ligands (Figure 1).[4] The dirhodium compounds $Rh_2(O_2CR)_4L_2$ (R = Me, Et, Pr; L = solvent) are the most well-investigated members of

* To whom correspondence should be addressed. E-mail: allison@cem.msu.edu; dunbar@mail.chem.tamu.edu.

† The Cancer Center. Present address Department of Chemistry, Texas A&M University, College Station, TX 77842-3012.

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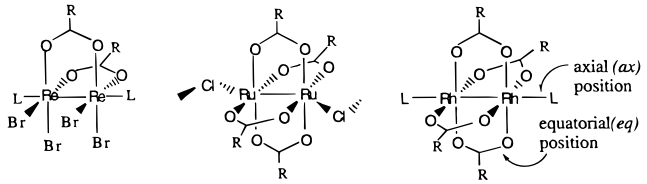

Figure 1. Dinuclear antitumor-active metal complexes with two possible binding sites.

this series. Numerous reports emerged in the 1970s that supported the carcinostatic activity of these dirhodium compounds against Erlich ascites and leukemia L1210 tumors in vivo.[5−7] Like cisplatin, dirhodium tetraacetate was found to be a potent inhibitor of cellular DNA synthesis with little effect on RNA or protein synthesis.[5,7,8] In terms of DNA binding, several researchers reported facile reactions with adenine but no guanine binding was observed. These reactivity differences were rationalized on the assumption that the purine coordination modes would be limited to the axial site, a situation that permits favorable hydrogen bonding contacts between carboxylate O atoms and the NH_2 group of adenine, but which introduces steric repulsions with the ketone O6 of guanine.[9] Findings from our laboratories have unequivocally established that adenine and also guanine bases form thermodynamically stable substitution products with $Rh_2(OAc)_4$ that involve an unprecedented equatorial bridging interaction for the purines.[10] Contrary to conventional wisdom, the substitution pathway involves displacement of equatorial carboxylate ligands rather than axial solvent molecules. Guanine and adenine (as well as guanosine and adenosine) adducts of $Rh_2(OAc)_4$ have been characterized in solution by NMR spectroscopy and by X-ray crystallography, and a variety of dimetal compounds containing bridging and chelating 9-ethylguanine and 9-ethyladenine are now in hand.[10] Taken together with our recent studies of amino acid reactions of dirhodium tetraacetate, these results offer new insights into the possible role of key cellular "ligands" in the metabolism of dirhodium antitumor complexes.[11] The detection of "$Rh_2(OAc)_2$" units bound to duplex DNA with{GpG} sequences by NMR spectroscopy[12] and the X-ray structures of various M_2 complexes containing bridging 9-ethylguanine and adenine as well as chelating adenines have revealed new potential binding modes of antitumor complexes with DNA.

This paper presents matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) data that support the conclusion that the dirhodium bis-acetate unit binds to small oligonucleotides containing a single GG or AA binding site. The combination of enzymatic digestion[13] experiments with MALDI analysis of the products provides confirmation of the binding site. This valuable information has not been easily available through NMR experiments and X-ray crystallographic studies to date, and is not available from mass spectrometric methods if conventional approaches and matrices are used. The results presented here suggest that the dirhodium/DNA complexes are less robust than the platinum adducts but that, under appropriate experimental conditions, they survive the transition to the gas phase. Most importantly, it has been found that the dinuclear metal core with two coordinated acetate ligands remain intact.

Experimental Section

Materials. $Rh_2(O_2CCH_3)_4(H_2O)_2$ and $Pt(NH_3)_2(OH)_2$ were obtained from Pressure Chemical Co. (Pittsburgh, PA) and used without further purification. Sodium acetate, potassium chloride, and TRIS buffer (tris-[hydroxymethyl]aminomethane) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Acetonitrile (HPLC grade) was obtained from VWR Scientific (Battavia, IL) and the water was purified using a MilliQ purification system. $[Rh_2(O_2CCH_3)_2(CH_3CN)_6][BF_4]_2$ was synthesized according to literature procedures.[14]The DNA 12-mer oligonucleotide of sequence 5′-CCTCTGGTCTCC-3′ (-GG- strand), 5′-CCTTCAACTCTC-3′ (-AA-strand), and the 11-mer 5′-TCTCTAATCTC-3′ were purchased from the Keck Oligonucleotide Synthesis Facility at Yale University. For mass spectrometric analysis, 3-hydroxypicolinic acid (3-HPA), anthranilic acid, and nicotinic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Diammonium citrate was purchased from J. T. Baker (Phillipsburg, NJ) and spermine was obtained from Fluka (Milwaukee, WI).

