Mechanistic information on the reaction of model palladium(II) complexes with purine nucleosides and 5′-nucleotides in reference to the antitumor activity of related platinum complexes

M. Shoukry*, H. Hohmann and R. van Eldik**
Institute for Inorganic Chemistry, University of Witten/Herdecke, Stockumer Strasse 10, 5810 Witten (FRG)

Abstract

The reaction of $Pd(Me_4en)Cl_2$ ($Me_4en = N,N,N',N'$-tetramethylethylenediamine) with inosine and 5′-inosine monophosphate was studied as a function of chloride concentration and pH. Evidence for the formation of a 1:1 complex with these nucleophiles is presented, and the kinetic data indicate that $Pd(Me_4en)(Cl)H_2O^+$ is the only reactive species under the selected experimental conditions. The results are compared to earlier data for the reactions of $Pd(R_4en)(H_2O)_2^{2+}$ (R = H, Me, Et) with a series of nucleosides and 5′-nucleotides. The dependencies studied in this investigation allow some tentative extrapolations to conditions relevant for the antitumor activity of related Pt(II) complexes.

Introduction

The antitumor activity of *cis*-Pt(II)(diamine) complexes has stimulated significant interest in the interaction of such species with DNA and its constituents in efforts to contribute towards a better understanding of the mechanism of the antitumor activity [1]. Most of the performed investigations involved the structural identification of the reaction products, such that a good understanding of the bonding modes of the DNA constituents to the metal center has been achieved. However, considerably less is known about the mechanistic details of these interactions and this topic requires further attention from kineticists.

In the first work we performed in this area [2], model diethylenetriamine (dien) complexes of Pd(II) were employed, for which the fundamental substitution behaviour had been studied in great detail before [3]. In these complexes three coordination sites are blocked by the dien ligand and only one coordination site is available for complex formation with a series of nucleic bases, nucleosides and 5′-nucleotides. The rate-determining step in all cases was found [2] to be the substitution of $Pd(dien)H_2O^{2+}$ by the DNA constituents. Addition of Cl^- to the reaction mixture caused a significant decrease in the observed rate constants due to the formation of $Pd(dien)Cl^+$ which did not react directly with the DNA constituents, but only via solvolysis, i.e. the formation of $Pd(dien)H_2O^{2+}$, followed by the mentioned reaction. A similar trend can be expected when the pH is increased from *c.* 4 to 7 due to the formation of inert $Pd(dien)OH^+$ species [4]. In this work [2] it was possible to distinguish between the reactivity of nucleic bases, nucleosides and 5′-nucleotides.

More recently we initiated a series of studies on model ethylenediamine (en) and N-substituted en complexes of Pd(II) in order to investigate the reactivities of the species $Pd(R_4en)Cl_2$, $Pd(R_4en)(H_2O)Cl^+$ and $Pd(R_4en)(H_2O)_2^{2+}$ (R = H, Me, Et) with typical purine nucleosides and 5′-nucleotides. The reactions of these species can be considered as a reference system for the corresponding *cis*-$Pt(NH_3)_2Cl_2$ species. The introduction of steric hindrance on the en ligand enables a kinetic control over the lability of these complexes and allows an extrapolation of the data to the less labile Pt(II) complexes. The quoted Pd(II) and Pt(II) complexes exhibit very similar thermodynamic properties in terms of complex formation and acid dissociation constants, although their reactivity differs by up to 5 orders of magnitude [5, 6]. The reactions of $Pd(R_4en)(H_2O)_2^{2+}$ with the purine nucleosides and 5′-nucleotides have now been studied in detail and the main results will be summarized here, although detailed accounts of the individual investigations are given elsewhere [7, 8]. In addition, we have undertaken a systematic study of the chloride concentration and pH dependence of the reactions of $Pd(Me_4en)Cl_2$ with

*On leave from the Department of Chemistry, Faculty of Natural Sciences, Cairo University, Cairo, Egypt.
**Author to whom correspondence should be addressed.

