Substitution and Reduction of Platinum(IV) Complexes by a Nucleotide, Guanosine 5′-Monophosphate

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A series of Pt^{IV} anticancer complexes with different reduction potentials has been investigated for their reactivity toward 5′-guanosine monophosphate (5′-GMP). The Pt^{IV} complexes studied were Pt^{IV}(trans-*d*,*l*)(1,2-$(NH_2)_2C_6H_{10}$)-Cl_4 (tetraplatin, Pt^{IV}(dach)Cl_4; dach = diaminocyclohexane), *cis*,*trans*,*cis*-[Pt^{IV}($(CH_3)_2CHNH_2)_2(OH)_2Cl_2$] (iproplatin, $Pt^{IV}(ipa)_2(OH)_2Cl_2$; ipa = isopropylamine), *cis*,*trans*,*cis*-[Pt^{IV}(en)$(OH)_2Cl_2$] (Pt^{IV}(en)$(OH)_2Cl_2$; en = ethylenediamine), Pt^{IV}(en)Cl_4, and *cis*,*trans*,*cis*-[Pt^{IV}(en)$(OCOCH_3)_2Cl_2$] (Pt^{IV}(en)$(OCOCH_3)_2Cl_2$). The reactivity was monitored by the decreased ^{1}H NMR peak intensity at 8.2 ppm due to H8 of free 5′-GMP and the increased intensity of a new peak around 8.6 ppm due to H8 of 5′-GMP bound to Pt^{II}. The reactivity followed the order of cathodic reduction potentials of the Pt^{IV} complexes: Pt^{IV}(dach)Cl_4 (−90 mV) ≫ Pt^{IV}(en)Cl_4 (−160 mV) > Pt^{IV}(en)$(OCOCH_3)_2Cl_2$ (−546 mV) > $Pt^{IV}(ipa)_2(OH)_2Cl_2$ (−730 mV). The most reactive complex, Pt^{IV}(dach)-Cl_4, showed an additional weak peak at 9.2 ppm due to H8 of the 5′-GMP bound to the Pt^{IV} complex, indicating the existence of a Pt^{IV} intermediate. ^{1}H NMR, UV/visible absorption spectra, and high-performance liquid chromatograms suggest that the final product is Pt^{II}(dach)(5′-GMP)(ox5′-GMP), where ox5′-GMP is oxidized 5′-GMP. A plausible mechanism is that there is an initial substitution of one Pt^{IV}/ligand by a 5′-GMP molecule, followed by a two-electron reduction, and finally a second substitution by another 5′-GMP. In the presence of excess 5′-GMP (at least 20-fold), ox5′-GMP seems to be replaced by 5′-GMP to form Pt^{II}(dach)$(5'\text{-GMP})_2$. UV/visible absorption spectroscopy shows that the formation of the Pt^{IV} intermediate by substitution is a very slow process followed by reduction. The reduction is characterized by a relatively fast exponential decay. The addition of a small amount of *cis*-[$Pt^{II}(NH_3)_2Cl_2$] shortened the slow formation time of the intermediate, implicating the occurrence of a Pt^{II}-assisted substitution reaction. These reactions may lead to a better understanding of the anticancer activity of Pt^{IV} complexes.

Introduction

There is a growing interest in six-coordinate Pt^{IV} complexes because of their anticancer activity, especially since these complexes are toxic to tumors that are resistant to cisplatin.[1] The anticancer activity mechanism of these Pt^{IV} complexes has not been studied in detail. It is generally believed that since Pt^{IV} compounds are inert in ligand substitution reactions relative to their Pt^{II} analogues,[2] they must be reduced to Pt^{II} species before binding to DNA. However, there are numerous experimental results that cannot be entirely explained by simple Pt^{IV} reduction to the active Pt^{II} analogue prior to DNA binding. Some Pt^{II} analogues do not show any of the activity or selectivity of their Pt^{IV} counterparts.[3a,b] For a series of different Pt^{IV} complexes that upon reduction putatively yield the same Pt^{II} analogue, there is an 800-fold range in activity.[3c] There is no strong correlation between reduction rate and cytotoxicity toward cisplatin-sensitive L1210/0 cells among eight Pt^{IV} complexes with varying reduction potential.[4] Metabolites of *cis*,*trans*,*cis*-[$Pt^{IV}(NH_3)(C_6H_{11}$-$NH_2)(OCOCH_3)_2Cl_2$] (JM-216) are not only its Pt^{II} analogue, *cis*-[$Pt^{II}(NH_3)(C_6H_{11}NH_2)Cl_2$], but also several Pt^{IV} complexes such as *cis*,*trans*,*cis*-[$Pt^{IV}(NH_3)(C_6H_{11}NH_2)(OCOCH_3)_2(OH)_2$], *cis*,*trans*,*cis*-[$Pt^{IV}(NH_3)(C_6H_{11}NH_2)(OCOCH_3)_2$(OH)Cl], and *cis*,-*trans*,*cis*-[$Pt^{IV}(NH_3)(C_6H_{11}NH_2)(OCOCH_3)_2$Cl(OH)].[5] Some Pt^{IV} complexes can bind to DNA fragments, guanosine 5′-monophosphate (5′-GMP), methylhypoxanthine, or methylxanthine without reducing agents.[6,7] A recent paper by Roat et al. has demonstrated that reactions between 9-methylxanthine and Pt^{IV} complexes with ammine, organic amine, and chloro ligands are speeded up dramatically by additions of small amounts of the