(a) Resins. DEAE Cellulose resin was purchased from Sigma Chemical Co. (St. Louis, MO). Source Q 15 anion-exchange resin (HPLC) and Sephadex G-25 were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Chelex-100 chelating ion-exchange resin was obtained from BIO-RAD (Hercules, CA).

(b) HPLC. Dirhodium-modified DNA strands were purified via anion exchange chromatography with a self-packed source Q 15 column (1.6 cm × 10 cm) on a Perkin-Elmer HPLC instrument equipped with a LC 235 diode array detector. DNA products were concentrated to dryness using a Centrivap concentrator (LABCONCO, Kansas City, MO) at 75 °C.

Procedures. (a) Synthesis of $Rh_2(O_2CCH_3)_2$·(TCTCTAATCTC). A 2 μmol aliquot of the 11-mer (TCTCTAATCTC) was dissolved in 400 μL of degassed, doubly distilled H_2O. To this was added 65 μL of a 0.04 M degassed solution of $[Rh_2(O_2CCH_3)_2(CH_3CN)_6][BF_4]_2$ (1:1.3 ratio). The solution, which immediately turned orange, was maintained at 37 °C for 72 h. At the end of the reaction time, the solution was a reddish-purple color.

(b) HPLC Purification. The reacted 11-mer was purified by anion exchange HPLC using an acetate buffer system monitored at 265 and 365 nm simultaneously. Eluent A: 0.2 M NaO_2CCH_3, 20% CH_3CN. Eluant B: 0.2 M NaO_2CCH_3, 1.2 M KCl, 20% CH_3CN. The product was purified by the following solvent program: 5 min gradient 0−28% B, 30 min gradient 28−33% B, 5 min gradient 33−100% B, 5 min

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isocratic 100% B, 5 min gradient 100−0% B, for a total protocol time of 50 min at a flow rate of 4.0 mL/min.

(c) Concentration and Desalting. Diethylaminoethyl (DEAE) cellulose resin was used to concentrate the sample. The HPLC fraction containing the product was diluted four times with 10 mM TRIS at pH 7.0. This dilute solution was then loaded onto a 10 cm × 1.5 cm (i.d.) column. The metalated 11-mer product was eluted from the column with 1 mL aliquots of a 10 mM TRIS, pH 7.0, 1.0 M NaCl solution. The fractions were screened for absorbance at 265 nm. Desalting of the concentrated sample was performed on a G-25 Size Exclusion Sephadex column (25 cm × 2.0 cm) (VWR Scientific, Battavia, IL). The concentrated sample was loaded onto the column and eluted with doubly distilled H_2O, and 1 mL fractions were collected and screened at 265 nm. The sample was then concentrated to dryness in a Centrivap prior to mass spectrometric analyses. Overall yield for the reddish colored 11-mer product was 65%.

(d) Synthesis of $Pt(NH_3)_2(OH)_2$·(CCTCTGGTCTCC). The metalated DNA oligomer was synthesized by reacting the purified 12-mer (CCTCTGGTCTCC) with $Pt(NH_3)_2(OH)_2$ in an oligo:Pt ratio of 1:1.3, at 37 °C. The metalated strand was purified by ion-exchange HPLC with a 72% A/28% B to 68% A/32% B 40 min gradient. In DNA purification HPLC experiments, buffer A is 200 mM NaCl and 10 mM NaOH, and buffer B is 1 M NaCl and 10 mM NaOH. The isolated product was desalted following the same procedures as described above and dried prior to mass spectrometric analyses.

