Steric and Nucleophilic Control over the Versatile Complex-Formation Kinetics of Model *cis*-Bis(amine)palladium(II) Complexes with Inosine and Inosine 5'-Monophosphate

Surapong Suvachittanont[1] and Rudi van Eldik*

Institute for Inorganic Chemistry, University of Witten/Herdecke, Stockumer Strasse 10, 58448 Witten, Germany

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A detailed kinetic study was undertaken of the complex-formation reactions of $Pd(en)Cl_2$ and $Pd(Me_4en)Cl_2$ (Me_4en = *N,N,N',N'*-tetramethylethylenediamine) with inosine and inosine 5'-monophosphate as a function of nucleophile and chloride concentrations. Two consecutive reaction steps could be observed for the Me_4en complex. The kinetic data clearly demonstrate a changeover from steady-state to pre-equilibrium behavior on changing steric hindrance and the nature of the nucleophile. All complex-formation reactions proceed via the formation of reactive intermediates of the type $[Pd(R_4en)(Cl)H_2O]^+$ and $[Pd(R_4en)(Nu)H_2O]^{2+}$. The results are compared to available data for the corresponding Et_4en system and allow a detailed discussion of the steric and nucleophilic control over the kinetics of such complex-formation reactions with representative DNA constituents.

Introduction

We have in recent years developed an interest in the mechanistic behavior of model *cis*-bis(amine)palladium(II) complexes in their reactions with purine nucleosides and 5'-nucleotides in reference to the antitumor behavior of related platinum complexes.[2-6] Our kinetic studies have revealed a richness of mechanistic versatility for ligand displacement and complex-formation reactions in these systems[6] that could be of fundamental importance to the understanding of the antitumor activity. Intensive efforts of various research groups, mainly focusing on the structural identification of the bonding modes of the DNA constituents to the metal center,[7] have contributed significantly toward an improved understanding of the antitumor activity of *cis*-bis(amine)platinum(II) complexes. However, it is generally accepted that such binding processes may be kinetically controlled,[7] which calls for a detailed mechanistic clarification of these interactions.

In some earlier work on a series of (diethylenetriamine)palladium(II) complexes it was found that various kinetic rate laws applied depending on the reactivity ratio of the various reaction steps involved in their interaction with nucleic bases, nucleosides, and 5'-nucleotides.[8-10] This work was recently extended to a series of ethylenediamine (en) complexes of the type $Pd(R_4en)Cl_2$, where R represents the substituent on the N-donor atoms and can be H, Me, or Et.[2-6] Such complexes exhibit rapid spontaneous solvolysis reactions to produce $[Pd(R_4en)(Cl)H_2O]^+$ and $[Pd(R_4en)(H_2O)_2]^{2+}$, such that the free chloride concentration in solution will determine the speciation of the complexes.[2] Furthermore, the substituent R allows a steric control over the ligand substitution and complex-formation reactions of these complexes with typical DNA constituents, viz. inosine (Ino) and inosine 5'-monophosphate (IMP), selected for this study. In the case of the most sterically hindered $Pd(Et_4en)Cl_2$ complex it was found that the first complex-formation step involves the $Pd(Et_4en)(Cl)H_2O^+$ species and follows steady-state kinetics, whereas the second complex-formation step involves a rapid preequilibrium of the 1:1 complex followed by a rate-determining substitution of the aqua complex to produce the 1:2 product species.[6] We have now performed similar detailed kinetic studies on the $Pd(Me_4en)Cl_2$ and $Pd(en)Cl_2$ systems, and the data allow us to comment on the steric and nucleophilic control over the versatile complex-formation kinetics of such complexes with Ino and IMP.

Experimental Section

Materials. The $Pd(en)Cl_2$ and $Pd(Me_4en)Cl_2$ complexes (Me_4en = *N,N,N',N'*-tetramethylethylenediamine) were prepared and characterized as described before.[2] Inosine and inosine 5'-monophosphate were obtained from Sigma and used without further purification. The pH of the test solutions was adjusted with $HClO_4$ and NaOH and measured before and after the reactions. 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 8452A 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. 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.