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inosine and the 5′-monophosphate of inosine, and the results are presented here. These data enable an extrapolation of our earlier work on the diaqua systems [7, 8] to more relevant conditions in terms of the chloride concentration and pH found in biological systems.

Experimental

All materials used in this study were prepared and obtained as described before [6–8]. The ionic strength of the test solutions was adjusted with $NaClO_4$ and the pH with $HClO_4$. Spectral measurements and stopped-flow kinetic studies were performed on the instrumentation outlined before [6–8]. All kinetic measurements were performed under pseudo-first-order conditions, i.e. an excess of the nucleoside or nucleotide and chloride (where appropriate) was employed. In the case where the absorbance–time trace was not a single exponential function, the OLIS KINFIT (OLIS, Jefferson, GA 30549, USA) set of programs was employed for the analysis of the data.

Results and discussion

Reactions in the absence of chloride

Our earlier work on the reactions of $Pd(dien)Cl^+$ and $Pd(dien)H_2O^{2+}$ with various DNA constituents [2] clearly demonstrated the important role played by the higher reactivity of the aqua complex. For this reason we first studied the substitution behaviour of $Pd(R_4en)(H_2O)_2^{2+}$ with the purine nucleosides adenosine and inosine (for R = H and Et), and with ribose-, adenosine-, inosine- and guanosine-5′-monophosphate (for R = H, Me and Et). In all cases the complex formation reactions occur in two consecutive steps, each of which depends on the nucleophile (Nu) concentration according to the rate law of eqn. (1).

$$k_{obs} = k_a + k_b[Nu] \quad (1)$$

From a detailed analysis of the kinetic traces [7] it was concluded that the consecutive steps represent the formation of 1:1 and 1:2 substitution products in eqn. (2), for which k_a and k_b represent the reverse aquation and complex formation rate constants, respectively. A comparison of the values of k_1 to k_4 for the investigated nucleosides and 5′-nucleotides with the corresponding data for chloride is given in Table 1.

$$Pd(R_4en)(H_2O)_2^{2+} + Nu \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Pd(R_4en)(Nu)H_2O^{2+} + H_2O$$

$$Pd(R_4en)(Nu)H_2O^{2+} + Nu \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Pd(R_4en)(Nu)_2^{2+} + H_2O \quad (2)$$

A comparison of the complex formation rate constants k_1 and k_3 for the series of nucleophiles in Table 1, demonstrates that the nucleosides are less reactive than chloride, whereas the 5′-monophosphates increase in their reactivity along the series RMP < AMP < IMP < GMP. The observation that k_1 for the reaction with RMP is slower than for the reaction with inosine, but faster than for the reaction with adenosine is important. In addition, the introduction of a monophosphate group on the nucleosides causes a remarkable increase in both k_1 and k_3, indicating that this group must have a significant transition state stabilization effect in order to favour these complex formation reactions. Similar observations have been reported before and could be in line with the suggestion that the phosphate group interacts via hydrogen bonding with the leaving solvent molecule and the axial amine group [1, 9–11]. The data in Table 1 clearly demonstrate that Cl^- can be a serious competitor during such complex formation reactions (see 'Discussion'). All the investigated reactions exhibit a significant decrease in rate constant with increasing steric hindrance on the en ligand, which is in agreement with the operation of an associative substitution mechanism [3]. A similar trend is seen in that k_3 increases less along the series AMP, IMP and GMP than k_1, especially for the more hindered complexes, since the presence of one nucleotide in the coordination sphere sterically hinders the entrance of the second. Activation parameters ($\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta V^{\neq}$) were determined for these reactions where possible [7, 8], and their values further support the above conclusions.