(e) Synthesis of $Rh_2(O_2CCH_3)_2$·(CCTTCAACTCTC) and $Rh_2(O_2CCH_3)_2$·(CCTCTGGTCTCC). The metalated DNA oligomer was synthesized by reacting with $[Rh_2(O_2CCH_3)_2(CH_3CN)_6][BF_4]_2$ with the purified 12-mer strands CCTCTGGTCTCC or CCTTCAACTCTC in an oligo:Rh_2 ratio of 1:1.3 at 37 °C. Once the purple solution of the compound was added to the DNA sample, the color immediately changed to a bright orange hue. Small aliquots of the reaction mixture were periodically analyzed by HPLC to monitor the progress and extent of the reaction by simultaneous detection of the DNA and $[Rh_2(O_2CCH_3)_2(CH_3CN)_6]^{2+}$ chromophores at 260 and 365 nm, respectively. The best yields for these reactions, obtained after 10 days, are 40% and 60% of Rh_2−GG and Rh_2−AA adducts, respectively. The metalated strands were purified by ion-exchange HPLC with a 72% A/28% B to 68% A/32% B 40 min gradient to give a light green solution. The isolated product was desalted following procedures described above, and concentrated to dryness for mass spectrometric analyses.

(f) Digestion of $Pt(NH_3)_2(OH)_2$·(CCTCTGGTCTCC). A 200 pmol sample of 5′-CCTCTGG{$Pt(NH_3)_2(OH)_2^{2+}$}TCTCC-3′ was digested with 2×10^{-3} units of snake venom phosphodiesterase (VPD) (Sigma Chemical Co., St. Louis, MO) in 110 mM TRIS buffer (pH 9.4) dissolved in water. The digestion, which takes place from the 3′ end, was performed at 37 °C for 15 min.

(g) Digestion of $Rh_2(O_2CCH_3)_2$·(CCTCTGGTCTCC). A 100 pmol sample of 5′-CCTCTGG{$Rh_2(O_2CCH_3)_2^{2+}$}TCTCC-3′ was digested with a mixture of $\sim 2 \times 10^{-3}$ units of VPD in 110 mM TRIS buffer (pH 9.4) at 37 °C for 15 min.

(h) Digestion of $Rh_2(O_2CCH_3)_2$·(TCTCTAATCTC). A 60 pmol sample of 5′-TCTCTAA{$Rh_2(O_2CCH_3)_2^{2+}$}TCTC-3′ was digested with a mixture of $\sim 2 \times 10^{-3}$ units of VPD in 110 mM TRIS buffer (pH 9.4) at 37 °C for 15 min. Another digestion of 60 pmol of the same complex was performed using a mixture of 2×10^{-3} units of VPD and 3×10^{-3} units of calf spleen phosphodiesterase (SPD) (Boehringer Mannheim, Indianapolis, IN) in 110 mM Tris buffer (pH 9.4) at 37 °C for 15 min.

Mass Spectrometry of Dirhodium−DNA Adducts. Samples were prepared for mass spectrometric experiments as follows: for the cisplatin−DNA adduct, the digestion of cisplatin−DNA adduct, and one experiment with a Rh_2−DNA adduct, the matrix solution that was used is saturated 3-hydroxypicolinic acid (3-HPA) in 1:1 acetonitrile/water containing 25 mM $(NH_4)_2HCit$. For experiments with Rh_2 adducts of 5′-TCTCTAATCTC-3′, 5′-CCTCTGGTCTCC-3′, and 5′-CCTTCAACTCTC-3′, and the digestion products of the Rh_2−DNA adducts, the matrix was saturated 80% anthranilic acid/20% nicotinic acid (hereafter referred to as 80/20) in 1:1 acetonitrile/water containing 12.5 mM spermine. Linear MALDI mass spectra were recorded on a PerSeptive Biosystems (Framingham, MA) Voyager Elite delayed extraction, time-of-flight reflectron mass spectrometer equipped with a nitrogen laser (337 nm, 3 ns pulse). For all samples, the accelerating voltage was 23 kV, the delay time was 50 ns, the grid voltage was set to 93.0% of the accelerating voltage, and the guide wire was set to 0.25% of the accelerating voltage. The mass spectrometer was calibrated using unmetalated 5′-CCTCTGGTCTCC-3′ as a mass standard, with a resulting mass accuracy of $<\pm 1$ Da for peaks below *m*/*z* 4000. Typically, 64−128 laser shots were averaged for each spectrum. The sample stage used was a gold sample plate. All spectra were collected in the negative ion mode.