Results and Discussion

When $Pd(R_4en)Cl_2$ is dissolved in slightly acidic aqueous solution (pH ≈ 4.5), it undergoes spontaneous solvolysis to produce $[Pd(R_4en)(Cl)H_2O]^+$ and $[Pd(R_4en)(H_2O)_2]^{2+}$, where the equilibrium distribution will depend on the chloride concentration in solution. The overall reactions are given in (1) and (2), for

$$Pd(R_4en)Cl_2 + H_2O \overset{K_1}{\rightleftharpoons} [Pd(R_4en)(Cl)H_2O]^+ + Cl^- \quad (1)$$

$$[Pd(R_4en)(Cl)H_2O]^+ + H_2O \overset{K_2}{\rightleftharpoons} [Pd(R_4en)(H_2O)_2]^{2+} + Cl^- \quad (2)$$

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(1) On leave from the Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkla 90112, Thailand.
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which the equilibrium constants have the values $K_1 = (7.7 \pm 0.8) \times 10^{-3}$, $(9.5 \pm 1.3) \times 10^{-3}$, $(1.9 \pm 0.3) \times 10^{-2}$ M and $K_2 = (2.5 \pm 0.1) \times 10^{-4}$, $(3.1 \pm 0.5) \times 10^{-4}$, $(3.5 \pm 0.2) \times 10^{-4}$ M for R = H, Me, and Et, respectively, at 25 °C and 0.10 M ionic strength.[2] On reaction of the dichloro and monochloro complexes with Ino and IMP, the chloride concentration in solution will change during the reaction and will cause a change in the speciation of the reactive species. In order to prevent this and to maintain a well-defined speciation, an excess of chloride was employed at such a level that only equilibrium 1 has to be taken into account; i.e., the amount of $[Pd(R_4en)(H_2O)_2]^{2+}$ can be neglected. This was done on purpose since the reactions of the latter species with Ino and IMP have been studied in detail before and rate data for the two subsequent complex-formation reactions are available.[3,4]

A further possible complication that must be taken into account is the deprotonation of the aqua complexes produced in (1) and (2) to form unreactive hydroxo complexes. The pK_a values for $[Pd(R_4en)(H_2O)_2]^{2+}$ and $[Pd(R_4en)(Cl)H_2O]^+$ were determined before[2] and have the values 5.6 ± 0.2, 5.4 ± 0.2, 5.8 ± 0.2 and 7.3 ± 0.2, 7.0 ± 0.2, 7.7 ± 0.2 for R = H, Me, and Et, respectively. It follows that at the selected pH of 4.5 no significant interference of the aqua hydroxo and chloro hydroxo species is expected. Furthermore, this pH will prevent protonation of the N7 and deprotonation of the N1 coordination sites of Ino and IMP, such that coordination is expected to occur at N7.[11-13] Protonation of the dianionic phosphate moiety in IMP occurs at $pK_a \approx 6$ [14] such that a monoanionic phosphate group will exist under the selected experimental conditions.

Reaction of Pd(en)Cl$_2$ with Ino and IMP. The reactions of Pd(en)Cl$_2$ in the presence of varying [Cl$^-$] with Ino and IMP are accompanied by significant spectral changes similar to those reported for the corresponding Et$_4$en system.[6] These consist of an absorbace increase at 305 nm and an absorbance decrease at 380 nm. The kinetic traces at these wavelengths gave the best reproducible data when they were fitted with single-exponential functions. A comparison of the kinetic data recorded at the two wavelengths did indicate some significant differences, which could only be assigned to a minor influence of a second reaction step that would affect the quality of the fit. However, the expected second step showed such small absorbance changes that the kinetic traces could not be fitted with two exponential functions as done before.[6]

The values of the pseudo-first-order rate constant, k_{obs}, exhibit a nonlinear dependence on the nucleophile concentration and decrease with increasing chloride concentration for both Ino and IMP at both wavelengths. Typical data for the reaction with Ino recorded at 380 nm and with IMP recorded at 305 nm are shown in Figures 1 and 2, respectively. These trends are similar to those found before for the corresponding reactions of Pd(Me$_4$en)Cl$_2$ and Pd(Et$_4$en)Cl$_2$[5,6] and are in agreement with the general mechanism outlined in reactions 1 and 3 under the condition that

$$Pd(R_4en)Cl_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Pd(R_4en)(Cl)H_2O]^+ + Cl^- \quad (1)$$

$$[Pd(R_4en)(Cl)H_2O]^+ + Nu \xrightarrow{k_3} [Pd(R_4en)(Nu)Cl]^+ + H_2O \quad (3)$$

the steady-state approximation applies to the aquachloro complex. The corresponding rate law is given in (4) , which predicts a linear relationship between k_{obs}^{-1} and [Cl$^-$]/[Nu]. Such relationships are shown for the reaction with both nucleophiles