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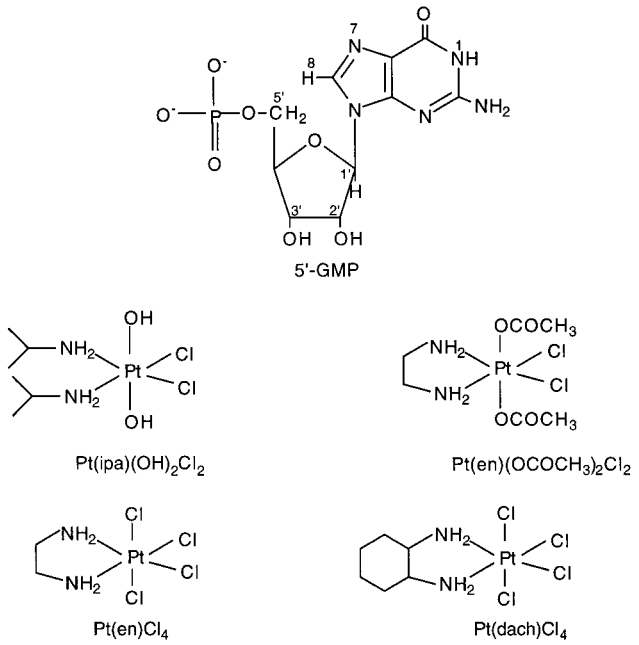

Figure 1. Molecular structures of 5′-GMP and Pt^{IV} complexes studied.

analogous Pt^{II} complexes.[7d] These experimental results suggest that not every Pt^{IV} complex behaves the same way in its reactivity. Depending on the nature of the ligands and reaction conditions, some Pt^{IV} complexes may undergo a reasonably fast substitution reaction, especially in the presence of Pt^{II} complexes. To address this possiblity, we have compared the reactivities of various Pt^{IV} complexes with 5′-GMP.

The Pt^{IV} complexes we have chosen to study (Figure 1) have a wide range of cathodic reduction potentials.[4] The final products seemed to be Pt^{II} adducts, and the reactivity followed the order of cathodic reduction potentials (E_c) of the Pt^{IV} complexes: $Pt^{IV}(dach)Cl_4$ (−90 mV) ≫ $Pt^{IV}(en)Cl_4$ (−160 mV) > $Pt^{IV}(en)(OCOCH_3)_2Cl_2$ (−546 mV) > $Pt^{IV}(ipa)_2(OH)_2Cl_2$ (−730 mV). The most reactive complex, $Pt^{IV}(dach)Cl_4$, has been studied in more detail with and without the Pt^{II} complex, *cis*-$[Pt^{II}(NH_3)_3Cl_2]$ (cisplatin). Its reaction with 5′-GMP showed the existence of a Pt^{IV} intermediate, whose formation was catalyzed by cis-$[Pt^{II}(NH_3)_3Cl_2]$.

Experimental Section

Materials. The Pt^{IV} complexes studied are shown in Figure 1. The $Pt^{IV}(en)(OCOCH_3)_2Cl_2$ and $Pt^{IV}(en)Cl_4$ complexes were synthesized following literature procedures.[3c] They were characterized by elemental analysis (Atlantic Micro Lab, Norcross, GA), IR spectroscopy (Mattson Cygnus 100), and ^{13}C NMR in D_2O solution (GE GN-Omega 300 MHz). IR spectra were obtained in diffuse reflectance mode using KBr as a diluant. The $Pt^{IV}(ipa)_2(OH)_2Cl_2$ (iproplatin) and $Pt^{IV}(dach)Cl_4$ (tetraplatin) complexes were obtained from the National Cancer Institute, Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment. $Pt^{II}(dach)Cl_2$ was obtained by reducing $Pt^{IV}(dach)Cl_4$ with a 10-fold excess of ascorbic acid.[8] Cisplatin and the sodium salt of 5′-GMP were obtained from Sigma. Deuterium oxide (99.9%) and all other chemicals were obtained from either Sigma or Aldrich and were used as supplied.

pH Measurements. The pH values of the solutions were adjusted with NaOD and DCl using a pH meter (Orion Research 960) equipped with an Ingold combination pH microelectrode. The pH was read directly from the pH meter without correction for deuterium isotope effects and was designated as pH*. The reported pH values are at the beginning of the reaction unless stated otherwise.

Reaction of Pt^{IV} Compounds with 5′-GMP. Buffers were not used in order to avoid complications arising from buffer coordination to platinum.[9] Solutions (5 mM) of Pt^{IV} compound for ^1H NMR spectra were prepared by dissolving 5 μmol of Pt^{IV} compound in 1 mL of D_2O in an amber vial (at least 12 h at 37 °C). After the Pt^{IV} compound was dissolved, an appropriate amount of 5′-GMP was added to give the desired final concentration. Time of 5′-GMP addition was recorded as the beginning of the reaction. Initial pH* of the solution was 8.3 and was not adjusted for most of the experiments. The reactions were also studied at pH* 6.1 and 7.1, but there were no significant differences. All reaction solutions were filtered using a syringe-end filter disk (Gelman Acrodisk 0.45-μm pore size).

Synthesis of the Pt^{II} Compounds with 5′-GMP. For reference purposes, $Pt^{II}(dach)(5'\text{-GMP})_2$ was made by reacting 2 equiv of 5′-GMP with 1 equiv of $Pt^{II}(dach)Cl_2$ at 37 °C for 24 h.[7c]

Physical Methods. Filtered solutions were tranferred to 5-mm NMR tubes fitted with PFTE (Teflon) vacuum valves. The NMR tubes were covered with aluminum foil when not in the NMR probe to avoid photodegradation of the reagents. The solutions were deoxygenated via a vacuum pump, and pressure was equilibrated with argon gas. ^1H NMR spectra were obtained at 37 °C using a 300-MHz GE Omega GN-300 NMR instrument equipped with Omega Patch 6.0.2.2 software. ^1H NMR spectra were externally referenced to the deuterated chloroform peak at 7.26 ppm. The solvent presaturation sequence was employed to decouple H_2O and HDO peaks at 4.75 ppm. The decoupling pulse was set at 80 MHz. Presaturation time was 1.5 s, pulse delay time between the decoupling pulse and the observation pulse was 0.5 s, and the radio frequency (rf) at 90° pulse width for the H8 proton was set at 10.5 MHz. Typical acquisition conditions were as follows: 300−304 transients, gain of 1000, and 2K data points. Free induction decay spectra of each sample were saved. Spectra were manipulated by manual phasing. To gather the kinetic data, a pulse sequence was used in which a spectrum was taken every 30 min for the first 24 h and subsequently every 12 h over a 5-day period. All samples were incubated at 37 °C between observation scans. Bound and unbound peaks were integrated by the software in relation to the initial free 5′-GMP and $Pt^{IV}(dach)Cl_4$ peaks.

