Versatile Complex-Formation Kinetics Observed for the Reactions of a Model *cis*-Bis(amine)palladium(II) Complex with DNA Nucleosides and Nucleotides

S. Suvachittanont,[1a,b] H. Hohmann,[1a] R. van Eldik,*[,1a] and J. Reedijk[1c]

Institute for Inorganic Chemistry, University of Witten/Herdecke, Stockumer Strasse 10, 58448 Witten, Germany, and Department of Chemistry, Gorlaeus Laboratories, Leiden University, 2300 RA, Leiden, The Netherlands

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The complex-formation reactions of $Pd(Et_4en)Cl_2$ (Et_4en = *N,N,N',N'*-tetraethylethylenediamine) with inosine and inosine 5'-monophosphate were studied as a function of nucleophile and chloride concentration. Two consecutive reaction steps could be observed under all experimental conditions. A detailed kinetic analysis revealed that $[Pd(Et_4en)(Cl)H_2O]^+$ is the reactive species in the first complex-formation step, for which a steady-state approximation can be used. In the case of the second step, a rapid preequilibrium $[Pd(Et_4en)(Nu)Cl]^+ + H_2O \rightleftharpoons [Pd(Et_4en)(Nu)H_2O]^{2+} + Cl^-$ is followed by rate-determining substitution of the aqua complex to produce the 1:2 product species. The complex-formation reaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with the dinucleotide d(GpG) was studied as a function of nucleophile concentration, temperature and pressure. Two consecutive reaction steps were observed in which the first exhibits a dependence on the d(GpG) concentration and the second is concentration independent. The negative $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$ values for the first reaction step support an associative complex-formation reaction, which is followed by the slower ring-closure reaction.

Introduction

Intensive efforts of various research groups in recent years have contributed significantly toward an improved understanding of the mechanism of the antitumor activity of *cis*-bis(amine)-platinum(II) complexes.[2] Most of these studies focused on the structural identification of the bonding modes of the DNA constituents to the metal center;[2,3] however, significantly less is known about the mechanistic details of these interactions.[2,4] Our interest in the interaction of model diethylenetriamine and ethylenediamine complexes of Pd(II) with nucleic bases, nucleosides, and 5'-nucleotides has resulted in a series of kinetic studies that revealed a richness of mechanistic versatility for ligand displacement and complex-formation reactions in these systems that could be of fundamental importance to understand the antitumor activity of related Pt(II) complexes. We preferred to use model Pd(II) complexes in our earlier studies[4] due to the very similar overall thermodynamic behavior, but orders of magnitude higher reactivity than the corresponding Pt(II) complexes. For instance, it was shown in the case of the diethylenetriamine complexes that the only reactive species, even in the presence of a large excess of Cl^-, was the monoaqua complex, and various rate laws applied depending on the reactivity ratio of the various reaction steps involved.[5] In the case of complexes of the type $[Pd(R_4en)(H_2O)_2]^{2+}$, reactions with DNA nucleosides and nucleotides clearly indicated two consecutive substitution reactions,[4m,n] of which the second could be suppressed by addition of an excess of chloride.[4o]

We have now extended this work to a lower chloride concentration range more relevant for biological conditions and have been able to resolve the detailed kinetics of the two subsequent chloride substitution reactions of $Pd(Et_4en)Cl_2$ with inosine (Ino) and inosine 5'-monophosphate (IMP). Surprisingly, for both nucleophiles these subsequent chloride substitution reactions exhibit very different kinetic behaviour and involve the changeover from a steady-state to a preequilibrium behavior. In addition, we have investigated the complex-formation of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with the dinucleotide d(GpG) in order to resolve the nature of the subsequent ligand substitution reactions. The d(GpG) unit is known to be the major target for the binding of *cis*-Pt on the DNA strand.[2,4a,j] In this way the versatility of ligand displacement reactions by DNA fragments on model Pd(II) complexes could be studied more thoroughly.