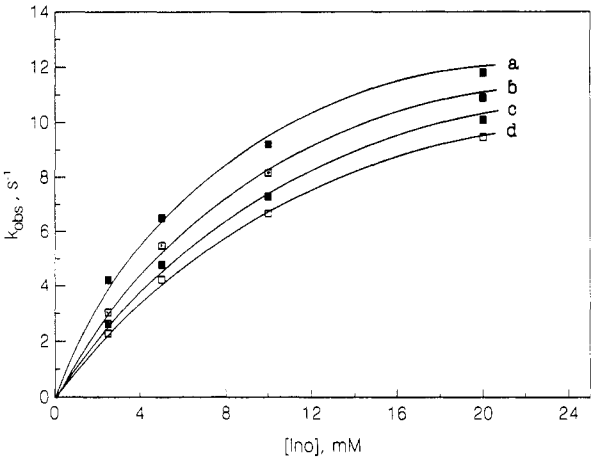

Figure 1. Plots of k_{obs} versus [nucleophile] for the reaction of Pd(en)Cl$_2$ with inosine as a function of [Cl$^-$]: (a) 0.01 M Cl$^-$; (b) 0.015 M Cl$^-$; (c) 0.02 M Cl$^-$; (d) 0.025 M Cl$^-$. Experimental conditions: [Pd(II)] = 2.5 × 10^{-4} M; pH = 4.5; ionic strength = 0.1 M; T = 25.1 °C; wavelength = 380 nm.

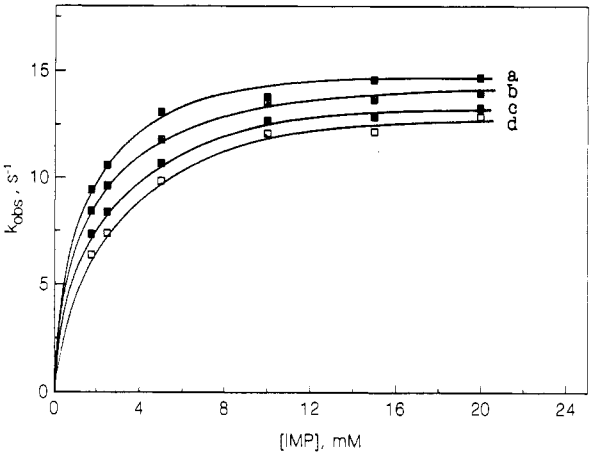

Figure 2. Plots of k_{obs} versus [nucleophile] for the reaction of Pd(en)Cl$_2$ with inosine 5′-monophosphate as a function of [Cl$^-$]: (a) 0.01 M Cl$^-$; (b) 0.015 M Cl$^-$; (c) 0.02 M Cl$^-$; (d) 0.025 M Cl$^-$. Experimental conditions are as in Figure 1 but at wavelength = 305 nm.

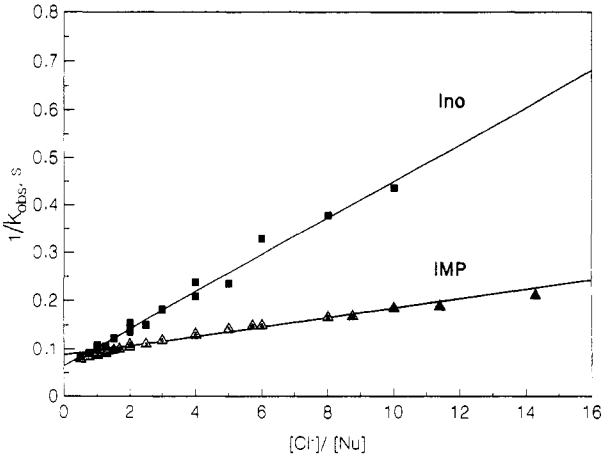

Figure 3. Plots of k_{obs}^{-1} versus [Cl$^-$]/[nucleophile] for the reaction of Pd(en)Cl$_2$ with inosine and inosine 5′-monophosphate studied at 380 nm.