Results and Discussion

If a heavy metal atom is bound to an oligonucleotide, its presence can be detected using mass spectrometry (MS). An illustration of the success of mass spectrometry in this area is the characterization of platinum−oligonucleotide complexes using desorption/ionization methods that generate gas phase ions for MS analysis directly from condensed phase targets. Using a combination of electrospray (ESI) MS and matrix-assisted laser desorption/ionization (MALDI) MS, coupled with enzymatic degradation, kinetic rate constants for platination of small oligonucleotide chains have been determined.[15] Such approaches work quite well since the enzymes used to degrade the DNA do not recognize the bound Pt, and therefore the digestion stops when it reaches the platinated site.[16] A number of excellent reviews are available that describe the techniques involved and the structures of Pt−DNA complexes.[1]

In the early 1990s, Costello and Lippard reported the characterization of Pt(II) oligonucleotide complexes by fast atom bombardment (FAB) MS techniques.[17] Later it was demonstrated that the MALDI MS spectrum of a single stranded DNA adduct of $Pt(NH_3)_2(OH)_2$ survives intact in the gas phase, with only the loss of the two hydroxyl groups being observed.[18] To obtain some experience in this area of application of mass spectrometry, and as a point of reference with which to compare data on dirhodium complexes, we performed a MALDI MS analysis of a 12-mer adduct of $Pt(NH_3)_2(OH)_2$. For these experiments, a typical matrix combination of 3-hydroxypicolinic acid and diammonium citrate was used, and the negative ion spectrum was obtained (Figure 2a). The intense, high mass peak observed at *m*/*z* 3770 represents the anionic form of the complex $[M + Pt(NH_3)_2 - 3H]^-$ where M is the neutral form of 5′-CCTCTGGTCTCC-3′. Note, the formation of this singly charged anionic species requires the loss of three protons from the neutral species; the resulting −3 species, coupled to a +2 metal, exhibits an overall −1 charge. This charged state can be generated and detected in the MALDI experiment. It is important to note that more than 97% of the oligonucleotide is observed in the platinated form, as there is only a very small peak representing the free strand, which appears in its anionic form as the $[M - H]^-$ ion at *m*/*z* 3546.

After establishing that cisplatin was bound to the oligonucleotide, the next step was to generate specific structural information. The binding site of cisplatin was determined by performing

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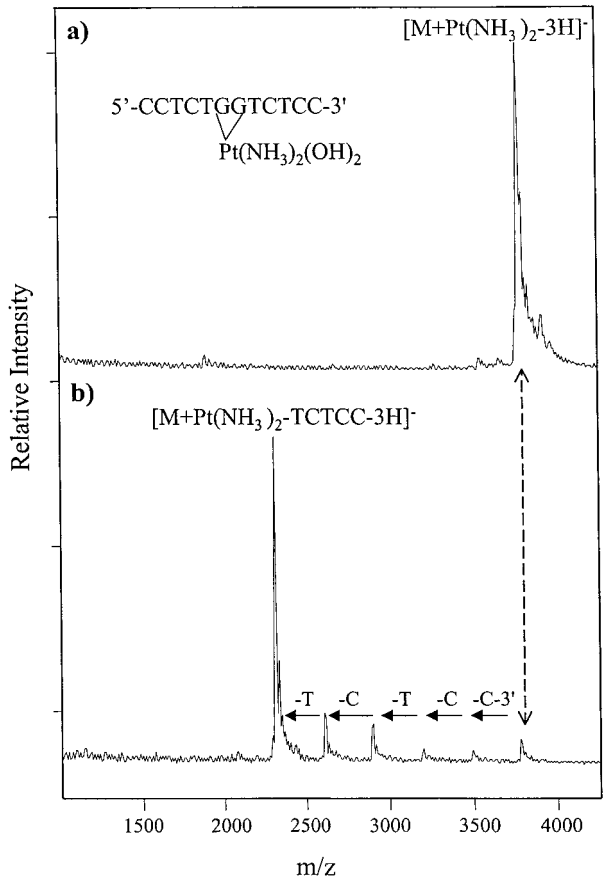

Figure 2. (a) MALDI mass spectrum of cisplatin bound to single-stranded DNA. (b) Digestion of the cisplatin-DNA complex using 3′ snake venom phosphodiesterase.