Experimental Section

Materials. The $Pd(Et_4en)Cl_2$ complex (Et_4en = *N,N,N',N'*-tetraethylethylenediamine) was prepared and characterized as described before.[6] The dichloro complex was converted in solution to the diaqua complex

(1) (a) University of Witten/Herdecke. (b) On leave from the Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkla 90112, Thailand. (c) Leiden University.

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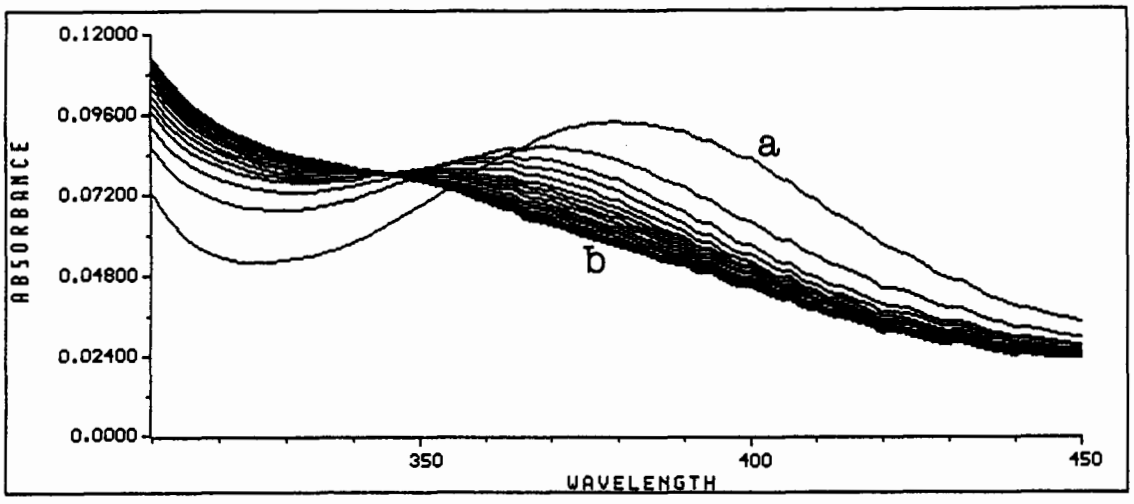

Figure 1. Repetitive scan spectra for the reaction of $Pd(Et_4en)Cl_2$ with inosine. Experimental conditions: [Pd(II)] = 2.5×10^{-4} M; [Cl^-] = 2.5×10^{-2} M; [Ino] = 2.0×10^{-2} M; pH = 4.5; ionic strength = 0.1 M; optical path length = 0.88 cm; Δt = 10 s; T = 25.0 °C. Key: (a) first spectrum; (b) final spectrum.

