Reactivity of Glutathione and Cysteine toward Platinum(II) in the Presence and Absence of Guanosine 5′-Monophosphate

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Reactions of L-cysteine (CySH) and glutathione (GSH) with PtI(dien)$^+$ and Pt(dien)$(H_2O)^{2+}$ were carried out in the presence and absence of guanosine 5′-monophosphate (GMP) at neutral pH. In the absence of GMP, reactions with the iodo complex afforded monomeric (thiolato)(dien)platinum(II) complexes initially. The platinum-195 chemical shifts of the cysteine and glutathione complexes are −3155 and −3217 ppm. The products are formed predominantly through direct reaction between the starting platinum complex and the ligands. The aquation pathway contributed insignificantly. The second-order rate constants for the cysteine and glutathione reactions were evaluated to be 1.3 ± 0.1 and 0.66 ± 0.08 M^{-1} s^{-1} at 40 °C. Reactions of Pt(dien)$(H_2O)^{2+}$ with CySH and GSH lead to the formation of the same products. The second-order rate constants for the formation of the platinum−cysteine complex lie in the range 1.9 to 114 M^{-1} s^{-1} within the pH range 1.5 to 7.9 at 25 °C. HPLC and ^{13}C NMR data indicate that a bis(cysteine) complex is formed by slow secondary reactions through complete deligation of dien. Reactions of the thiols in the presence of excess GMP did not yield any GMP coordinated products at neutral pH. The products were the same as observed in the absence of the nucleotide. The kinetic preference of the aquaplatinum complex at neutral pH is unequivocally toward thiols, but not toward the nucleotide. The higher reactivity of thiols at neutral pH is presumably due to an efficient proton transfer from the thiols to the coordinated hydroxo−Pt(dien) complex.

Introduction

The platinum substrate *cis*-diamminedichloroplatinum(II) is a widely used chemotherapeutic agent for the treatment of a variety of cancers.[1] It is generally believed that DNA is the cellular target for this drug.[2] *In vitro* experiments utilizing various DNAs, have demonstrated that intrastrand metallation takes place predominantly through the two adjacent guanine and guanine-adenine[3−5] bases. X-ray structure and NMR characterizations of short oligonucleotide−Pt complexes reveal that N7 sites of the purine rings are involved in coordination.[6−7] Platinum(II) has remarkable affinity toward sulfur donor atoms, and there is substantial thiol concentration in the cell attributed to cysteine, glutathione, and various cytosolic proteins. In fact, direct covalent bond formation through sulfur sites of a variety of plasma proteins is attributed to nephrotoxic effects.[8−10] Recently, we have reported that eukaryotic DNA polymerase-α activities are tremendously inhibited[11] in the presence of $PtCl_2$-(en) and hydrolyzed products of *cis*-DDP. We have shown that the platinum−DNA polymerase-α complex does not exhibit any activity toward cell extension reactions utilizing calf thymus DNA as a template. Furthermore, the uptake of the platinum complex by the DNA polymerase is accompanied by the loss of Zn from the enzyme. Since the zinc-finger domain of the enzyme is thought to coordinate Zn(II) by four cysteine residues,[12] direct[11] platination at these cysteine sites may be responsible for the inactivity of the enzyme. Therefore, detailed understanding of the rates of reactions of sulfur containing amino acids and nucleotides with *cis*-DDP is necessary in order to evaluate kinetic preference toward nucleic acid over the key replication enzyme. Recently, Reedijk and co-workers[13−15] reported a series of studies related to the reactions of glutathione, *S*-methylglutathione, and methionine etc. toward the monofunctional platinum substrate Pt(dien)Cl$^+$. Utilizing equimolar or excess platinum complex over the amino acids, these authors concluded that at pHs below 7, sulfur-bridged dinuclear complexes formed rapidly. This ligation follows a rate limiting mononuclear complexation.[13,14] Furthermore, on the basis of the experiments in acidic solutions, it was concluded that the guanosine 5′-monophosphate enjoys kinetic preference toward aquaplatinum complexes over thiols and thioethers.[13] These authors contended that since the *cis*-DDP reaction with DNA is primarily limited by the rate of aquation, DNA would be able