UV/visible spectra were obtained in 1-mm-path length cells on an Olis-14 spectrophotometer with Olis kinetic assay software. The absorbances at 360 nm (λ_{max} of $Pt^{IV}(dach)Cl_4$) were monitored over 10 h. The plots of $\ln(A - A_\infty)$ vs time were obtained using Kaleidagraph. Kinetic experiments were replicated at least in triplicate. The sample temperature was maintained by circulating water through the jacketed cuvette holders from a RM 6 Lauda Brinkmann bath.

High-pressure liquid chromatographic (HPLC) traces were obtained with a reversed-phase Vydac C_{18} column (Vydac 218TP54) and Vydac C18 guard column using isocratic H_2O elution at a flow rate of 1 mL/min on a Hewlett-Packard (HP) 1090 liquid chromatograph with an HP 1040A photodiode array detector. The detection wavelengths were set at 200, 260, and 360 nm.

Results and Discussion

Reaction of Pt^{IV} Complexes with 5′-GMP. To see the effect of ligands on reactivity, several Pt^{IV} complexes were reacted with 5′-GMP. Figure 2 displays the downfield NMR spectra of the reaction solutions of Pt^{IV} complexes (5 mM) with 5′-GMP (5 mM) after 1 week. The signals at 8.2 and 8.6 ppm were assigned to H8 of free and Pt^{II}-bound 5′-GMP at the N7 position, respectively.[7] All three Pt^{IV} complexes show an 8.6 ppm peak except $Pt^{IV}(ipa)_2(OH)_2Cl_2$, indicating that these complexes reacted with 5′-GMP to produce Pt^{II} adducts. This is consistent with the results of Reedijk's group, who first recognized the reduction of Pt^{IV} compounds by 5′-GMP.[7a,c] They proposed that the electrons needed for the reduction come from the sugar

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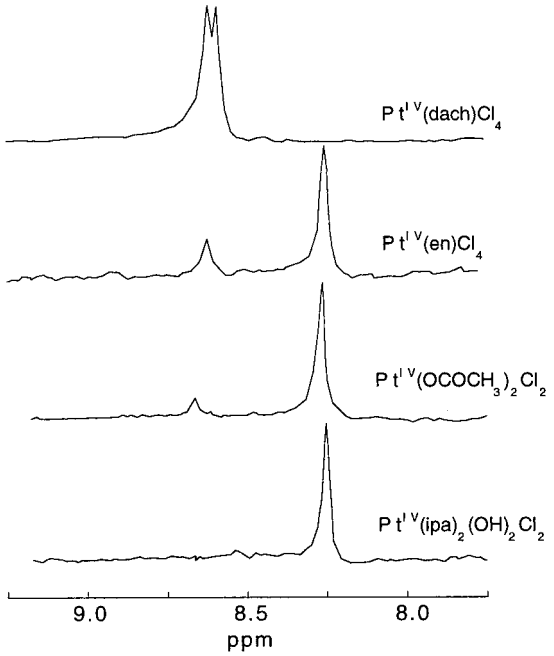


Figure 2. ^{1}H NMR spectra of Pt^{IV} complexes (5 mM) and 5′-GMP (5 mM) at pH 8.3 after 1 week of reaction at 37 °C.

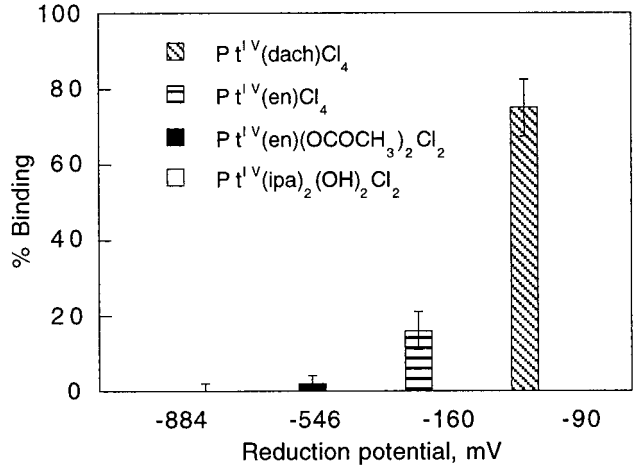


Figure 3. Percent binding of 5′-GMP Pt^{IV} complexes vs reduction potential of Pt^{IV} complexes.