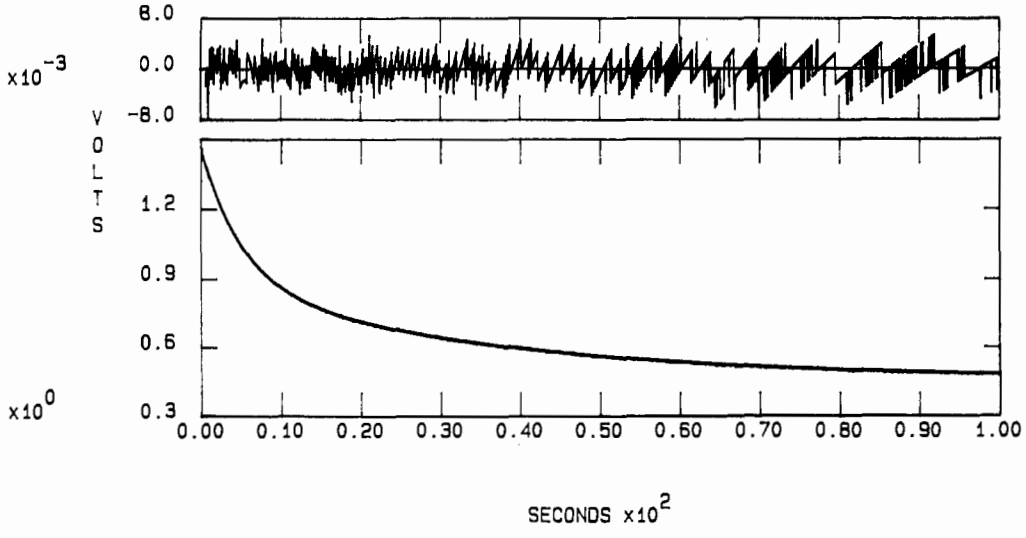

Figure 2. Typical kinetic trace recorded for the reaction of $Pd(Et_4en)Cl_2$ with inosine. Experimental conditions: [Pd(II)] = 2.5×10^{-4} M; [Cl^-] = 1.0×10^{-2} M; [Ino] = 2.0×10^{-2} M; pH = 4.5; ionic strength = 0.1 M; T = 25.0 °C; wavelength = 390 nm; 10 V = 1 absorbance unit. The difference between the experimental and fitted trace is given in the top part of the figure.

by treating it with $AgClO_4$ as described elsewhere for the corresponding ethylenediamine complex.[7] Inosine and inosine 5′-monophosphate were obtained from Sigma and used without further purification. d(GpG) was synthesized via an improved phosphotriester method and used as its sodium salt.[8] The pH of the test solutions was adjusted with $HClO_4$ and NaOH and measured before and after the reactions. Samples used for pH measurements were rejected subsequently in order to prevent any contamination by Cl^- in the cases where the diaqua complex was studied. The reference electrode of the pH meter was filled with NaCl instead of KCl to prevent the precipitation of $KClO_4$, since $NaClO_4$ was used to adjust the ionic strength of all test solutions to 0.10 M. Millipore water was used in the preparation of all solutions.

Measurements. UV–vis spectra were recorded on Shimadzu UV 250 and Hewlett-Packard diode-array spectrophotometers. Kinetic measurements were performed on a Durrum D110 stopped-flow unit attached to an on-line data acquisition system with which the kinetic traces were evaluated, using the OLIS KINFIT (Jefferson, GA) set of programs. Kinetic measurements at elevated pressure were performed on a homemade high-pressure stopped-flow instrument thermostated to ±0.1 °C.[9] All kinetic measurements were performed under pseudo-first-order conditions; i.e. at least a 10-fold excess of nucleophile was used. More details on the data-fitting procedure are given in the following section for the appropriate cases.

Results and Discussion

Reaction of $Pd(Et_4en)Cl_2$ with Inosine and Inosine 5′-Monophosphate. When $Pd(Et_4en)Cl_2$ is dissolved in slightly acidic aqueous solution it undergoes spontaneous solvolysis to produce $[Pd(Et_4en)(Cl)H_2O]^+$ and $[Pd(Et_4en)(H_2O)_2]^{2+}$, and the product distribution at equilibrium will depend on the chloride concentration in solution. The overall reactions are given in (1) and (2),

$$Pd(Et_4en)Cl_2 + H_2O \rightleftharpoons [Pd(Et_4en)(Cl)H_2O]^+ + Cl^- \quad K_1 \quad (1)$$

$$[Pd(Et_4en)(Cl)H_2O]^+ + H_2O \rightleftharpoons [Pd(Et_4en)(H_2O)_2]^{2+} + Cl^- \quad K_2 \quad (2)$$

for which $K_1 = (1.9 \pm 0.3) \times 10^{-2}$ and $K_2 = (3.5 \pm 0.2) \times 10^{-4}$M at 25 °C and 0.1 M ionic strength.[6] When the dichloro system is reacted with Ino and IMP, the chloride concentration will change during the reaction and will cause a change in the equilibrium distribution as defined by reactions 1 and 2. In order to prevent this and to maintain a well-defined speciation, an excess of chloride

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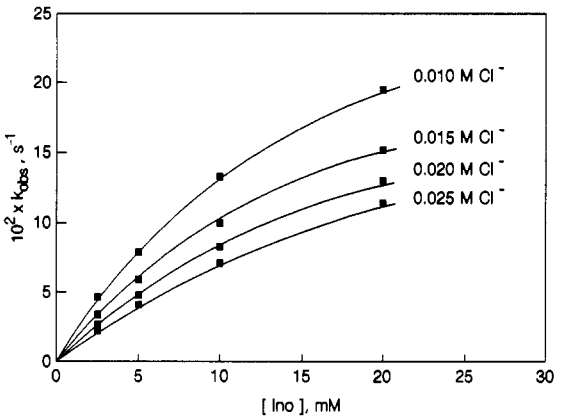