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to compete with S-donor biomolecules in the cell. Here we report the reaction of cysteine and glutathione with Pt(dien)I^+ and its hydrolyzed products in the presence and absence of GMP near physiological pH, along with the characterization of products utilizing ^{13}C and ^{195}Pt NMR spectroscopy. We find that neither the parent complex nor its hydrolyzed product exhibits any kinetic preference toward the nucleotide over the thiols at physiological pH, even if excess nucleotide is employed. The monofunctional complexes provide insight into the initial complexation reaction of *cis*-DDP, since the reactions of the latter complex with sulfur-containing amino acids and peptides are often complicated by the formation of polymeric complexes[15,16] and are frequently accompanied by the release of coordinated ammonia.[17-20]

Experimental Section

Reagents. Cysteine, DNO_3, NaOD, and D_2O were obtained from Sigma and used without further purification. Sodium perchlorate was prepared by neutralizing Na_2CO_3 with perchloric acid. The platinum complex Pt(dien)I^+ was synthesized following the literature method.[21] The corresponding aqua complex was generated *in situ* by adding stoichiometric amounts of $AgNO_3$ to the latter. For NMR measurements, the pH was adjusted to desired values adding either dilute DNO_3 or NaOD.

NMR Measurements. NMR spectra were recorded on GE 300 MHz (GN 300) instrument equipped with variable temperature broad band probes. Platinum-195 spectra were obtained in 10 mm sample tubes while the proton and ^{13}C resonances were recorded in 5 mm tubes. A deuterium lock was used. Chemical shifts are with respect to K_2PtCl_4 (set at −1624 ppm). Proton spectra are with respect to the H−O−D signal at 4.67 ppm. Typical data acquisition parameters were as follows: (^{195}Pt), 30 μs pulse width (90°), 8−16K data points, 300 ms pulse delay, 160 ms data acquisition time, 40 000−60 000 Hz spectral width, and 10 000−20 000 accumulations. A line broadening factor of 50 Hz for platinum-195 spectra was used before Fourier transformations. Carbon-13 spectra were recorded with an 11 μs pulse, 8K data points, 0.5 s pulse delay, and 20 000 Hz spectral width. Usually 2000 accumulations were required for a 40 mM cysteine solution in order to observe signals with $S/N > 10$. A line broadening factor of 2 Hz was introduced before Fourier transformation. INEPT experiments for ^{13}C were carried out following the conventional pulse sequences:

$$^{1}H: \left(\frac{\pi}{2}\right)_x - \tau - \pi_x - \tau - \left(\frac{\pi}{2}\right)_y$$

$$^{13}C: \pi_x - \left(\frac{\pi}{2}\right)_x - \text{acquire}$$

The magnitude of τ was selected to be 1.7 ms.

Rate Measurements. Rates of slow reactions were measured spectrophotometrically on a Perkin-Elmer Lambda 600 instrument interfaced with an Epson Equity 1^+ computer. For fast reactions, a Durram-110 stopped-flow spectrophotometer was employed. Reactions were carried out with at least 10-fold excess ligand over the platinum complex. An ionic strength of 0.5 M was maintained by $NaClO_4$. The pH of the ligand solutions was first adjusted to 7.0 with NaOH and then Bis-Tris or phosphate buffer (25 mM)[22] was added to maintain a constant pH. The solutions of iodo complex were freshly prepared in $NaClO_4$ and used immediately in order to minimize aquation. Dilute solutions (0.2−0.5 mM) of aquaplatinum complex were kept at pH <3.0 to avoid dimerization and were unbuffered. For the stopped-flow experiments, absorbance−time traces were initially captured on an oscilloscope (Tektronix 2211), which were then transferred to an IBM compatible PC by using the Grabber software.[23] A typical data acquisition includes 4000 data points among which 1000 points represent the base line in the pretriggered state. The post-triggered data were then read from the computer and the rate constants were evaluated from the computer fits of the appropriate rate expressions.

Table 1. Rate Data for the Reaction of Pt(dien)I^+ (0.1−1 mM) and Glutathione in the Presence and Absence of GMP in Bis-Tris[a] Buffer (10 mM) at μ = 0.5 M ($NaClO_4$), T = 40 °C, and pH = 7.0

[GSH], mM	[GMP], mM	$k_0 \times 10^3$, s^{-1}
1.0		1.3
10.0		10
15.0		13
25.0		18
30.0		21
1.0	30.0	1.7
3.0	30.0	3.2
5.0	30.0	3.7
10.0	30.0	9.9
10.05	10.0	12
10.05	31.85	12
10.05	50.0	11

[a] Bis-Tris = (bis[2-hydroxyethyl]amino)tris(hydroxymethyl)methane.