Reactions in the presence of chloride

When complexes of the type $Pd(R_4en)Cl_2$ are dissolved in slightly acidic aqueous solution they undergo spontaneous solvolysis to produce $Pd(R_4en)(Cl)H_2O^+$ and $Pd(R_4en)(H_2O)_2^{2+}$, and the product distribution at equilibrium will depend on the chloride concentration in the medium. These spontaneous solvolysis reactions, i.e. the reverse of the reactions in eqn. (2), have been studied in detail before [5, 6]. In addition, the acid dissociation constants of the produced aqua complexes are also known, and thus enable a complete speciation of the system.

Kinetic measurements on the reaction of $Pd(R_4en)Cl_2$ with nucleosides and 5′-nucleotides in the absence of added chloride are complicated by the fact that the chloride concentration in solution steadily increases during the reaction and causes an increasing contribution of a non-pseudo-first-order reverse process. In order to resolve this difficulty, experiments in this study were always performed in excess chloride, where the speciation could be well predicted and no significant change in chloride concentration would occur during

TABLE 1. Summary of kinetic data at 25 °C for the complex formation reactions of $Pd(R_4en)(H_2O)_2^{2+}$ according to the general scheme outlined in eqn. (2)[a]

Parameter	R	Nu[b]						
		Ino	Ado	RMP	AMP	IMP	GMP	Cl^-
k_1 (M^{-1} s^{-1})	H	1.4×10^4	1.1×10^3	4.8×10^3	1.2×10^4	[c]	[c]	2.9×10^4
	Me	[d]	[d]	1.0×10^3	5.1×10^2	3.8×10^4	[c]	1.5×10^4
	Et	2.4×10^2	6.3	99	57	8.6×10^2	2.2×10^3	1.6×10^3
k_2 (s^{-1})	H	*c.* 0	9.3	*c.* 0	*c.* 0	[c]	[c]	7.5
	Me	[d]	[d]	15.2	0.2	*c.* 0	[c]	*c.* 0
	Et	1.3	1.3×10^{-2}	2.0	0.8	*c.* 0	*c.* 0	*c.* 0
k_3 (M^{-1} s^{-1})	H	9.3×10^2	3.7×10^2	[d]	7.1×10^2	1.4×10^4	3.2×10^4	1.3×10^3
	Me	[d]	[d]	[d]	6.6	2.6×10^2	5.5×10^2	2.9×10^2
	Et	12.4	4.7×10^2	[d]	7.0	9.7	25.5	13.3
k_4 (s^{-1})	H	8.6	1.5	[d]	6.4	*c.* 0	*c.* 0	9.7[e]
	Me	[d]	[d]	[d]	0.11	*c.* 0	*c.* 0	2.8[e]
	Et	*c.* 0	*c.* 0	[d]	0.037	*c.* 0	*c.* 0	0.29[e]

[a]pH = 4 to 5; 0.1 M ionic strength; detailed kinetic data are reported elsewhere [5–8]. [b]Abbreviations: Ino = inosine; Ado = adenosine; 5′-XMP = ribose-(R), adenosine-(A), inosine-(I) and guanosine-(G)-5-monophosphate. [c]Too fast to be measured using stopped-flow methods. [d]Not studied. [e]From base hydrolysis data for $Pd(R_4en)Cl_2$ [6].

the substitution process. Based on the available complex formation constants for the $Pd(Me_4en)Cl_2$ system [6], the [total Cl^-] was varied between 0.020 and 0.30 M for a [Pd(II)] of 5×10^{-4} M to ensure that only $Pd(Me_4en)Cl_2$ and $Pd(Me_4en)(Cl)H_2O^+$ were present in solution. Under these conditions, the reactions with Ino and IMP revealed only one kinetic step, that is accompanied by a strong decrease in absorbance at 380 nm, an increase in absorbance at *c.* 315 nm and an isosbestic point between 345 and 355 nm depending on the [total Cl^-] of the medium. At [total Cl^-] below 5×10^{-3} M a significant percentage of the Pd(II) complex is in the diaqua form, and under such conditions two consecutive reaction steps could be observed in the absorbance–time traces. However, the kinetic analysis of these steps was complicated by the variation in Cl^- concentration during the reaction as mentioned above. We conclude from the above observations that in the presence of an excess Cl^- only a 1:1 complex formation to produce $Pd(Me_4en)(Cl)Nu^+$ occurs. Chloride is an effective competitor in such substitution reactions (see Table 1) and thus prevents the formation of a 1:2 complex with Ino and IMP under such conditions.