$$k_{obs} = k_1k_3[Nu]/\{k_{-1}[Cl] + k_3[Nu]\} \quad (4)$$

recorded at 380 and 305 nm in Figures 3 and 4, respectively. The experimental data in Figures 3 and 4 nicely conform to the expected behavior and do not show a major difference for the two wavelengths used to study the reactions. Thus the interference of the second substitution reaction to produce $[Pd(R_4en)(Nu)_2]^{2+}$

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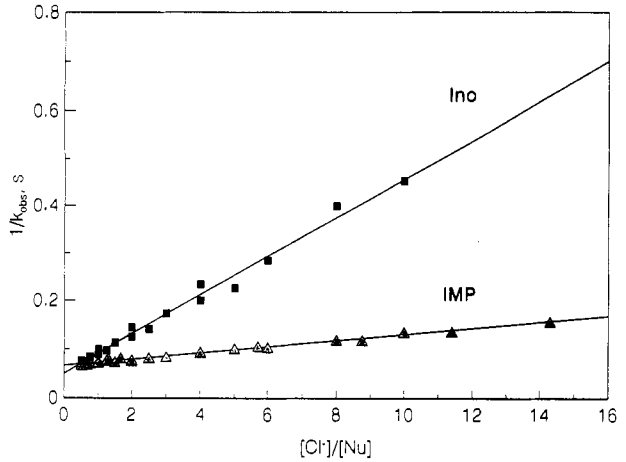

Figure 4. Plots of k_{obs}^{-1} versus [Cl⁻]/[nucleophile] for the reaction of $Pd(en)Cl_2$ with inosine and inosine 5′-monophosphate studied at 305 nm.

Table 1. Summary of Rate/Equilibrium Constants for the Reactions of $Pd(R_4en)Cl_2$ with Inosine and Inosine 5′-Monophosphate in the Presence of an Excess of [Cl⁻] [a]

R	rate/equil consts	Nu = Ino	Nu = IMP
H	k_1, s^{-1}	19.7 ± 2.1 (at 305 nm)	14.9 ± 0.2 (at 305 nm)
		15.4 ± 1.3 (at 380 nm)	11.4 ± 0.3 (at 380 nm)
		15.7 ± 0.8;[b]	9.7 ± 0.1[b]
	k_3/k_{-1}	1.2 ± 0.2 (at 305 nm)	10.6 ± 0.4 (at 305 nm)
		1.7 ± 0.2 (at 380 nm)	8.9 ± 0.5 (at 380 nm)
	k_{-1}, M^{-1} s^{-1}	1340 ± 20[b]	
	k_3, M^{-1} s^{-1}	(1.7 ± 0.2) × 10^3	(1.42 ± 0.07) × 10^4
		(2.2 ± 0.3) × 10^3	(1.20 ± 0.09) × 10^4
Me	k_1, s^{-1}	4.0 ± 0.4	2.85 ± 0.07
		2.9 ± 0.1;[b]	2.8 ± 0.1[b]
	k_3/k_{-1}	1.4 ± 0.2	10.8 ± 0.8
	k_{-1}, M^{-1} s^{-1}	286 ± 8[b]	
	k_3, M^{-1} s^{-1}	(4.0 ± 0.6) × 10^2	(3.1 ± 0.3) × 10^3
	K_4, M		(3.5 ± 0.1) × 10^{-2}
	k_5, M^{-1} s^{-1}		37.3 ± 0.4
	k_{-5}, s^{-1}		(3.74 ± 0.07) × 10^{-2}
	k_4, s^{-1}	2.8 ± 0.4	
	k_5/k_{-4}	0.6 ± 0.1	
Et[c]	k_1, s^{-1}	0.27 ± 0.01	0.26 ± 0.01
		0.32 ± 0.01;[b]	0.29 ± 0.01[b]
	k_3/k_{-1}	0.86 ± 0.06	9.5 ± 0.9
	k_{-1}, M^{-1} s^{-1}	13.3 ± 0.5[b]	
	k_3, M^{-1} s^{-1}	11 ± 1	126 ± 19
	K_4, M	(5 ± 1) × 10^{-3}	(14 ± 1) × 10^{-3}
	k_5, M^{-1} s^{-1}	4.0 ± 0.6	2.8 ± 0.2
		12.4[d]	9.7[d]
	k_{-5}, s^{-1}	(9 ± 2) × 10^{-2}	(24 ± 2) × 10^{-4}