The reactions of cysteine and glutathione with Pt(dien)I^+ were monitored by following exponential decays in absorbance at 300 and 260 nm. Reactions of the aqua complex Pt(dien)$(H_2O)^{2+}$ were followed at 280 nm. The rate constants for reactions under pseudo-first order conditions using excess thiols were evaluated from an iterative computer fit according to

$$D = (D_0 - D_\infty)e^{-k_0 t} + D_\infty \quad (1)$$

where D_0, D, and D_∞ are absorbances at time $t = 0$, at time t, and at infinite time. The reproducibility of the rate constants is within 6%.

Reactions of cysteine with Pt(dien)$(H_2O)^{2+}$ were also followed under second order conditions. The absorbance−time traces can be adequately described by the second-order rate expression

$$D = D_0 + \frac{k[B](D_\infty - D_0)e^{([B]-[A])kt} - 1}{k[B]e^{([B]-[A])t} - k[A]} \quad (2)$$

where k is the second-order rate constant and [A] and [B] are the concentrations of the platinum complex and cysteine.

Chromatographic Separations. Separations of the reactants, intermediates and products were performed on a ternary gradient HPLC system (ISCO) utilizing an analytical C-18 reversed phase column. A UV−visible detector tuned at 254 nm was employed to detect the eluants. Phosphate buffers (0.1 M, pH 6.8−7.2) were used as the mobile phase, and a 5 μL sample was injected in the column in each separation. Usually isocratic separations using 1−2 mL/min flow rates were performed. Retention times of the reactants were determined independently. Peaks due to the unreacted materials in the reaction mixtures were ascertained by matching the retention times of the reactants.

Results

A. Reaction of Glutathione with Pt(dien)I^+ and Pt(dien)$(H_2O)^{2+}$. The reaction of the iodo complex with the excess tripeptide exhibited first order absorbance-time traces. Rate constants obtained at several [GSH] are listed in Table 1. In accordance to the established substitution kinetics of square planar platinum complexes, a two-term rate law[24]

$$k_0 = k_a + k_2[GSH] \quad (3)$$

is expected. In this rate law, k_a is the first order rate constant for the aquation process and k_2 is the second-order rate constant for the direct reaction of the thiol with the iodo complex. The plot of k_0 vs [GSH] is linear with negligible or zero intercept

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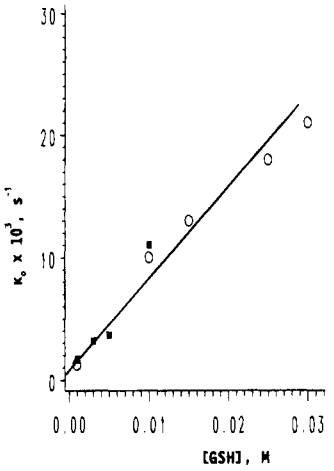

Figure 1. k_0 vs [GSH] plot for the reaction between Pt(dien)I^+ and glutathione in the absence (open circle) and presence (filled rectangle) of guanosine 5′-monophosphate at 40 °C, μ = 0.5 M ($NaClO_4$), and pH = 7.0 (Bis-Tris buffer). Concentrations of GMP are indicated in Table 1.

(Figure 1). The second-order rate constant, 0.66 ± 0.08 M^{-1} s^{-1} can be evaluated by using $k_0 = k_2$[GSH]. The rate constant for the aquation of the iodo complex[24] is at least three orders of magnitude smaller than the lowest value of k_0 measured in this system. Therefore, such a small intercept (k_a) cannot be considered significant. The magnitude of the slope indicates that the thiol complexation takes place predominantly through the direct reaction with the iodo complex.

HPLC separations of the reaction mixture containing 25.0 mM GSH and 2.0 mM iodo complex utilizing phosphate buffer (0.5 M) as the mobile phase is shown in Figure 2. The broad signal, IV is for the parent platinum complex, and I is for the counteranion, I^-. As the reaction proceeds, a new peak III grows with time with concomitant decrease in the intensity of peak IV. The intensity of signal I also increased with time since iodide is released from the coordination sphere as the tripeptide is coordinated. The iodo complex was completely depleted and the product peak had grown to its maximum intensity in 45 min at room temperature. A new peak, V, appeared slowly ($t_{1/2} \simeq 2$ days) in the chromatogram at the expense of the initial product peak, III. Peak III, however, did not completely vanish within a week.