Figure 3. Plots of k_{obs} versus [nucleophile] for the first reaction step of $Pd(Et_4en)Cl_2$ with inosine as a function of $[Cl^-]$. Experimental conditions: [Pd(II)] = 2.5×10^{-4} M; pH = 4.5; ionic strength = 0.1 M; T = 25.1 °C.

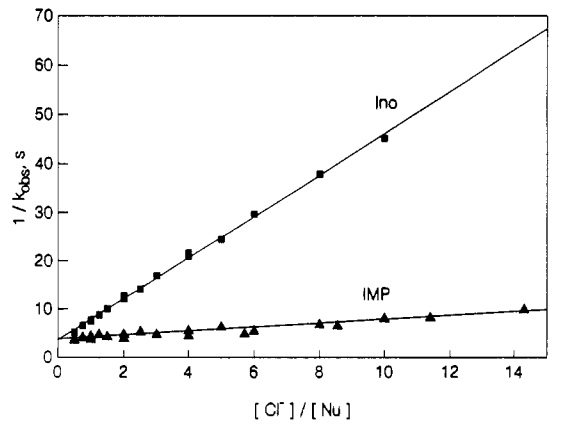

Figure 4. Plots of k_{obs}^{-1} versus $[Cl^-]/[nucleophile]$ for the first reaction step of $Pd(Et_4en)Cl_2$ with inosine and inosine 5′-monophosphate. For experimental conditions, see Figure 3.

was employed at such a level that only equilibrium 1 has to be taken into account; i.e. the amount of $[Pd(Et_4en)(H_2O)_2]^{2+}$ can be neglected. The repetitive scan spectra recorded under such conditions (see Figure 1) clearly show two consecutive reaction steps, of which the second slower step is characterized by an isosbestic point at 345 nm.

These two reaction steps can be separated from the absorbance decrease at 390 nm as illustrated in Figure 2, in which the experimental trace was fitted with two consecutive exponential functions. The values of the pseudo-first-order rate constant, k_{obs}, for the first reaction step for both nucleophiles exhibit a nonlinear dependence on the nucleophile concentration and decrease with increasing chloride concentration (see Figure 3). These trends are similar to those found before for the reaction of Ino and IMP with $Pd(Me_4en)Cl_2$[40] and are in agreement with the mechanistic scheme outlined in reactions 1 and 3 under the

$$Pd(Et_4en)Cl_2 + H_2O \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [Pd(Et_4en)(Cl)H_2O]^+ + Cl^- \quad (1)$$

$$[Pd(Et_4en)(Cl)H_2O]^+ + Nu \xrightarrow{k_3} [Pd(Et_4en)(Nu)Cl]^+ + H_2O \quad (3)$$

condition that the steady-state approximation applies to the aquachloro complex species. The corresponding rate law is given in (4), which predicts a linear relationship between k_{obs}^{-1} and

$$k_{obs} = k_1k_3[Nu]/\{k_2[Cl^-] + k_3[Nu]\} \quad (4)$$

Table I. Summary of Rate and Equilibrium Constants for the Reactions of $Pd(Et_4en)Cl_2$ with Inosine and Inosine 5′-Monophosphate in the Presence of an Excess of Cl^- [a]

rate/equilibrium const	value at 25 °C			ref
	Nu = Ino		Nu = IMP	
k_1, s^{-1}	0.275 ± 0.003		0.260 ± 0.011	*b*
		0.32 ± 0.01		6
		0.29 ± 0.01		6
k_2, M^{-1} s^{-1}		13.3 ± 0.5		6
k_3, M^{-1} s^{-1}	11.3 ± 1.2		126 ± 19	*b,c*
K_4, M	$(5.3 \pm 1.0) \times 10^{-3}$		$(13.8 \pm 1.5) \times 10^{-3}$	*b*
k_5, M^{-1} s^{-1}	4.0 ± 0.6		2.8 ± 0.2	b
	12.4		9.7	4n
k_6, s^{-1}	$(9.3 \pm 2.5) \times 10^{-4}$		$(24 \pm 2) \times 10^{-4}$	b