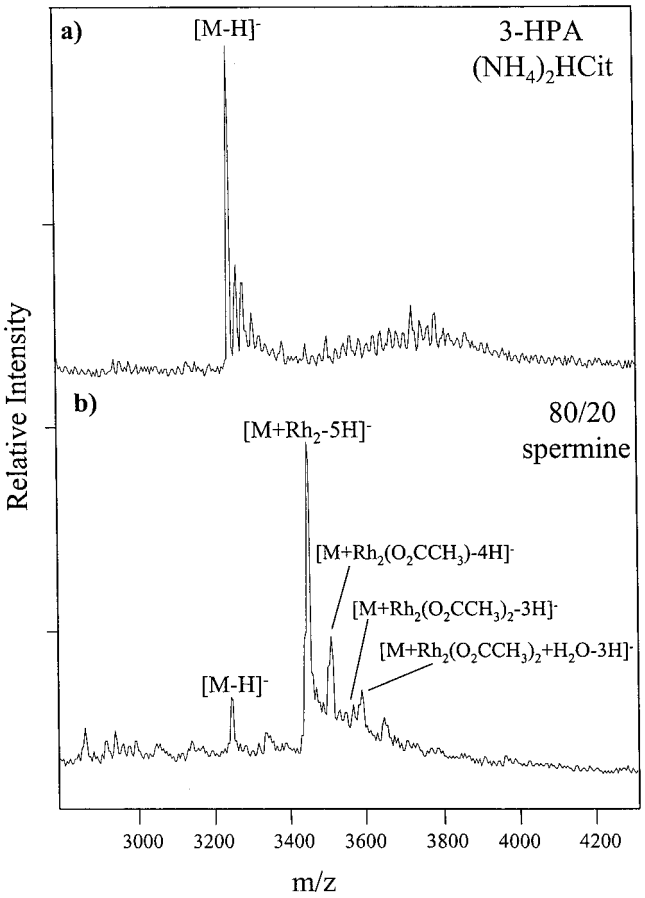

Figure 3. A portion of the (a) MALDI mass spectrum of $Rh_2(O_2CCH_3)_2$·(TCTCTAATCTC) using 3-hydroxypicolinic acid with diammonium citrate and (b) the MALDI mass spectrum of the same compound using 80% anthranilic acid/20% nicotinic acid with spermine.

an enzymatic digestion from the 3′ end of the metalated strand with the use of snake venom phosphodiesterase (VPD). When the digestion mixture was analyzed directly using MALDI MS in the negative ion mode, the spectrum shown in Figure 2b was obtained. As before, the highest *m*/*z* peak occurred at 3770, which is the intact metalated strand. In this case, however, the intensity is very low since most of the original complex was consumed in the digestion process. The spectrum contains a number of other low intensity peaks due to consecutive removal of mononucleotides, which occurs until the enzyme encounters the Pt unit on the DNA strand. At this point the enzyme is inhibited and the digestion stops. The peak at *m*/*z* 2294 represents the digestion product $[5'\text{-CCTCTGG}\{Pt(NH_3)_2^{2+}\}\text{-}3H]^-$, which indicates that cisplatin is bound to the GG portion of this DNA sequence.

Unfortunately the detection of dirhodium−DNA complexes by MALDI MS proved to be much less straightforward than the analysis of the corresponding platinum/DNA adducts. A systematic investigation of numerous matrix and additive combinations was undertaken in an attempt to detect an intact dirhodium/DNA adduct in the gas phase. Figure 3a depicts the negative ion MALDI mass spectrum of $Rh_2(O_2CCH_3)_2$· (TCTCTAATCTC) using the common matrix combination of 3-HPA with DAHC. The dirhodium−DNA interactions are being destroyed in the course of the MALDI experiment, as evidenced by our observation of a free DNA peak at *m*/*z* 3242 and a complete absence of peaks representing the intact complex. When the matrix was changed to 80% anthranilic acid/20% nicotinic acid in combination with spermine as the matrix additive, however, a peak at *m*/*z* 3443 was observed in the resulting spectrum (Figure 3b). This peak represents the naked Rh_2^{4+} core attached to the DNA strand, viz., $[M + Rh_2 - 5H]^-$. Apparently these conditions allow for the fundamental dimetal/oligonucleotide interactions to be retained. Of even greater importance is the observation of higher mass peaks at *m*/*z* 3502 and 3561 that represent the mono- and di-acetate forms, $[M + Rh_2(O_2CCH_3) - 4H]^-$ and $[M + Rh_2(O_2CCH_3)_2 - 3H]^-$, respectively. The matrix combination of 80/20 with spermine (a MALDI additive developed in our laboratories) stabilizes the dirhodium−DNA complex that had been synthesized. We have found that the low *m*/*z* peak in the series with only the Rh_2^{4+} unit attached is always the most intense peak, and the higher *m*/*z* acetate-containing components are less abundant. This cluster of peaks is the signature for the presence of the dirhodium moiety, and they can be easily observed in the spectra. As an additive for oligonucleotides, spermine has been shown to greatly reduce alkali cation adducts and enhance sensitivity.[19] Spermine, in combination with 80/20, gives a Na^+ and K^+ free spectrum of the intact metal−DNA complex. That is, with spermine present, negatively charged phosphates are bound to protons, rather than to alkali ions. A minor peak is observed at *m*/*z* 3242 which represents $[M - H]^-$, an indication that the dirhodium−DNA interactions are disrupted to a very small extent. Note that the $[M - H]^-$ peak does not possess a set of higher mass "satellite peaks" (as does the *m*/*z* 3443 peak), since the *m*/*z* 3242 peak does not contain dirhodium.