A platinum-195 NMR spectrum recorded within an hour of the reaction using 25 mM Pt complex and 100 mM GSH, exhibited only one peak at −3155 ppm. This resonance has been assigned to the thiolato complex,[13] Pt(GS)(dien). When pH was lowered, the chemical shift remained unchanged. The same product peak was also observed when the reaction was carried out at pH 4.0, and no other new peaks were detected in −1500 to −4000 ppm region. Platinum-195 NMR spectra recorded at regular time intervals up to 1 week at pH 7.0 did not show any new resonances. The signal intensity, however, decreased considerably over time. Comparison of intensities was made based on an equal number of FID acquisitions.

B. Reaction of Cysteine with Pt(dien)I^+ and Pt(dien)-(H_2O)$^{2+}$ Complexes. The reaction of cysteine with Pt(dien)I^+ also follows two distinct phases. The initial phase of the reaction was over within 10 min (at 40 °C), but the second phase required several days. The absorbance−time traces for the first phase exhibit a first-order kinetic profile utilizing excess cysteine. The pseudo-first-order rate constants (k_0) evaluated at various [CySH] are listed in Table 2. The rate constants (k_0) exhibit the similar dependence on [CySH] as observed for the

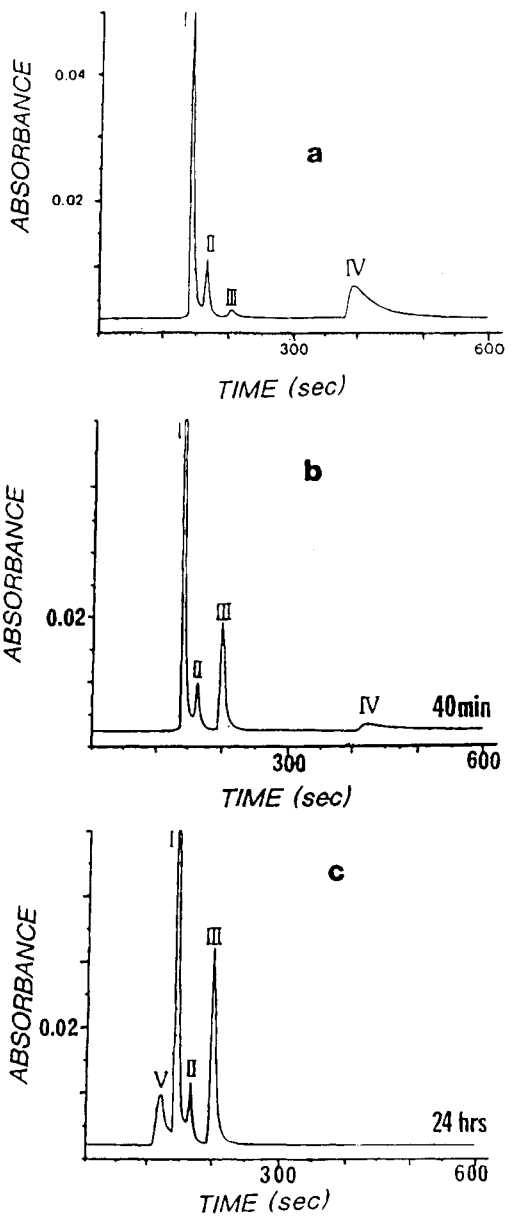

Figure 2. Reversed phase HPLC separations of products from Pt-(dien)I^+ (20 mM)−GSH (112 mM) reaction mixture. Chromatograms a, b, and c are recorded immediately after mixing, after 40 min, and after 24 h at ambient temperature (~22 °C). Peaks are detected at 254 nm using phosphate buffer (pH 6.8, 50 mM) as the mobile phase with 1 mL/min flow rate. The peak assignments are as follows: I, I^- counteranion; II, an impurity in GSH; III, Pt(dien)(GS); IV, Pt(dien)-I^+; V, secondary product of Pt(dien)GS.

glutathione reaction. The value of k_2 obtained from a linear least-squares fit (Figure 3) is 1.3 ± 0.1 M^{-1} s^{-1}. Note that the magnitude of k_2 is much higher than k_a. Therefore, the product is formed predominantly through the direct reaction between cysteine and the iodo complex.