portion of 5′-GMP.[7c] The 8.6 ppm peak in the spectrum of Pt^{IV}(dach)Cl$_4$ is a doublet. This is due to the different environments above and below the platinum coordination plane created by the cyclohexane ring in Pt^{IV}(dach)Cl$_4$.[9] The percent binding of 5′-GMP to each Pt^{IV} compound was calculated by dividing the intensity of the signal near 8.6 ppm by that near 8.2 ppm at t = 0. This is plotted against its reduction potential[4] in Figure 3. The binding increases as the reduction potential increases: Pt^{IV}(ipa)$_2$(OH)$_2$Cl$_2$ (−730 mV) < Pt^{IV}(en)(OCOCH$_3$)$_2$Cl$_2$ (−546 mV) < Pt^{IV}(en)Cl$_4$ (−160 mV) < Pt^{IV}(dach)Cl$_4$ (−90 mV). Pt^{IV}(dach)Cl$_4$, with the highest reduction potential, exhibits 75% binding under our experimental conditions while Pt^{IV}(ipa)$_2$(OH)$_2$Cl$_2$, with the lowest reduction potential, shows no binding. The variation in the reactivity of different Pt^{IV} compounds was also recognized by previous researchers;[7b] *trans,cis,cis*-Pt^{IV}(NH$_3$)(c-C$_6$H$_{11}$NH$_2$)Cl$_2$(OCOCH$_3$)$_2$ reacted with 5′-GMP and 9-methylxanthine producing Pt^{II} adducts, but *trans,trans,trans*-Pt^{IV}(NH$_3$)(c-C$_6$H$_{11}$NH$_2$)Cl$_2$(OH)$_2$ did not react at all. The *trans,trans,trans*-Pt^{IV}(NH$_3$)(c-C$_6$H$_{11}$NH$_2$)Cl$_2$(OH)$_2$ compound with hydroxyl axial ligands is expected to have a very low reduction potential[4] and hence may show no reactivity.

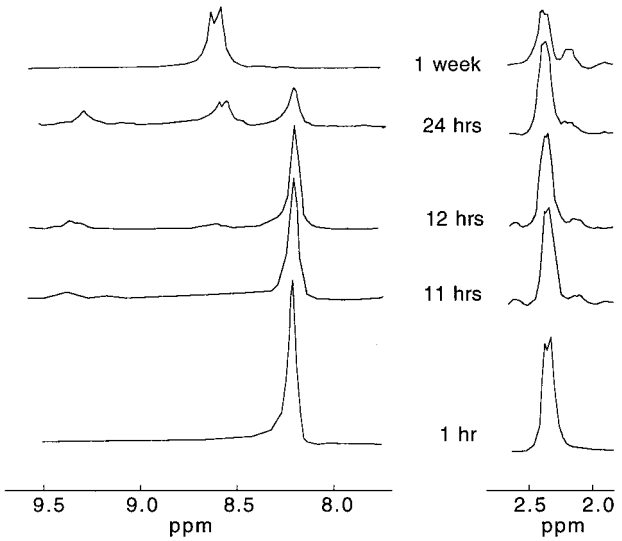


Figure 4. Time-dependent ^{1}H NMR spectra of the reaction at 37 °C between Pt^{IV}(dach)Cl$_4$ (5 mM) and 5′-GMP (5 mM) at pH* 8.3.

Reaction of Pt^{IV}(dach)Cl$_4$ with 5′-GMP (5 mM/5 mM). Since Pt^{IV}(dach)Cl$_4$ showed relatively high reactivity, it was chosen for a more detailed mechanistic study. The time course of the reaction between Pt^{IV}(dach)Cl$_4$ (5 mM) and 5′-GMP (5 mM) at 37 °C and pH 8.3 was monitored by ^{1}H NMR spectroscopy for 1 week. Figure 4 displays the NMR spectra in the 8−9.5 ppm and 2−2.5 areas. These two areas are the most sensitive to the degree of reaction. The peaks in 8−9.5 ppm are due to H8 of 5′-GMP.[7] It is noted that the peak at 8.2 ppm moves around (±0.1 ppm) and broadens during the reaction. The former is due to the pH change of the reaction (vide infra), and the latter is due to exchange of 5′-GMP H8 with D_2O.[7c] The peaks in 2−3 ppm arise from the protons of Pt^{IV}(dach)Cl$_4$ and are suspected to arise from the ring protons of dach**.** At the start of the reaction, the peaks at 8.2 and 2.3 ppm are due to free 5′-GMP and free Pt^{IV}(dach)Cl$_4$, respectively. After 11 h of reaction, multiplets around 9.2 and 2.2 ppm appear. The multiplet peak around 9.2 ppm has been assigned to H8 of Pt^{IV}-bound 5′-GMP.[7] Its intensity remained constant for 3 days, after which time it disappered. The peak at 2.2 ppm concurrently reflects the binding status of platinum. When dach is bound to Cl$^-$-bound platinum, its ring protons resonate at 2.3 ppm. But when it is bound to N7 of 5′-GMP-bound platinum, its ring protons resonate at 2.2 ppm. This upfield shift may arise from the deshielding effect of N compared to Cl$^-$. After 12 h of reaction, another new peak near 8.6 ppm due to H8 of Pt^{II}-bound 5′-GMP appears and its intensity continuously grows with gradual disappearance of the signal near 8.2 ppm due to H8 of free 5′-GMP. The peak at 2.3 ppm due to free Pt^{IV}(dach)Cl$_4$ decreases in intensity while the new peak at 2.2 ppm, due to bound platinum (Pt^{IV} and Pt^{II}) grows in its intensity as the reaction progresses.

Reaction of Pt^{IV}(dach)Cl$_4$ with 5′-GMP (5 mM/100 mM). The time course of the reaction between 5 mM Pt^{IV}(dach)Cl$_4$ and 100 mM 5′-GMP at 37 °C and pH 8.3 was monitored by ^{1}H NMR spectroscopy for 16 h. The concentrations of bound 5′-GMP to Pt^{IV} and Pt^{II} were calculated by multiplying 100 mM by the areas of the 9.2 and 8.6 ppm relative to that of 8.2 ppm at t = 0, respectively. The concentration of bound platinum was calculated by multiplying 5 mM by the area of 2.2 ppm relative to that of 2.3 ppm at t = 0. These are displayed in Figure 5 as a function of time. The appearance and disappearance of the peak at 9.2 ppm suggests that the Pt^{IV} adduct is an intermediate