[a] Ionic strength = 0.1 M; pH ~ 4.0. [b] This study. [c] k_3 was calculated from the value of k_2/k_3 obtained in this study and k_2 from the literature.[6]

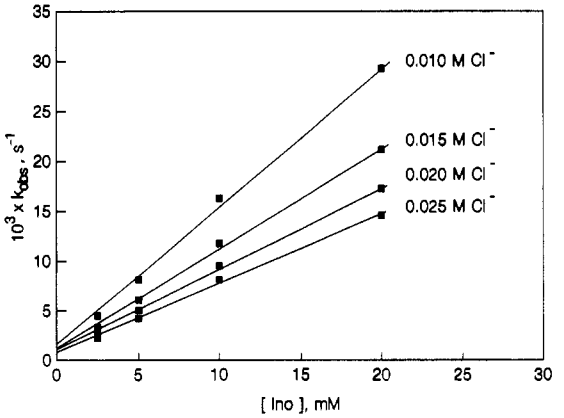

Figure 5. Plots of k_{obs} versus [nucleophile] for the second reaction step of $Pd(Et_4en)Cl_2$ with inosine as a function of $[Cl^-]$. For experimental conditions, see Figure 3.

$[Cl^-]/[Nu]$. The experimental data in Figure 3 nicely conform to this requirement (see Figure 4), from which the values of k_1 and and k_2/k_3 could be determined. These are summarized along with available literature values in Table I.

In contrast to the first reaction step, the second exhibits a completely different dependence on the nucleophile concentration as shown in Figure 5. These data are in line with the reaction scheme outlined in (5) and (6), where a rapid preequilibrium

$$[Pd(Et_4en)(Nu)Cl]^+ + H_2O \overset{K_4}{\rightleftharpoons} [Pd(Et_4en)(Nu)H_2O]^{2+} + Cl^- \quad (5)$$

$$[Pd(Et_4en)(Nu)H_2O]^{2+} + Nu \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [Pd(Et_4en)(Nu)_2]^{2+} + H_2O \quad (6)$$

reaction preceeds the rate-determining substitution step. According to the corresponding rate law in (7), plots of k_{obs} versus

$$k_{obs} = k_6 + k_5K_4[Nu]/\{K_4 + [Cl^-]\} \quad (7)$$

[Nu] should be linear with a common intercept k_6 and a slope $k_5K_4/\{K_4 + [Cl^-]\}$. From the $[Cl^-]$ dependence of the slope it is possible to estimate k_5 and K_4 as shown in Figure 6. The results are also included in Table I.

A comparison of the data obtained in this study with those reported for some of the reactions elsewhere[4m,n,6,7] reveals some important new information. First of all the limiting rate constant for the first reaction of $Pd(Et_4en)Cl_2$ with Ino and IMP reached at high nucleophile concentration and low $[Cl^-]$, i.e. k_1 obtained from the intercepts in Figure 4, is in good agreement with the

Table II. Summary of Observed Rate Constants and Activation Parameters for the Reaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with d(GpG)[a]