The reaction of cysteine with the aqua complex is first order with respect to each of the reactants. The second-order rate constant increases with the increase in pH. Since kinetic curves above pH 8.0 were irreproducible and often exhibit multiphasic features including S-shaped appearances, evaluation of rate constants above this pH was not possible.

HPLC chromatograms for the Pt(dien)I^+−cysteine reaction mixtures exhibited features similar to those observed for the GSH reactions but with different retention times. Initially, a product peak grew and then very slowly (up to several days) decayed. Concomitantly, a new peak with a much shorter retention time appeared in the chromatogram. The latter peak

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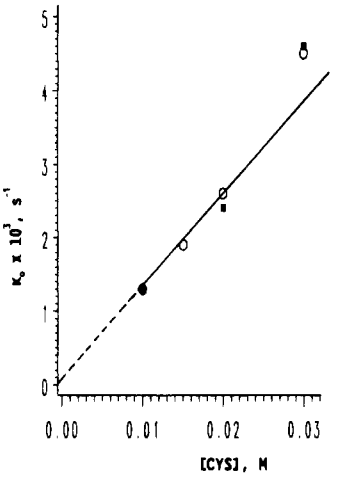

Figure 3. Plot of k_0 vs [cysteine] for the reaction between Pt(dien)I^+ and cysteine at 40 °C, pH = 7.0, and μ = 0.5 M ($NaClO_4$) in the absence (open circle) and presence (filled rectangle) of GMP. Concentrations of GMP are indicated in Table 2.

Table 2. Rate Data for the Reaction of Pt(dien)I^+ (1 mM) and L-Cysteine in the Presence or Absence of GMP in Bis-Tris[a] Buffer (10 mM) at μ = 0.5 M ($NaClO_4$), T = 40 °C, and pH 7.0

[L-CYS], mM	[GMP], mM	$k_0 \times 10^2$, s^{-1}
10.0		1.3
15.0		1.9
20.0		2.6
30.0		4.5
10.0	30.0	1.3
20.0	30.0	2.4
30.0	30.0	4.6

[a] Bis-Tris = (bis[2-hydroxyethyl]amino)tris(hydroxymethyl)methane.

must then represent a product due to a slow secondary reaction. Furthermore, a pale yellow precipitate formed slowly.

Platinum-195 NMR spectra of a cysteine−Pt(dien)I^+ reaction mixture, containing 25 mM platinum complex and 0.12 M cysteine, recorded at a regular time interval exhibited a single resonance at −3217 ppm due to the formation of a cysteine adduct. The peak initially grew in intensity and no appreciable change in intensity was observed over 5−8 h. The chemical shift of this product peak did not change with time. No new resonances were observed. Similar observations were made when the aqua complex, Pt(dien)$(H_2O)^{2+}$, was employed in the reaction.

Products of cysteine−platinum(II) reaction were also examined by proton and carbon-13 NMR spectroscopy. At pH 7.0, free cysteine exhibits three ^{13}C resonances at 24.8 (β-carbon), 55.2 (α-carbon), and 209.7 (carboxylic) ppm, whereas Pt(dien)-$(H_2O)^{2+}$ shows two signals at 50.4 and 54.2 ppm. A ^{13}C NMR spectrum of the reaction mixture containing 40 mM cysteine and 20 mM Pt(dien)$(H_2O)^{2+}$ was recorded at pH 7.0 2 h after mixing. The spectrum exhibited peaks at 53.2, 51.2, 45.1, and 37.6 ppm, in addition to the peaks for the unreacted cysteine. Since no peaks for the unreacted starting platinum complex were detected, platinum was completely complexed with cysteine during this time. When the reactants were mixed in a 1:1 mole ratio (20 mM each), no signals for unreacted cysteine or the dien complex were observed after 2 h. The signal at 37.6 ppm can be taken as the signal for the β-carbon of cysteine since it is expected to exhibit a significant change in chemical shift due to coordination by the sulfur atom. An INEPT experiment reveals two antiphase peaks of equal intensity centered at 53.2 ppm with a separation of 145 Hz. The peak at 53.2 ppm can be assigned to the α-carbon of cysteine. A small change, 2 ppm, in chemical shift can be noticed for this carbon. This is consistent with the observation that thiol coordination influences the α-carbon insignificantly. The remaining two peaks at 51.1 and 45.1 ppm, are assigned to the sets of two equivalent carbon of the dien. We suspect that the downfield peak, 45.1 ppm, is for the two $-CH_2$ groups adjacent to the middle nitrogen, trans to the Pt−S bond.