The observed pseudo-first-order rate constants for the reactions with Ino and IMP exhibit a non-linear dependence on the nucleophile concentration at constant [Cl^-], and decrease with increasing [Cl^-] at constant nucleophile concentration (see Fig. 1). In these experiments the nucleophile concentration was varied between 5.0×10^{-3} and 2.5×10^{-2} M and the chloride concentration was varied between 0.020 and 0.30 M at 0.6 M ionic strength. A typical set of experimental data for the reaction with Ino is presented in Fig. 1. The fact that k_{obs} decreases significantly with increasing [Cl^-] suggests that $Pd(Me_4en)Cl_2$ (mainly present at high [Cl^-]) cannot react effectively with the attacking nucleophile. The experimental data are in good agreement with the mechanism outlined in eqn. (3) in which the $Pd(Me_4en)(Cl)H_2O^+$ complex is considered to be the only reactive species, similar to that found in our earlier study of the corresponding dien system [2].

$$Pd(Me_4en)Cl_2 + H_2O \underset{k_3}{\overset{k_4}{\rightleftharpoons}} Pd(Me_4en)(Cl)H_2O^+ + Cl^-$$

$$k_5 \downarrow \quad +Nu$$

$$Pd(Me_4en)(Cl)Nu^+ + H_2O \qquad (3)$$

The rate law for this mechanism is given in eqn. (4), which can be rewritten as shown in eqn. (5) and requires that all the experimental data for a particular nucleophile should exhibit a linear correlation between k_{obs}^{-1} and [Cl^-]/[Nu].

$$k_{obs} = k_4k_5[Nu]/\{k_3[Cl^-] + k_5[Nu]\} \qquad (4)$$

$$k_{obs}^{-1} = k_3[Cl^-]/k_4k_5[Nu] + k_4^{-1} \qquad (5)$$

Indeed the data for both Ino and IMP conform to this requirement as seen from the plots in Figs. 2 and 3, respectively, from which k_4 and k_3/k_4k_5 could be determined. These experiments were repeated at different temperatures and the results are summarized in Table 2. The values of k_4 obtained from the two sets of data (Ino and IMP) are similar within the experimental error limits, but differ considerably from that reported in