temp, °C	pressure, MPa	[d(GpG)], mM	first reaction step			second reaction step
			$10\,k_{obs}$,[b] s^{-1}	$10\,k_a$, s^{-1}	$10^{-2}\,k_b$, $M^{-1}\,s^{-1}$	$10^2\,k_{obs}$,[b] s^{-1}
25.3	0.1	0.50	2.95 ± 0.17	1.48 ± 0.33	3.36 ± 0.32	6.8 ± 1.0
		0.73	4.15 ± 0.21			6.6 ± 0.4
		1.11	5.32 ± 0.21			7.2 ± 1.0
		1.49	6.37 ± 0.20			7.9 ± 0.4
15.8	0.1	0.50	1.95 ± 0.06	0.69 ± 0.14	2.51 ± 0.26	3.7 ± 0.2
		1.11	3.48 ± 0.05			3.4 ± 0.3
35.5	0.1	0.50	4.51 ± 0.44	2.14 ± 0.41	4.74 ± 0.51	13.6 ± 1.2
		1.49	9.20 ± 0.41			15.3 ± 2.4
$\Delta H^{\ddagger}$, kJ mol^{-1}				40 ± 8	21.4 ± 1.1	50 ± 3
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}				−127 ± 29	−125 ± 4	−98 ± 9
24.8	10	1.11	5.9 ± 0.4			7.7 ± 1.8
	50		6.3 ± 0.5			6.8 ● 2.4
	100		6.7 ± 0.5			6.7 ± 1.2
	135		7.2 ± 0.6			7.2 ± 1.5
$\Delta V^{\ddagger}$, cm^3 mol^{-1}			−3.8 ± 0.2			+1 ± 2

[a] Experimental conditions: [Pd(II)] = 1×10^{-4} M; pH = 4.0; ionic strength = 0.1 M ($NaClO_4$); wavelength = 310 nm. [b] Mean value of at least four kinetic runs.

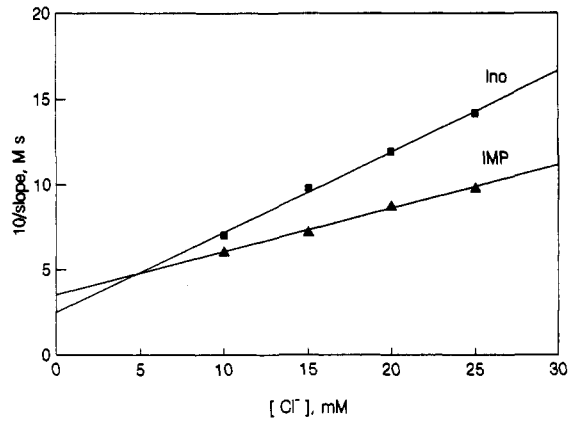

Figure 6. Plots of $(slope)^{-1}$ versus $[Cl^-]$ for the second reaction step of $Pd(Et_4en)Cl_2$ with inosine and inosine 5′-monophosphate according to the rate expression given in (7). For experimental conditions see Figure 3. Data taken from Figure 5 for the reaction with inosine.

values for k_1 measured directly.[6] The values of k_2 and k_3 are such that the nucleophilicity order is IMP > Ino ≈ Cl^-, which is in good agreement with similar trends reported earlier for the diaqua complex.[4n] It follows that Cl^- is a very efficient competitor for the binding of the model Pd(II) complexes to the DNA fragments. The values of K_4 indicate that the hydrolysis constant is significantly larger for the IMP than for the Ino chloro complex. The values of k_5 for the second substitution reaction are very similar for Ino and IMP, but ca. 3 times smaller than found in the direct study (starting with the diaqua complex) before.[4n] It should be kept in mind that the values of k_5 are extrapolated in an indirect manner in the present study, which may partially account for this apparent discrepancy. We repeated a few experiments starting with the dichloro complex in the absence of added Cl^- and found apparent values for k_5 to be 9.11 and 2.85 M^{-1} s^{-1} for Ino and IMP, respectively. Under these conditions the chloroaqua complex should be the main reactive species in solution, and the apparent values of k_5 are of the same order of magnitude as those quoted in Table I. At present we cannot offer a reasonable explanation for the observed deviations and can only conclude that the various procedures do result in values for k_5 of the same order of magnitude. The values of k_6 quoted in Table I demonstrate that the aquation of $Pd(Et_4en)(IMP)_2$ is faster than of the corresponding inosine complex, although the values are again of the same order of magnitude. In the direct study of the diaqua complex, the second complex formation step did not exhibit a significant intercept,[4n] which did not allow the determination of k_6. The formation constant for the 1:2 complex in reaction 6, i.e. k_5/k_6, is ca. 10^4 and 10^3 for Ino and IMP, respectively, which means that the inosine complex is somewhat more stable than the inosine 5′-monophosphate complex.