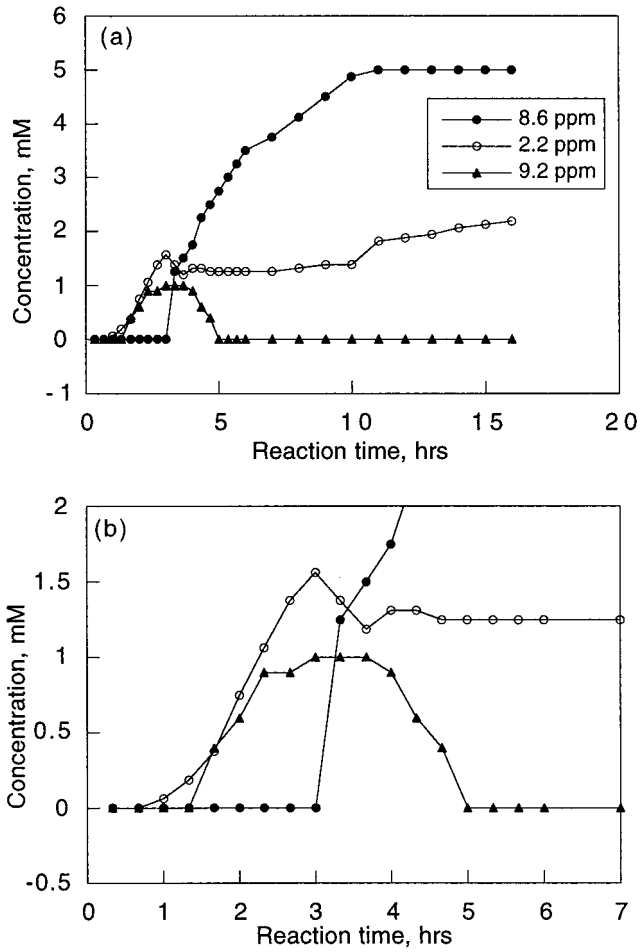


Figure 5. (a) Intensity variation of the H8 signal of 5′-GMP at 9.3 and 8.6 ppm and the H of $Pt^{IV}(dach)Cl_4$ at 2.2 ppm with time for the reaction at 37 °C between $Pt^{IV}(dach)Cl_4$ (5 mM) and 5′-GMP (100 mM) at pH* 8.3. (b) Expansion of the 0−5-h region.

which is reduced to a Pt^{II} adduct. At $t = 3$ h, when there was no 8.6 ppm peak, approximately 1 mM Pt^{IV}-bound 5′-GMP (9.2 ppm) and approximately 1 mM bound platinum (2.2 ppm) appeared indicating that 1 mM 5′-GMP is bound to 1 mM platinum: the Pt^{IV} intermediate is proposed to be $Pt^{IV}(dach)Cl_3$(5′-GMP). We do not have clear evidence for the position of 5′-GMP, but we suspect it is trans to dach from steric considerations. At $t = 16$ h, when there was no 9.2 ppm peak, approximately 5 mM Pt^{II}-bound 5′-GMP (8.6 ppm) and approximately 2.5 mM bound platinum (2.2 ppm) appeared,

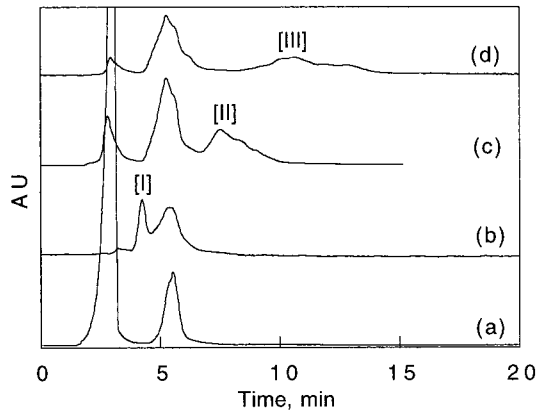
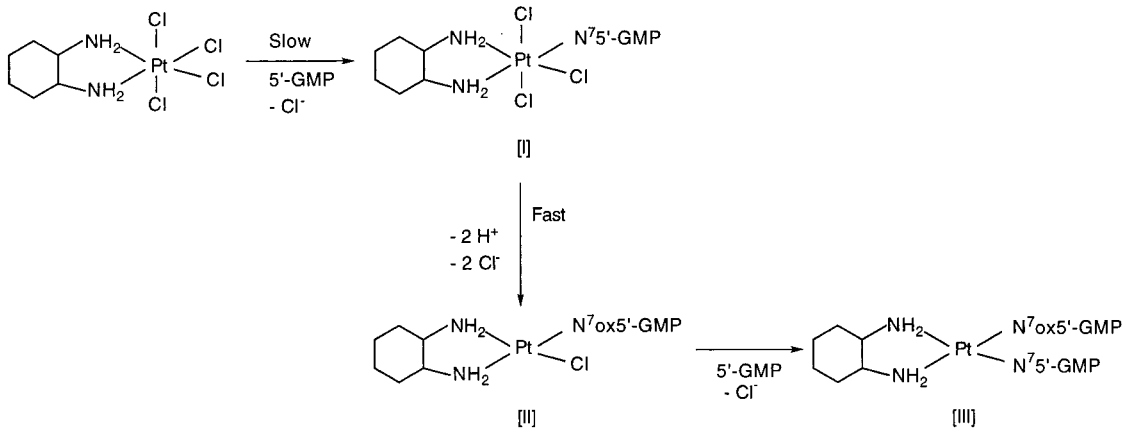


Figure 6. HPLC chromatograms for the reaction at 37 °C between $Pt^{IV}(dach)Cl_4$ and 5′-GMP at pH* 8.3. (a) $t = 0$; (b) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/100 mM) after 2 h, $\lambda_{det} = 360$ nm, (c) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/2.5 mM) after 6 days, $\lambda_{det} = 200$ nm; (d) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/2.5 mM) after 7 days, $\lambda_{det} = 200$ nm.

indicating that 5 mM 5′-GMP is bound to 2.5 mM platinum in a 2:1 stoichiometric ratio. Since the final product is Pt^{II} and there are no reactants other than 5′-GMP, this molecule should be the source of two electrons; 5′-GMP should be in the oxidized form. This is supported by ^1H NMR spectra and HPLC (vide infra). We propose the final Pt^{II} adduct to be $Pt^{II}(dach)$(5′-GMP)-(ox5′-GMP) (oxGMP = oxidized 5′-GMP). A tentative reaction mechanism is proposed in Scheme 1.