[a] [Pd(II)] = 2.5 × 10^{-4} M; ionic strength = 0.1 M; pH = 4.5; T = 25.1 °C. [b] Data taken from ref 2. [c] Data taken from ref 6. [d] Data taken from ref 4.

seems to be minor. The data in Figures 3 and 4 were used to determine the values of k_1 and k_{-1}/k_3, which are summarized along with available literature data in Table 1. The literature value for k_{-1} was employed to estimate the value of k_3.

Reaction of $Pd(Me_4en)Cl_2$ with Ino and IMP. These reactions were studied before in a significantly higher chloride concentration range and higher ionic strength than selected in the present study.[5] Under those conditions the kinetic traces only exhibited one exponential function and it was concluded that the effective competition of Cl⁻ prevented the formation of the 1:2 complex with Ino and IMP. However, under the conditions selected in this study, kinetic traces clearly exhibited evidence for two exponential decays as shown in Figure 5. The data for the first reaction step with Ino and IMP indicate exactly the same behavior as described above for the reactions with $Pd(en)Cl_2$ and as found before.[5] A typical set of data for the reaction with IMP is shown in Figure 6, and the resulting plots of k_{obs}^{-1} versus [Cl⁻]/[Nu] are given in Figure 7. It follows that the first reaction step can be assigned to the formation of the 1:1 complex as shown in (1) and (3), for which the steady-state treatment is applicable.

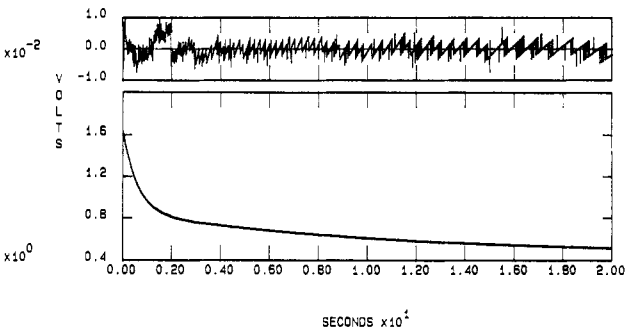

Figure 5. Typical kinetic trace recorded for the reaction of $Pd(Me_4en)Cl_2$ with inosine 5′-monophosphate. Experimental conditions: [Pd(II)] = 2.5 × 10^{-4} M; [Cl⁻] = 2.0 × 10^{-2} M; [IMP] = 2.5 × 10^{-3} M; pH = 4.5; ionic strength = 0.1 M; T = 25.1 °C; wavelength = 390 nm. The difference between the experimental and fitted trace is given in the top part of the figure.

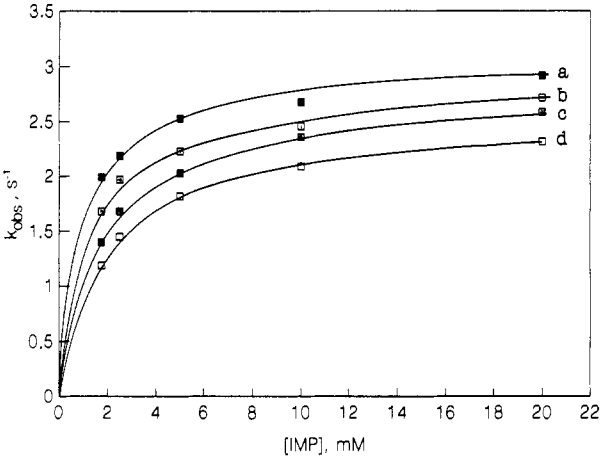

Figure 6. Plots of k_{obs} versus [nucleophile] for the first reaction step of $Pd(Me_4en)Cl_2$ with inosine 5′-monophosphate as a function of [Cl⁻]: (a) 0.01 M Cl⁻; (b) 0.015 M Cl⁻; (c) 0.02 M Cl⁻; (d) 0.025 M Cl⁻. Experimental conditions: [Pd(II)] = 2.5 × 10^{-4} M; pH = 4.5; ionic strength = 0.1 M; T = 25.1 °C; wavelength = 390 nm.