The most remarkable and important observation is the fact that different kinetic treatments, steady-state compared to a preequilibrium situation, must be used to fit the observed kinetic data for the two subsequent complex-formation reactions. In both cases it is an aqua complex that is the reactive species, and the ratio of the different rate constants determines whether a steady-state or a preequilibrium situation applies. Such a changeover in the kinetic treatment has been observed when the nature of the entering nucleophile is changed,[5] but not for two consecutive complex-formation reactions involving the identical nucleophile.

Reaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with d(GpG). The very limited availability of d(GpG) due to the time consuming and tedious preparation method,[8] restricted the number of kinetic experiments that could be performed. For this reason we preferred to perform the measurements with the diaqua complex, i.e. in the absence of free or added Cl^-, in order to simplify the system significantly. Furthermore, the data in the previous section and those obtained before[4n,o,p,5] clearly indicated that it is only an aqua complex that undergoes binding to the DNA constituents. On addition of d(GpG) to the diaqua complex there is a significant decrease in absorbance at 465 nm, a significant increase in absorbance at 310 nm and an isosbestic point at 350 nm. Kinetic traces at 310 nm, where the largest absorbance change occurs, clearly exhibited two consecutive exponential functions as can be seen from the typical trace in Figure 7. The kinetic data were recorded as a function of [d(GpG)], temperature, and pressure, for which the results are summarized in Table II.

The first reaction step exhibits a linear concentration dependence on [d(GpG)] according to the relationship $k_{obs} = k_a + k_b[d(GpG)]$, for which the values of k_a and k_b are included in Table II. The second reaction step exhibits, within the relatively large experimental error limits due to the small change in absorbance associated with this reaction step, no dependence on the [d(GpG)]. This is characteristic for a ring-closure reaction such that the overall complex-formation reaction can be formulated as shown in reactions 8 and 9. The rate law for the first

$$[Pd(Et_4en)(H_2O)_2]^{2+} + d(GpG) \underset{k_8}{\overset{k_7}{\rightleftharpoons}} [Pd(Et_4en)(d(GpG))H_2O]^{2+} + H_2O \quad (8)$$

$$[Pd(Et_4en)(d(GpG))H_2O]^{2+} \xrightarrow{k_9} [Pd(Et_4en)(d(GpG))]^{2+} + H_2O \quad (9)$$

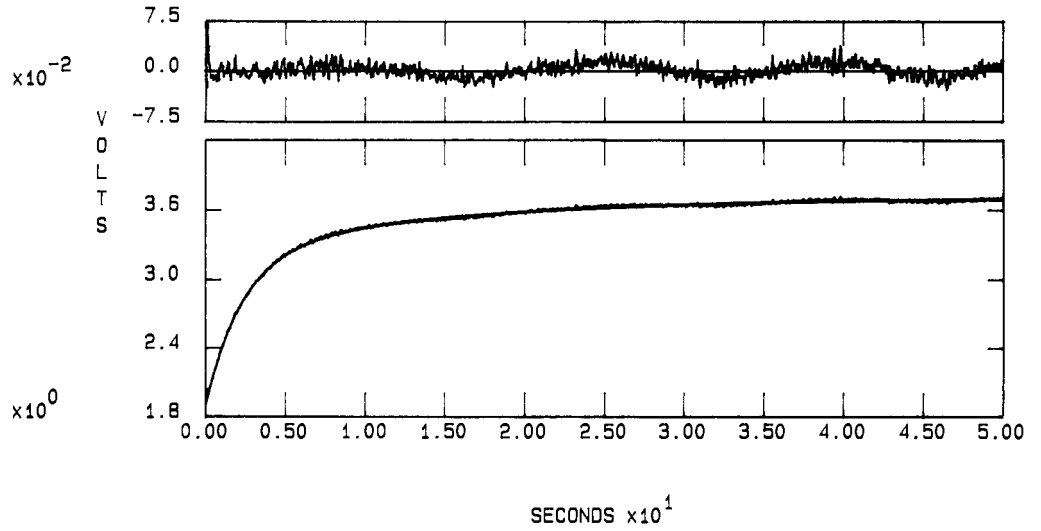

Figure 7. Typical kinetic trace for the reaction of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with d(GpG). Experimental conditions: [Pd(II)] = 1×10^{-4} M; [d(GpG)] = 7.3×10^{-4} M; pH = 4.0; T = 25.3 °C; ionic strength = 0.10 M; wavelength = 310 nm; 1 V = 1 absorbance unit. The difference between the experimental and fitted trace is given in the top part of the figure.