The reaction course of $Pt^{IV}(dach)Cl_4$ and 5′-GMP was monitored by HPLC to support the proposed mechanism. The HPLC chromatograms of the reaction at $t = 0$ (Figure 6a) show two peaks with retention times of 3 and 6 min due to free 5′-GMP and free $Pt^{IV}(dach)Cl_4$, respectively. After 3 h of reaction for the $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/100 mM) solution (Figure 6b), a new peak with a retention time of 4.2 min appears. The absorption maximums of this peak obtained directly from the HPLC diode array detector were not significantly different from those of free 5′-GMP except for a feature on the shoulder which shifted from 280 to 275 nm. We assign this to the arising of the Pt^{IV} intermediate, [I]. As the reaction progresses, the 4.2-min peak disappears and a new peak at 7.5 min appears in the reaction mixture of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/2.5 mM) (Figure 6c). The absorption maximum of this peak obtained directly from the HPLC diode array detector is 2 nm red-shifted from the 254 nm of free 5′-GMP, indicating that the 7.5-min peak contains bound oxidized 5′-GMP. We assign this peak to the second intermediate, [II]. When 5′-GMP is bound to Pt^{II}, the absorption maximum of 5′-GMP red shifts about 6 nm.[10]

Scheme 1



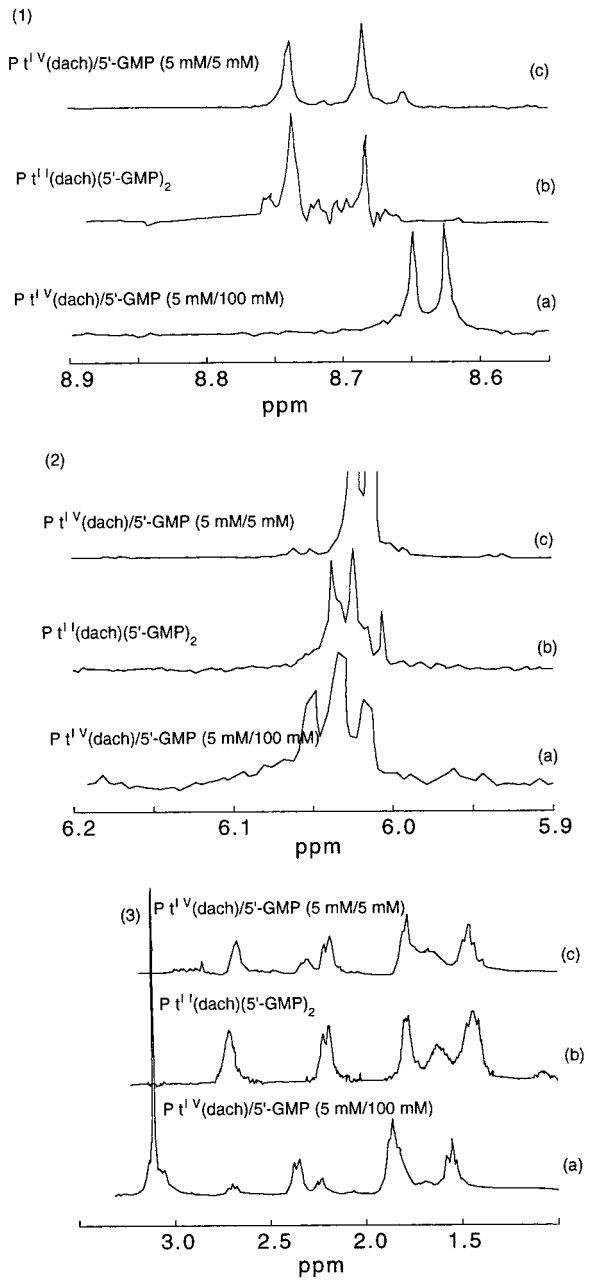


Figure 7. Comparison of the final reaction solution of $Pt^{IV}(dach)Cl_4$/5′-GMP with $Pt^{II}(dach)(5'\text{-GMP})_2$ by ^1H NMR spectra. (a) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) after 7 days; (b) $Pt^{II}(dach)(5'\text{-GMP})_2$; (c) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/100 mM) after 14 days.

After 7 days of reaction, the 7.5-min peak disappeared and a new peak around 10 min appeared (Figure 6d). The HPLC diode array detected absorption spectrum of this peak showed the absorption maximum of 260 nm, indicating the 10-min peak contains bound 5′-GMP. We assign this peak to [III].