Experimental results such as those shown in Figure 3 lead to the conclusion that, for these studies, the 80/20 matrix with spermine will allow for dirhodium−DNA complexes to be

(19) Asara, J. M.; Allison, J. *Anal. Chem.* **1999**, *71*, 2866.

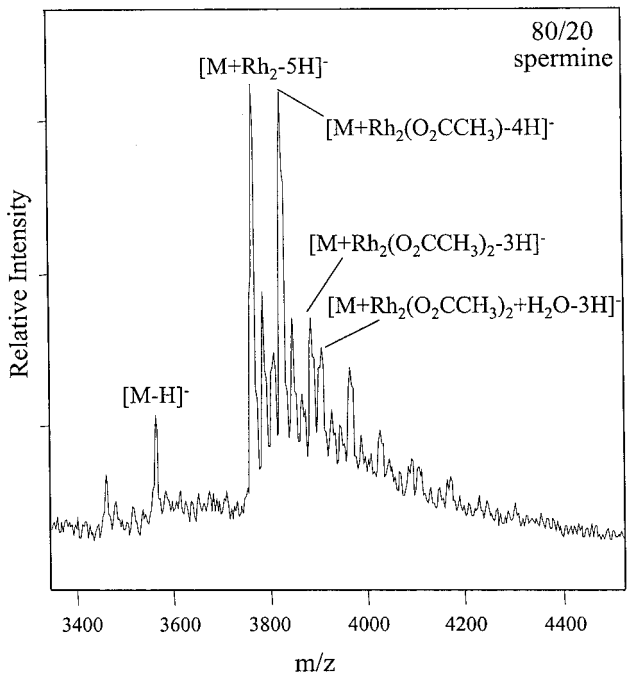

Figure 4. A portion of the MALDI mass spectrum of $Rh_2(O_2CCH_3)_2$·(CCTCTGGTCTCC) using 80% anthranilic acid/20% nicotinic acid with spermine.

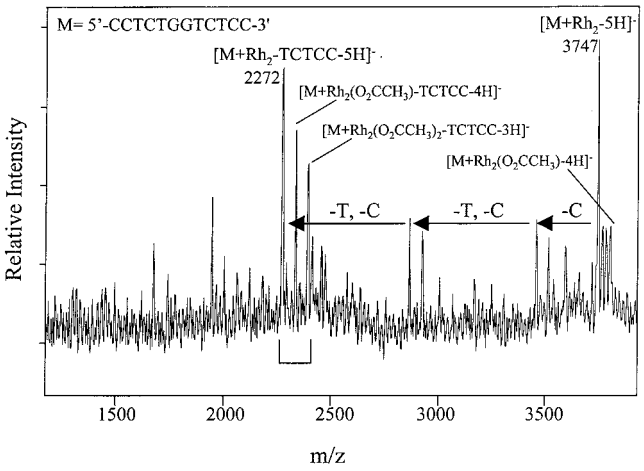

Figure 5. The MALDI mass spectrum of the digestion products of $Rh_2(O_2CCH_3)_2$·(CCTCTGGTCTCC) using 3′ snake venom phosphodiesterase.

detected using MALDI MS. When this matrix is used to analyze $Rh_2(O_2CCH_3)_2$(CCTCTGGTCTCC), the complex is successfully detected. The high *m*/*z* portion of the MALDI mass spectrum shown in Figure 4 shows the intact complex, and intense peaks representing acetate-containing forms as well. We have also detected the dirhodium complex of the same strand, with AA replacing the GG segment in the center, using the same matrix conditions (data not shown). While the results in Figure 4 support the conclusion that dirhodium binds to a GG-containing strand, it does not prove that GG is the binding site.