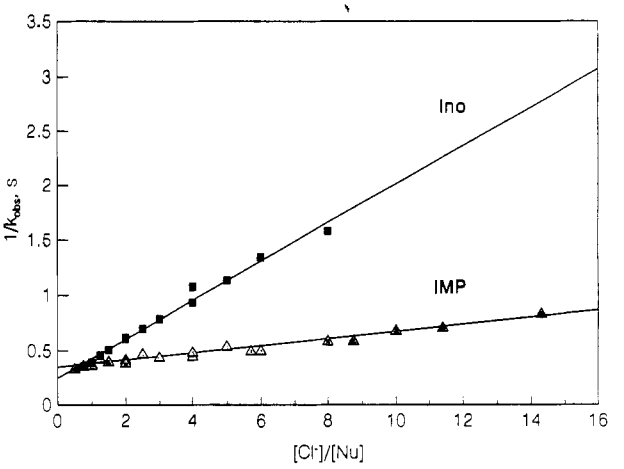

Figure 7. Plots of k_{obs}^{-1} versus [Cl⁻]/[nucleophile] for the first reaction step of $Pd(Me_4en)Cl_2$ with inosine and inosine 5′-monophosphate. For experimental conditions, see Figure 6.

A very surprising difference was observed in the kinetic data for the second reaction step involving Ino and IMP. The results reported in Figure 8 for the second reaction step with Ino follow the same pattern as for the first reaction and suggest that the formation of the 1:2 complex must occur according to reactions 5 and 6 . On application of steady-state conditions to [Pd(R_4-

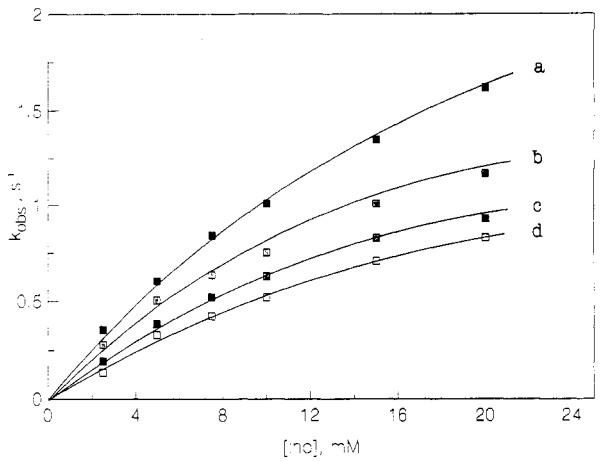

Figure 8. Plots of k_{obs} versus [nucleophile] for the second reaction step of $Pd(Me_4en)Cl_2$ with inosine as a function of $[Cl^-]$: (a) 0.01 M Cl^-; (b) 0.015 M Cl^-; (c) 0.02 M Cl^-; (d) 0.025 M Cl^-. For experimental conditions, see Figure 6

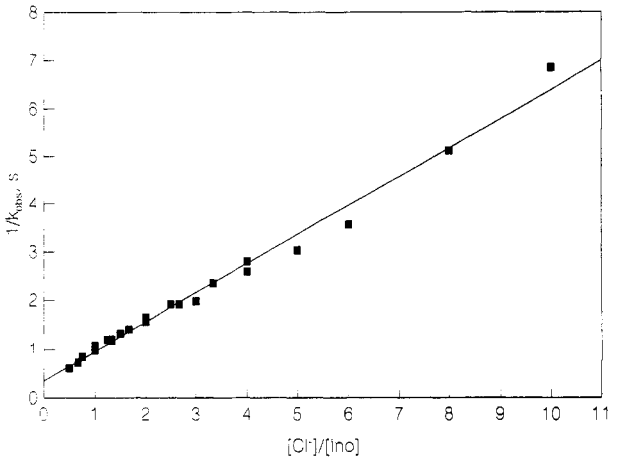

Figure 9. Plots of k_{obs}^{-1} versus $[Cl^-]/[\text{nucleophile}]$ for the second reaction step of $Pd(Me_4en)Cl_2$ with inosine. For experimental conditions, see Figure 6.