The spectrum of the 1:2 reaction mixture (Pt:cysteine) recorded after 2 days shows an additional peak at 38.7 ppm. Furthermore, the peaks at 37.6, 53.2, and 51.2 ppm also decreased in intensity. The intensities of the peaks for the unreacted excess cysteine gradually depleted, disappearing completely after 10 days. During this time a pale yellow precipitate also appeared. When the precipitate was removed by centrifuge, the supernatant solution afforded two peaks at 45.0 and 38.8 ppm. These two peaks match with those observed for free dien at pH 7.0. The NMR data indicate that the secondary reaction with cysteine leads to the complete deligation of dien and that the precipitate is most likely a bis-(cysteine) complex. Unfortunately, due to its poor solubility we were unable to record the spectrum of the precipitate. Carbon-13 NMR spectra of the 1:1 reaction mixture recorded over a week, did not show any new peak, nor did we observe any appreciable precipitate. These results indicate that the secondary product is formed in the presence of excess ligand.

The proton NMR spectra of the reaction mixture were also recorded at various time intervals up to 5 days. The resonances at 4.58 and 3.2 ppm for the cysteine were significantly broadened upon coordination to the platinum center. The peak at 4.58 merged with the H−O−D peak at 4.67 ppm and the methylene protons evolved into a single broad peak.

C. Reactions of the Platinum Complexes with Cysteine and Glutathione in the Presence of GMP. The reaction of cysteine with the platinum complex in the presence of GMP was carried with concentration Pt:CyS:GMP ratio of 1:10:10−50 employing 2.0 mM [Pt(dien)I]$^+$. When the reaction was followed by proton NMR spectroscopy, only one H8 resonance at 8.05 ppm for the free GMP was detected. Invariably, a GMP coordination to Pt(II) is accompanied by the appearance of a new H8 signal at about 0.3−0.8 ppm downfield from that of free GMP. No new H8 signals were apparent when the GMP concentration was exceeded up to 5-fold over cysteine. Furthermore, only a single platinum−195 resonance at −3217 ppm was observed at the end of the reaction utilizing 25 mM Pt, 50 mM cysteine, and 50 mM GMP. The chemical shift for this resonance is identical to that observed for the cysteine reaction in the absence of GMP. The reaction of the amino acid in the presence of GMP also exhibited a first order rate profile. The rate constants (Table 1) were the same as obtained for the reaction with the amino acid alone. Finally, HPLC chromatograms exhibited a peak due to free GMP in addition to the peaks shown in Figure 2. No GMP complexation was observed when glutathione was used as a substrate in the presence of GMP. When the hydrolyzed platinum complex Pt(dien)$(H_2O)^{2+}$ was employed in the mixture of the amino acids and nucleotide at neutral pH, no GMP coordination was observed in the proton and platinum-195 NMR spectra as well.

Discussion

The rate law for the cysteine and glutathione reactions with the iodo complex is consistent with the following mechanism:

$$\text{Pt(dien)}I^+ + \text{HS}-\text{R} \xrightarrow{k_2} \text{Pt(dien)}(\text{S}-\text{R})^+ + I^- + H^+ \quad (4)$$

$$\text{Pt(dien)}I^+ + H_2O \xrightarrow{k_a} \text{Pt(dien)}(H_2O)^{2+} + I^- \quad (5)$$

$$\text{Pt(dien)}(H_2O)^{2+} + \text{HSR} \xrightarrow{\text{fast}} \text{Pt(dien)}(\text{S}-\text{R}) + H_3O^+ \quad (6)$$

Here, HSR represents cysteine or glutathione. The reaction

predominantly proceeds through the direct reaction of the iodo complex with the thiols, as evident from the magnitudes of the second-order rate constants compared to the rate of aquation of this complex. The direct reactions of the aqua complex were more than an order of magnitude faster than that of the iodo complex as measured from independent reactions with the aqua complex. The ^{195}Pt NMR chemical shifts of the primary products agree with the values reported for Pt(GS)(dien) and Pt(CyS)(dien) complexes.[13,14] The formation of thiolato-bridged dimers in these reactions can be ruled out from the chemical shift data and the rate law.