To locate the position of the dirhodium complex on the oligonucleotide strand, the sample was subjected to enzymatic digestion followed by MALDI MS analysis of the resulting mixture. The metalated -GG- strand (M = 5′-CCTCTG-GTCTCC-3′) was digested using snake venom phosphodiesterase, an exonuclease that digests from the 3′ end. The spectrum in Figure 5 shows the result of the digestion. The peak at *m*/*z* 3747 represents the intact Rh_2−DNA complex. The peak

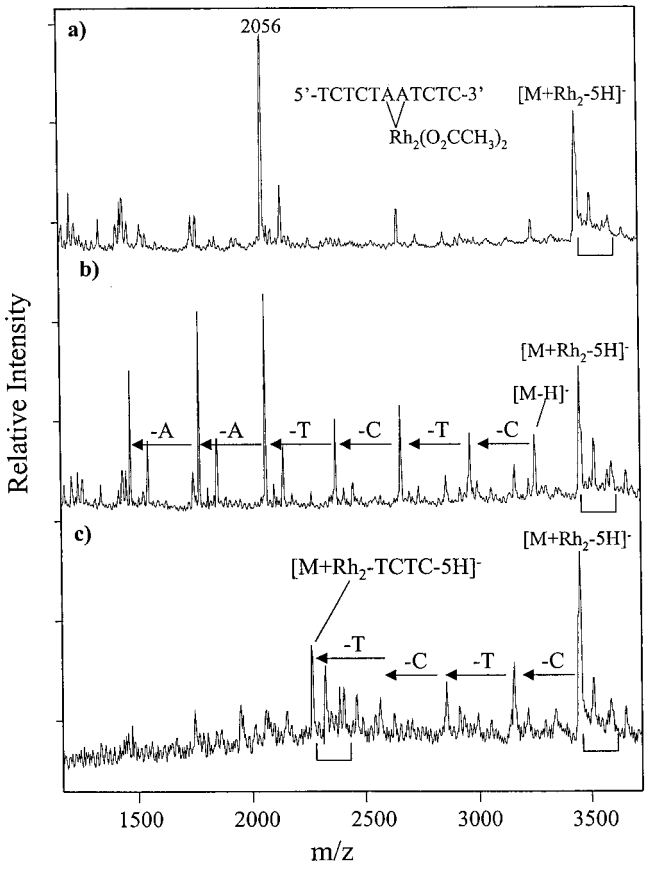

Figure 6. The MALDI mass spectrum of (a) $Rh_2(O_2CCH_3)_2$·(TCTCTAATCTC), (b) the digestion products of the same dirhodium−DNA complex using 3′ snake venom phosphodiesterase, and (c) the digestion of the dirhodium−DNA complex using the double enzyme digestion of 3′ snake venom phosphodiesterase and 5′ calf spleen phosphodiesterase (brackets identify the cluster of peaks indicative of dirhodium acetate bound to DNA).

at *m*/*z* 2272 represents $[M + Rh_2 - TCTCC - 5H]^-$; the digestion stops when the metal is encountered. Notice the signature peaks for the acetate ligands at *m*/*z* 2332 and 2392 which represent $[M + Rh_2(O_2CCH_3) - TCTCC - 4H]^-$ and $[M + Rh_2(O_2CCH_3)_2 - TCTCC - 3H]^-$, respectively. This is in contradiction to previous claims that dirhodium interactions with guanine bases do not occur.[5,7,8a−c] It is unusual that much of the $[M + Rh_2 - 5H]^-$ signal remains. It is most likely due to some inhibition of the enzyme in this particular system. One possible scenario is that the Rh_2−DNA complex exists in multiple forms of complexation with acetates in the solution. The $Rh_2(O_2CCH_3)_2$−DNA complex can be digested without metal interactions with the enzyme. However, the Rh_2−DNA complex may lead to metal−enzyme interactions that inhibit digestion. This may explain why, in Figure 5, the digestion product ion $[CCTCTGG - Rh_2 - 5H]^-$ shows the signature for the dirhodium satellite peaks due to one and two acetates, while the intact complex does not, suggesting that only the acetate-"protected" Rh_2−DNA complex is efficiently digested.