substitution reaction will be $k_{obs} = k_8 + k_7[d(GpG)]$, such that $k_7 = k_b$ and $k_8 = k_a$ in Table II, whereas k_{obs} for the ring-closure reaction will be k_9. The thermal activation parameters are such that they underline the operation of an associative substitution process for all reaction steps in (8) and (9). In addition, the kinetic experiments performed at elevated pressure do indicate that at relatively high [d(GpG)], where $k_{obs} \approx k_b[d(GpG)]$, the first reaction step does exhibit a negative ΔV^* value, rather characteristic for associative substitution reactions of square-planar complexes.[10] The absorbance changes for the second step are too small to obtain accurate kinetic data at elevated pressure as demonstrated by the large error limits of the data given in Table II. It follows that the diaqua complex reacts with the d(GpG) fragment via a fast water displacement reaction followed by a slower ring-closure step.

A comparison of the data in Table II with that reported[4n] for the subsequent substitution reactions of $[Pd(Et_4en)(H_2O)_2]^{2+}$ with guanosine 5′-monophosphate (GMP), reveals some interesting trends. The value of k_7 ($=k_b$) at 25 °C, viz. 336 M^{-1} s^{-1}, is ca. 6 times smaller than the value reported for the first substitution reactions with GMP, viz. 2.2×10^3 M^{-1} s^{-1} under similar experimental conditions, but ca. 13 times larger than the value found for the second substitution step, viz. 25.5 M^{-1} s^{-1}.[4n] This trend suggests that complex-formation of the diaqua complex with d(GpG) occurs at a rate intermediate to that for the first and second complex-formation reactions with GMP. Thus the nucleophilicity of d(GpG) seems to be somewhat lower than that of GMP, which may partly be related to the steric hindrance caused by the bulkiness of the d(GpG) structure. This effect is accompanied by a reverse reaction step k_8 ($=k_a$ in Table II) of 0.15 s^{-1} at 25 °C for the aquation of $[Pd(Et_4en)(d(GpG))H_2O]^{2+}$, something that was not observed for $[Pd(Et_4en)(GMP)H_2O]^{2+}$ and may reflect the weaker bonding of d(GpG) as a monodentate ligand than GMP.

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Conclusions

The results reported in this study demonstrate the richness of mechanistic information obtained for ligand substitution reactions of model antitumor complexes with DNA fragments. On the one hand the presence of chloride will affect the equilibrium distribution of the aquated species and so determine the rate of the substitution process. Under all our experimental conditions it is only the aqua complexes that undergo ligand substitution, presumably due to their significantly higher lability than the corresponding chloro complexes. Furthermore, in contrast to our earlier work on the $Pd(Me_4en)Cl_2$ complex where only the first substitution reaction with Ino and IMP in the presence of chloride could be observed, evidence for two subsequent substitution reactions could now be found for the corresponding Et_4en system. When a bidentate fragment such as d(GpG) is used, ring-closure is a more effective reaction step than the entrance of a second fragment into the coordination sphere of the complex. This accounts for the preferred binding of *cis*-platinum complexes to the GpG sequence in DNA.[2,3] The reversible nature of the first complex-formation reaction, (8), found for the binding of d(GpG) to the model $[Pd(Et_4en)(H_2O)_2]^{2+}$ species is most probably related to the high lability of Pd(II) complexes in general. This is not expected to occur in the case of the less labile Pt(II) complexes (see Introduction), in agreement with the irreversible binding of Pt(II) to G-N7 in DNA. In addition, Pt(II) antitumor complexes have NH groups available for H-bonding that affect the selectivity for the binding to G-N7 in DNA.

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