Comparison of Final Products of $Pt^{IV}(dach)Cl_4$/5′-GMP with $Pt^{II}(dach)(5'\text{-GMP})_2$. To ensure that the final product of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) is $Pt^{II}(dach)(5'\text{-GMP})$-(ox5′-GMP), we compared it with $Pt^{II}(dach)(5'\text{-GMP})_2$ using ^1H NMR spectra (Figure 7) and HPLC (Figure 8). For both products, the H8 peaks appear around 8.7 ppm as a doublet (Figure 7.1), indicating 5′-GMP is bound to Pt^{II} as mentioned earlier. However, there is a slight difference in the chemical shifts (0.1 ppm) along with the coupling constants: 12.3 Hz for the final products of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) (Figure 7.1a) and 28.2 Hz for $Pt^{II}(dach)(5'\text{-GMP})_2$ (Figure 7.1b). The peaks around 6.0 ppm due to H1′ are also slightly different in the chemical shifts and shapes: Figure 7.2a for the final products of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) and Figure 7.2b for $Pt^{II}(dach)(5'\text{-GMP})_2$. In their work on the reaction of 5′-GMP and *trans*-$Pt^{IV}(NH_3)_2Cl_4$, van der Veer et al.[7c] suggested the final product to be Pt^{II} adducts of 5′-GMP and the electrons needed to reduce Pt^{IV} to Pt^{II} are from the sugar portion of 5′-GMP. The line shape of the H1′ peak would be the most likely affected if this were the case, and our result is consistent with their suggestion. The significant spectral differences are also seen in the high-field region where the ring protons of dach resonate: Figure 7.3a for the final products of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) and Figure 7.3b for $Pt^{II}(dach)(5'\text{-GMP})_2$. The HPLC chromatograms of the final products of the Pt^{IV}-$(dach)Cl_4$/5′-GMP (5 mM/5 mM) and $Pt^{II}(dach)(5'\text{-GMP})_2$ are shown in Figure 8. The final product of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) elutes at 11 min (Figure 8a) and $Pt^{II}(dach)(5'\text{-GMP})_2$ elutes at 9 min (Figure 8b). These NMR and HPLC results support our speculation that the final product of the Pt^{IV}-$(dach)Cl_4$/5′-GMP (5 mM/5 mM) is not $Pt^{II}(dach)(5'\text{-GMP})_2$. We suggest it to be $Pt^{II}(dach)(5'\text{-GMP})$(ox5′-GMP).

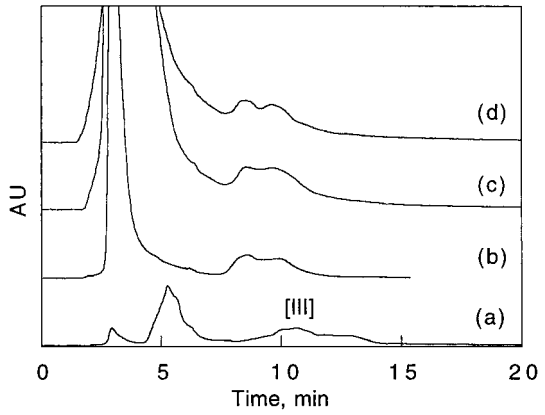


Figure 8. Comparison of the final reaction solution of $Pt^{IV}(dach)Cl_4$/5′-GMP with $Pt^{II}(dach)(5'\text{-GMP})_2$ by HPLC chromatograms, $\lambda_{det} = 200$ nm. (a) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) after 7 days; $\lambda_{det} = 360$ nm; (b) $Pt^{II}(dach)(5'\text{-GMP})_2$; (c) mixture for (a) + excess 5′-GMP (100 mM); (d) $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/100 mM) after 14 days. The peaks at 5 and 3 min are due to free 5′-GMP and $Pt^{IV}(dach)Cl_4$, respectively.

The differences in NMR spectral feature and HPLC retention time between the final product of the $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) and $Pt^{II}(dach)(5'\text{-GMP})_2$ disappeared when the reaction mixture contained excess 5′-GMP (i.e., $Pt^{IV}(dach)Cl_4$/5′-GMP, 5 mM/100 mM). The low-field NMR spectrum of the final product of the $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) showing the coupling constant of the 8.6 ppm peak to be 28 Hz (Figure 7.1c) is exactly the same as that of $Pt^{II}(dach)(5'\text{-GMP})_2$ (Figure 7.1b). And the high-field spectrum (Figure 7.3c) also became exactly the same as that of $Pt^{II}(dach)(5'\text{-GMP})_2$ (Figure 7.3b), except for the peak due to unreacted $Pt^{IV}(dach)$-Cl_4. The peak around 6.0 ppm could not be examined due to the presence of excess free 5′-GMP (Figure 7.2c).

The HPLC experiment was consistent with the NMR result. When excess 5′-GMP (100 mM) was added to the final solution of a reaction mixture of $Pt^{IV}(dach)Cl_4$/5′-GMP (5 mM/5 mM) (Figure 8c) or the reaction mixture that did initially have excess 5′-GMP (100 mM) (Figure 8d), the HPLC peak shows at 9 min, which is the retention time of $Pt^{II}(dach)(5'\text{-GMP})_2$ (Figure 8b).

(10) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352−1356.