$$[Pd(R_4en)(Nu)Cl]^+ + H_2O \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} [Pd(R_4en)(Nu)H_2O]^{2+} + Cl^- \quad (5)$$

$$[Pd(R_4en)(Nu)H_2O]^{2+} + Nu \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} [Pd(R_4en)(Nu)_2]^{2+} + H_2O \quad (6)$$

$en)(Nu)H_2O]^{2+}$, and in the absence of a back reaction k_{-5} since the plots in Figure 8 show no significant intercepts, the corresponding rate law is given in 7 . This is similar to (4) and

$$k_{obs} = k_4k_5[Nu]/\{k_{-4}[Cl^-] + k_5[Nu]\} \quad (7)$$

once again predicts a linear relationship between k_{obs}^{-1} and $[Cl^-]/[Nu]$, which is indeed the case as demonstrated by Figure 9. From the plots in Figures 7 and 9 the values of k_1 and k_3 for the first reaction steps with Ino and IMP, and the values for k_4 and k_5/k_{-4} for the second reaction step with Ino, can be determined and are summarized in Table 1.

The second reaction step for IMP as nucleophile exhibits a completely different concentration dependence, viz. a linear dependence with a common intercept, as shown in Figure 10. These data are in line with the reactions outlined in (5) and (6), on the assumption that (5) is a rapid pre-equilibrium, i.e. $K_4 = k_4/k_{-4}$, and reaction 6 is a reversible process with k_{-5} being the common intercept in Figure 10. A similar behavior was observed for the second reaction step of $Pd(Et_4en)Cl_2$ with both Ino and IMP.[6] The corresponding rate law given in (8) predicts a linear

$$k_{obs} = k_{-5} + k_5K_4[Nu]/\{K_4 + [Cl^-]\} \quad (8)$$

dependence of k_{obs} on [Nu] with a common intercept k_{-5} 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 11, and the results are included in Table 1 along with those available from the literature.

Overall Comparison of Data. All the available data for reactions 1, 3, 5, and 6, along with those determined before, are summarized in Table 1 for the three complexes studied. The first and second substitution reactions leading to the formation of the $[Pd(R_4en)(Nu)Cl]^+$ and $[Pd(R_4en)(Nu)_2]^{2+}$ species, respectively, exhibit a versatile kinetic behavior as summarized in Table 2. A changeover from a steady-state to a pre-equilibrium behavior was observed for the first and second substitution reactions of $Pd(Me_4en)Cl_2$ with IMP and of $Pd(Et_4en)Cl_2$ with both Ino and

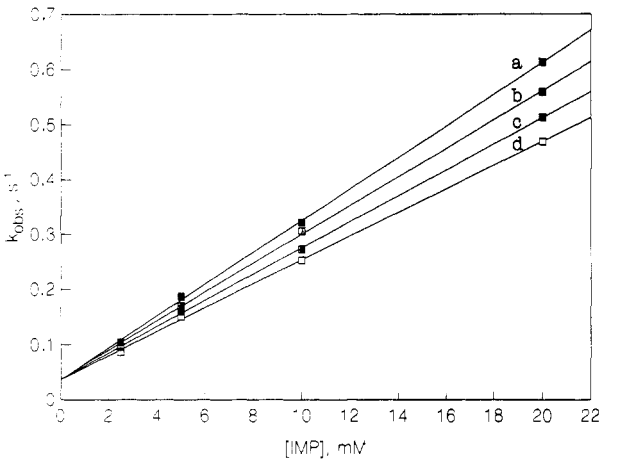

Figure 10. Plots of k_{obs} versus [nucleophile] for the second reaction step of $Pd(Me_4en)Cl_2$ with inosine 5′-monophosphate as a function of $[Cl^-]$: (a) 0.01 M Cl^-; (b) 0.015 M Cl^-; (c) 0.02 M Cl^-; (d) 0.025 M Cl^-. For experimental conditions see Figure 6.