In principle, the pH-dependent rate constants for the reaction between the aqua-platinum complex and cysteine can be described by the following equations:

$$Pt(dien)(H_2O)^{2+} + CySH \xrightarrow{k_1} Pt(dien)(CyS) + H_3O^+ \quad (7)$$

$$Pt(dien)(H_2O)^{2+} + CyS^- \xrightarrow{k_2} Pt(dien)(CyS) + H_2O \quad (8)$$

$$Pt(dien)(OH)^+ + CySH \xrightarrow{k_3} Pt(dien)(CyS) + H_2O \quad (9)$$

$$Pt(dien)(OH)^+ + CyS^- \xrightarrow{k_4} Pt(dien)(CyS) + OH^- \quad (10)$$

Here, CySH represents cysteine in which thiol is protonated. Since $Pt(dien)(H_2O)^{2+}$ exists predominantly below neutral pH, and CyS^- exists above neutral pH, the relative contribution by the reaction 8 is expected to be small compared to the other reactions (7, 9, and 10) unless k_2 is significantly higher than k_3 or k_4. Neglecting reaction 8, we obtain[25]

$$k = \frac{k_1[H^+]^2 + k_3Ka_1[H^+] + k_4Ka_1Ka_2}{(Ka_1 + [H^+])(Ka_2 + [H^+])} \quad (11)$$

where k is the observed second-order rate constants. Acid dissociation constants of the aqua complex and thiol functionality of cysteine are represented by Ka_1 and Ka_2. A nonlinear least-squares fit of the pH-rate data according to eq 11 yielded $k_1 = 1.9 \pm 0.2$ M^{-1} s^{-1}, $k_3 = 47 \pm$ M^{-1} s^{-1}, and $k_4 = 239 \pm 30$ M^{-1} s^{-1}. The values of Ka_1[25] (1.26×10^{-6})[26] and Ka_2 (5.01×10^{-9}) were kept invariant. The calculated values are listed in parentheses in Table 3.

Although HPLC separations indicate that a new product is formed by a slow secondary reaction ($t_{1/2} \approx 2$ days) at the expense of the Pt(dien)(thiol) complexes, we were unable to detect any new 195 Pt NMR signal. Utilizing higher [Pt]/[GSH] ratios (1:1 and 1:2) or by further reaction of the Pt(GS)(dien) complex with the initial platinum complex, Reedijk and co-workers[13-15] observed a thiolato-bridged dimeric complex. The ^{195}Pt chemical shift of this complex is reported to be at −3185 ppm. Since we do not observe such a signal in our ^{195}Pt NMR spectra, the formation of a dimer in the slow secondary reactions in our system can be ruled out. This is consistent with the mechanism proposed by Reedijk and co-workers that the dimer is formed by a reaction between the mono(thiolato) and the starting platinum complexes. Since [thiol] is much higher in our system, the specific rate, k_0[thiol] is much higher than the rate of dimer formation. The secondary products observed in the HPLC chromatograms can not be the oxidized thiols, cystine (GSSG) for which we observed markedly different retention times in the chromatograms. Furthermore, the peak is not due to free dien since it does not absorb significantly at 260 nm ($\epsilon_{max} < 5$ M^{-1} cm^{-1}). The small peak in the HPLC chromatogram for the secondary product is most likely due to the bis-(cysteinato) complex that exhibits a limited solubility and escapes ^{13}C and ^{195}Pt NMR detection. The amino acid may be coordinated in bidentate fashion presumably ligating through the sulfur and amine group. The formation of chelates through sulfur and nitrogen coordination by amino acids have been reported[16,27,28] for *cis*-dichloro− and *cis*-diaqua−Pt(II)−amine complexes.