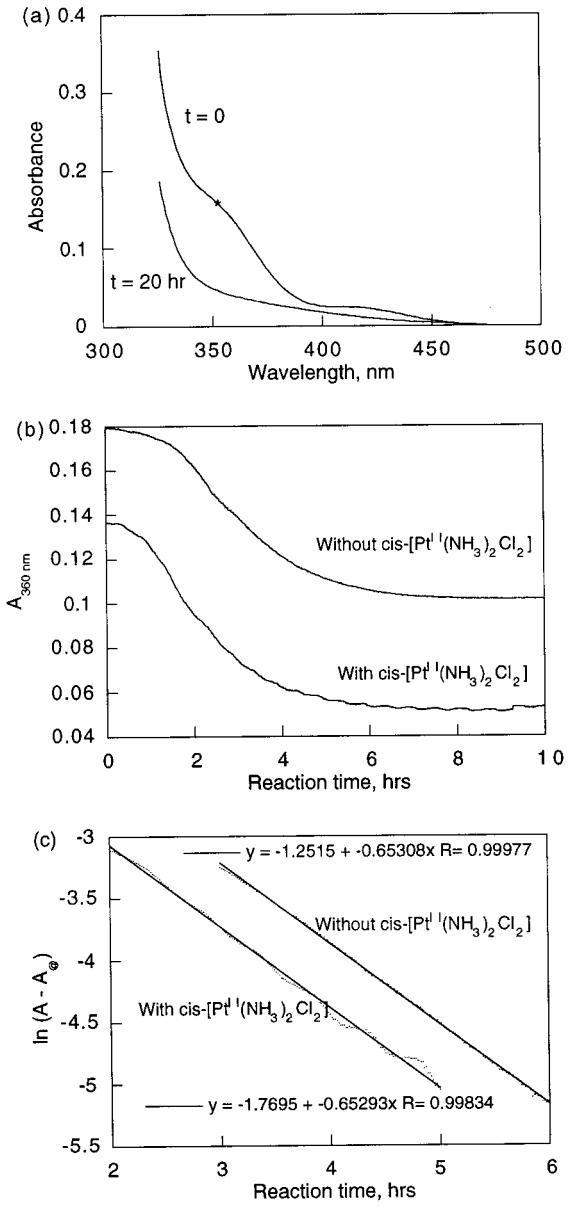


Figure 9. (a) UV/visible spectra for the reaction at 37 °C between Pt^{IV}(dach)Cl_4 (5 mM) and 5′-GMP (100 mM) at pH* 8.3. (b) Absorbance at 360 nm vs reaction time with and without *cis*-[$Pt^{II}(NH_3)_2Cl_2$] (0.5 mM). (c) $\ln(A_t - A_\infty)$ vs time with and without *cis*-[$Pt^{II}(NH_3)_2Cl_2$] (0.5 mM).

We suspect that excess 5′-GMP (at least 20-fold) forced replacement of ox5′-GMP forming Pt^{II}(dach)(5′-GMP)$_2$. This exchange reaction is not expected to be thermodynamically favorable since Pt−N(7) bond once formed should be stable. However, in the presence of a large excess of 5′-GMP, the forced equilibrium may happen.

As suggested by Scheme 1, two H^+ ions are released from 5′-GMP upon oxidation. The pH of the reaction solutions decreased from 8.3 to 6.3 over the course of the reaction, supporting Scheme 1. Roat and Reedijk also reported the lowering of pH (from pH 5.71 to pH 3.01) when the final products of mer-[Pt^{IV}(diethylenetriamine)Cl_3]Cl and 5′-GMP contained the Pt^{II} adduct.[7a] The pH of the initial and final Pt^{II}(dach)Cl_2/5′-GMP were the same within experimental error, which was around 8.3.

Reaction of Pt^{IV}(dach)Cl_4 and 5′-GMP in the Presence of *cis*-[$Pt^{II}(NH_3)_2Cl_2$]. The Pt^{II} complex *cis*-[$Pt^{II}(NH_3)_2Cl_2$] (cisplatin) was added to the reaction mixture of Pt^{IV}(dach)Cl_4 and 5′-GMP to see if the reaction rate was enhanced. The time course of the reaction among Pt^{IV}(dach)Cl_4 (5 mM), 5′-GMP (5 mM), and *cis*-[$Pt^{II}(NH_3)_2Cl_2$] (0.5 mM) at 37 °C and pH 8.3 was monitored by ^{1}H NMR and UV/visible absorption spectroscopies. The 9.2 ppm peak appeared after 8 h instead of 11 h without *cis*-[$Pt^{II}(NH_3)_2Cl_2$] (NMR spectra not shown), indicating that *cis*-[$Pt^{II}(NH_3)_2Cl_2$] catalyzes the first slow substitution reaction. Our result is consistent with the recent work by Roat et al., demonstrating that Pt^{II} complexes catalyze Pt^{IV} substitution reactions.[7d] In Roat's work, the catalyst Pt^{II} complex was the analogue of the Pt^{IV} complex, but our work shows that the Pt^{II} complex does not have to be the direct analogue of the Pt^{IV} complex.

The reactions of 5 mM Pt^{IV}(dach)Cl_4 and 100 mM 5′-GMP without and with 0.5 mM *cis*-[$Pt^{II}(NH_3)_2Cl_2$] were monitored by UV/visible absorption spectroscopy (Figure 9). Figure 9a displays the UV/visible spectra at $t = 0$ and $t = 20$ h. The absorption maximums at 420 and 360 nm at $t = 0$ are due to the Pt^{IV}(dach)Cl_4 reactant. The absorbance at 360 nm with and without *cis*-[$Pt^{II}(NH_3)_2Cl_2$] vs time is shown in Figure 9b. It is apparent that there are two major steps in the reaction: the very slow initial step and the relatively fast second step showing exponential decay. This is consistent with the proposed mechanism in Scheme 1. The slow substitution reaction is followed by a fast redox reaction. The addition of 0.5 mM *cis*-[$Pt^{II}(NH_3)_2Cl_2$] shortens the initial slow reaction from 2 to 1 h. The plots of $\ln(A_t - A_\infty)$ vs time during the exponential decay periods, with and without *cis*-[$Pt^{II}(NH_3)_2Cl_2$], are displyed in Figure 9c. Both are linear ($R^2 = 0.998$) with the same slope of -0.65, indicating the second step in the Scheme 1 is first order and is not affected by *cis*-[$Pt^{II}(NH_3)_2Cl_2$]: *cis*-[$Pt^{II}(NH_3)_2Cl_2$] only catalyzes the first slow substitution reaction, indicating that Pt^{II}-assisted Pt^{IV} substitution reactions do occur.[2]

Conclusion

We have shown that the reactivity of Pt^{IV} complexes toward 5′-GMP depends on their reduction potentials; the higher the reduction potential, the higher the reactivity. We propose a mechanism for the reaction of 5′-GMP and Pt^{IV}(dach)Cl_4. 5′-GMP slowly substitutes for one chloride ion of Pt^{IV}(dach)Cl_4 to produce an intermediate, Pt^{IV}(dach)(5′-GMP)Cl_3, which is exponentially converted to the Pt^{II} adduct. The source of two electrons is suggested to be a 5′-GMP which released two H^+ ions to the solution, becoming ox5′-GMP. The final product is suggested to be Pt^{II}(dach)(ox5′-GMP)(5′-GMP). The slow substitution reaction is catalyzed by *cis*-[$Pt^{II}(NH_3)_2Cl_2$].

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