These experiments are good examples of cases where mass spectrometry alone cannot be used to provide structure determination. The MALDI instrument used here has Post-Source Decay (PSD) capabilities for analyzing fragment ions of a selected precursor ion, but for metal−oligonucleotide complexes, PSD cannot be successfully used. Signal intensities are frequently too low to perform PSD and the loss of metal is usually the most energetically favorable fragmentation process in the gas phase.

Figure 6a depicts the complete MALDI spectrum of the dirhodium−DNA complex, a portion of which was shown in Figure 3b. In addition to the peak representing the intact dirhodium−DNA complex, there are lower *m*/*z* peaks, notably a prominent peak at *m*/*z* 2056. These peaks may represent fragment ions, formed in the mass spectrometry experiment, or may represent fragments of the oligonucleotide, formed in the solution during the MALDI target formation process. The *m*/*z* 2056 peak represents the $[M - H]^-$ ion for TCTCTAA, a fragment of the larger analyte. For this experiment, it is important to define the spectrum, Figure 6a, before digestion, so the new peaks formed in the digestion products can be clearly identified. Figure 6a sets this baseline. Figure 6a is shown as a reference for the spectrum in Figure 6b that represents the mixture obtained after digestion. The enzyme used was VPD, which digests from the 3′ end. The results of the digestion show a significant loss of the metal complex, which evidently occurs when the complex interacts with VPD, since the major series of digestion products are from the free DNA. The $[M - H]^-$ peak representing the free strand is observed at *m*/*z* 3242. There are minor peaks at *m*/*z* 3154 and 2850 which represent digestion products from the intact dirhodium−DNA complex, but the series is of low intensity and yields no information on the location of the dirhodium binding site. Thus, while the matrix and additive used here allow the metal core to remain bound to the oligonucleotide, the interaction with the enzyme appears to displace the metals at an early point in the digestion, unlike what was observed for GG binding in Figure 5. This particular approach is, therefore, ineffective for determining the site of metalation.

After our initial experiments with VPD digestion, a two-enzyme system was investigated, with the pH optimized to activate only one of the enzymes. The enzymes VPD and calf spleen phosphodiesterase (which is a 5′ enzyme) SPD were selected and used at pH 9.4, which is the optimum pH for VPD. In this manner it was possible to digest the sequence from the 3′ end until the $Rh_2(O_2CCH_3)_2^{2+}$ complex is encountered by the enzyme. As the spectrum in Figure 6c shows, the digestion products contain the intact dirhodium complex. Furthermore, the results indicate that the dirhodium complex is attached to the AA portion of the DNA strand. Additional confirmation of the dirhodium position is illustrated by the characteristic pattern of peaks representing acetate adducts at masses higher than *m*/*z* 2257 (indicated by a bracket in the figure). From close inspection it can be discerned that the peaks in Figure 6c all contain the dirhodium core, as indicated by satellite peaks at higher *m*/*z* values due to the presence of one and two acetate ligands. Clearly, if $[M - H]^-$ is present as in Figure 6b, digestion products of $[M - H]^-$ are observed. When $[M + Rh_2 - 5H]^-$ is observed, only digestion products of this metal−DNA complex are observed as in Figure 6c. All digestion products in Figure 6c contain the metal. It is believed that the digestion with VPD alone results in the removal of Rh_2 from the DNA strand by the enzyme. It is not obvious how the addition of SPD aids in the digestion of the intact dirhodium−DNA complex, but such results could not be obtained by using VPD alone. Thus, we assume that SPD, in some manner, stabilizes the complex while allowing VPD to digest the DNA. We are not necessarily suggesting the use of two enzymes as a general method; we simply report that this allowed for the digestion to be completed for this example. The dual enzyme system was not useful in digesting the GG system (Figure 5).

Conclusions

This study provides the first structural evidence for the binding of dirhodium complexes to DNA. Furthermore it has been shown that dirhodium bis-acetate binds to GG as well as the AA containing oligonucleotides in direct contradiction to earlier claims that guanine adducts of dirhodium complexes are not stable. Although the dirhodium−DNA interactions are weaker than corresponding cisplatin−DNA adducts, they are sufficiently strong to be observed in the gas phase when handled in a matrix that contains spermine. The ultimate importance of these findings is its extension to double-stranded DNA oligonucleotides, work that is in progress.[20]

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