Table 3. Second-Order Rate Constants for the Reaction of L-cysteine with $Pt(dien)(H_2O)^{2+}$ as a Function of pH at 25 °C (μ = 0.5 M $NaClO_4$)

pH	k, M^{-1} s^{-1}
1.5	1.9[a] (2.0)[b]
4.2	2.6 (2.8)
4.9	8.2 (6.1)
5.7	23 (20)
5.9	25 (25)
6.2	33 (33)
6.6	39 (43)
6.9	43 (50)
7.0	45 (52)
7.5	70 (72)
7.9	114 (101)

[a] No buffer was used. [b] The values in parentheses were calculated based on eq 11 utilizing $k_1 = 1.9$, $k_3 = 47$, $k_4 = 239$, $Ka_1 = 1.26 \times 10^{-6}$ and $Ka_2 = 5.01 \times 10^{-9}$.

The reaction of the aqua complex with a mixture of GMP and cysteine yielded a product that exhibits the ^{195}Pt NMR resonance with the same chemical shift as was observed for the cysteine reaction. In the absence of other resonances it can be concluded that GMP cannot effectively compete with the amino acid when both ligands are of equal concentrations. Further, in a competing situation the GMP complexation is also negligible even with 5-fold excess nucleotide over the amino acid, as long as a stoichiometric amount of the latter ligand is present.

It is informative to compare the second-order rate constants for the thiol coordinations with that of GMP toward the aqua complex, $Pt(dien)(H_2O)^{2+}$ in order to understand the kinetic preference of one group of biomolecules over the other. The rate constants listed in Table 3 clearly point to a kinetic preference toward the thiol ligands at neutral pH. This is also reflected in the competition reactions utilizing mixtures of the thiol and GMP. Proton and platinum-195 NMR data do not show any N7 coordination of GMP up to a 5-fold excess of GMP over thiol. Reedijk and co-workers[14] reported that in

(25) The inclusion of reaction (8) yielded the following expression:

$$k = \frac{k_1[H^+]^2 + k_2Ka_2[H^+] + k_3Ka_1[H^+] + k_4Ka_1Ka_2}{(Ka_1 + [H^+])(Ka_2 + [H^+])} \quad (12)$$

This can be written in the form

$$k = \frac{a[H^+]^2 + b[H^+] + c}{(Ka_1 + [H^+])(Ka_2 + [H^+])} \quad (13)$$

The value of b can be estimated as 5.9×10^{-6} from a nonlinear least squares fit. However, the value of b cannot be broken into two terms, i.e. $k_2Ka_2 + k_3Ka_1$ since the fit of eq 12 yielded a negative value of k_2. We suspect that either reaction 8 or 9, but not both, contributes significantly to the overall rate. Mathematically, the term $k_3Ka_1[H^+]$ can be replaced by $k_2Ka_2[H^+]$, neglecting reaction 9. In such case, the value of k_2 can be estimated as 1.2×10^4 M^{-1} s^{-1}, which seems unreasonably high for a platinum(II) substitution reaction.

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acidic solution, GMP coordination to the aqua platinum complex is preferred over GSH or *S*-methylglutathione. We concur with these workers that in acidic solutions GMP can effectively complete with thiol. Since in acidic solution the protonated thiol is not as good a nucleophile as the deprotonated N7 of the purine. However, we differ in that near neutral pH the kinetic preference is exclusively toward the thiol. Although the reaction of the deprotonated thiol is rapid, the reaction of $Pt(dien)(OH)^+$ with protonated cysteine is of importance at physiological pH due to their concentration distributions. The facile reaction between coordinated hydroxide and protonated thiol may be due to an efficient proton transfer from the thiol to the coordinated hydroxo group. This proton transfer step is presumably accomplished by the formation of a trigonal bipyramidal transition state in which the sulfhydryl group is coordinated to platinum and is in close proximity to the coordinated hydroxide group.

The present study clearly establishes that the aqua-platinum complex does not show kinetic preference toward nucleotides, as was hypothesized[14] to explain the binding of platinum drugs to DNA in the cell. Furthermore, since the available platinum concentration in the cell is much smaller than glutathione and other sulfhydryl groups, formation of thiolato-bridged species can be ruled out. Finally, it remains to be seen how platinum reacts with DNA since the cellular milieu contains high concentrations of sulfhydryl groups that include cysteine, glutathione, and cytosolic proteins. To this end, Sadler and coworkers[29] recently indicated that GMP can effectively replace coordinated methionine from the Pt(dien)(methionine) complex.

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