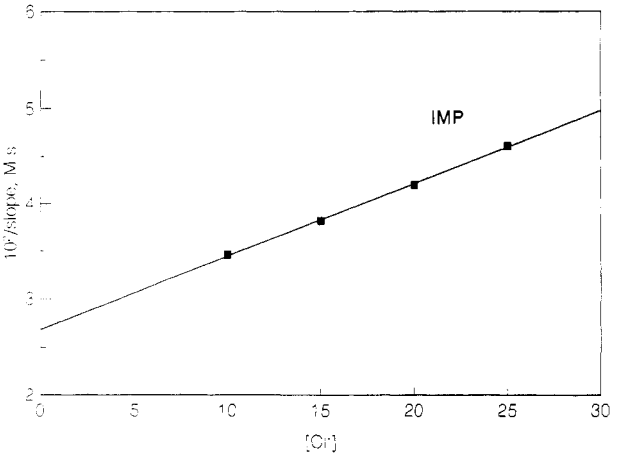

Figure 11. Plots of $(\text{slope})^{-1}$ versus $[Cl^-]$ for the second reaction step of $Pd(Me_4en)Cl_2$ with inosine 5′-monophosphate. For experimental conditions, see Figure 6. Data are taken from Figure 10.

IMP. In contrast, no such changeover was observed for the reactions of $Pd(Me_4en)Cl_2$ with Ino. This clearly demonstrates that the versatile kinetic behavior is controlled by steric hindrance on the ethylenediamine ligand and the nucleophilicity of the entering nucleophile. Increasing steric hindrance is expected to slow down, whereas increasing nucleophilicity is expected to speed up the ligand substitution reaction in terms of an associative mechanism. All available activation parameters support the

Table 2. Summary of the Type of Kinetic Data Treatment Employed for the Reactions of $Pd(R_4en)Cl_2$ with Ino and IMP

R	Nu	no. of reacn steps obsd	kinet treatment employed
H	both Ino and IMP	1	steady state
Me	Ino	2	1: steady state 2: steady state
	IMP	2	1: steady state 2: pre-equilibrium
Et	both Ino and IMP	2	1: steady state 2: pre-equilibrium

operation of an associative substitution mechanism.[3,4] This steric and electronic control affects the relative rates of the competing reactions in (5) and (6), and the relative values of k_4, $k_{-4}[Cl^-]$ and $k_5[Nu]$ will determine the steady-state or pre-equilibrium approximation. For the steady-state treatment these terms will be of the same magnitude, whereas k_4 and $k_{-4}[Cl^-]$ must be significantly larger than $k_5[Nu]$ for the pre-equilibrium treatment. Thus the coordination of one Ino or IMP molecule to the Et_4en complex in $[Pd(Et_4en)(Nu)Cl]^+$ causes a changeover to a pre-equilibrium situation as compared to a steady-state situation for the $Pd(Et_4en)Cl_2$ complex. A similar changeover occurs for the reactions of IMP with $Pd(Me_4en)Cl_2$ but not for the reactions of Ino with this complex. These observations clearly indicate that steric hindrance caused by the larger IMP molecule overrules the larger nucleophilicity usually observed for this ligand.

The data in Table 1 show some interesting trends. The values of k_1 determined in an indirect way in the present study are in close agreement with those determined in a direct way before.[2] The values of k_3 for all three complexes are approximately 10 times larger for the reaction with IMP than for the reaction with Ino, which must be related to the higher nucleophilicity of IMP observed before.[4] This is only the case for the first substitution reaction since the values of k_5 for the Et_4en complex are indeed very similar for both Ino and IMP. The values of k_5 determined in an indirect way in our earlier study[6] do differ by a factor of 3 from those measured directly,[4] but no reasonable explanation for this deviation could be offered.[6] Furthermore the data in Table 1 clearly demonstrate the effect of steric hindrance on the ethylenediamine ligand. There is a consistent decrease in k_3 and k_5 along the series en > Me_4en > Et_4en.

The results of this study once again clearly indicate that the aqua complexes, $[Pd(R_4en)(Cl)H_2O]^+$ and $[Pd(R_4en)(Nu)H_2O]^+$, are the reactive species in the ligand substitution reactions even in the presence of an excess of chloride. This must be related to their significantly higher lability than the corresponding chloro complexes. Substitution of a chloride ligand by water decreases the electron density on the metal center and makes it more reactive toward associative nucleophilic attack. A similar sequence of reactions can be expected to account for the binding of *cis*-$Pt(NH_3)_2Cl_2$ to two adjacent guanine sites on DNA[7] and will involve the intermediates *cis*-$[Pt(NH_3)_2(Cl)H_2O]^+$ and *cis*-$[Pt(NH_3)_2(G\text{-}N7)H_2O]^+$.

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