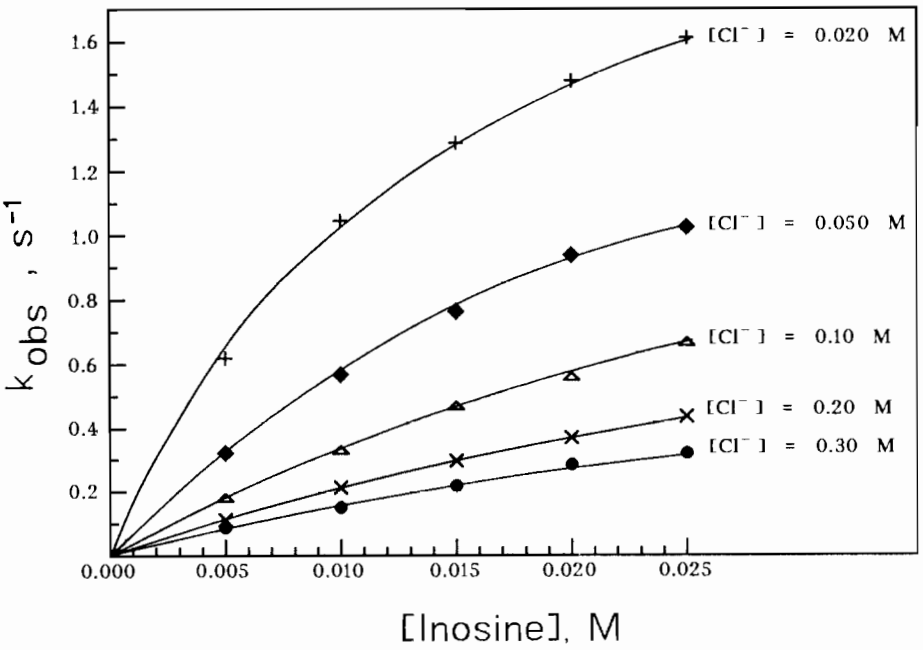

Fig. 1. Plots of k_{obs} vs. [inosine] as a function of [Cl^-] for the overall reaction of eqn. (3). The experimental conditions are given in Table 2, temperature = 25.4 °C.

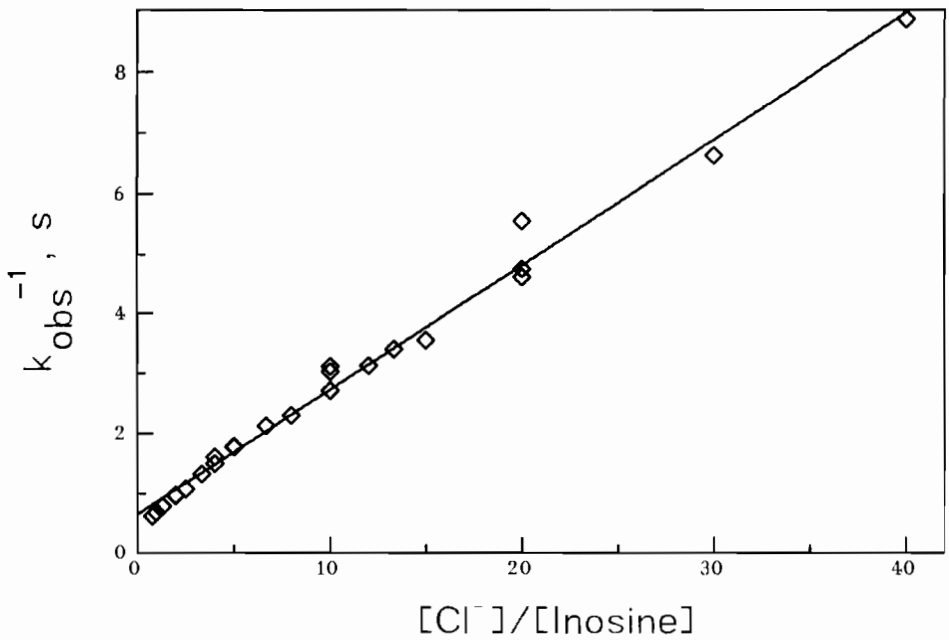

Fig. 2. Plot of k_{obs}^{-1} vs. [Cl^-]/[inosine] for all the data in Fig. 1 according to eqn. (5).

Table 1. This is ascribed to the difference in ionic strength, viz. 0.6 compared to 0.1 M, respectively.

In order to be able to resolve the value of k_5, it was necessary to determine k_3 at 0.6 M ionic strength. For this purpose the anation of $Pd(Me_4en)(Cl)H_2O^+$ (mainly present in solution when $Pd(Me_4en)Cl_2$ is dissolved in water) by chloride was studied as a function of [Cl^-] over the range 0.050–0.20 M, similar to the concentration range selected for the above reported study. The resulting values of k_3 were found to be 80 ± 2, 200 ± 4 and 376 ± 4 M^{-1} s^{-1} at 15.3, 25.5 and 34.5 °C, respectively. The intercepts of plots of k_{obs} versus [Cl^-] are subjected to large error limits under these experimental conditions (high [Cl^-] range) and do not give accurate values for k_4. These data now enabled the calculation of k_5 as indicated in Table 2. The rate and activation parameters for the substitution of $Pd(Me_4en)(Cl)H_2O^+$ by Cl^-, Ino and IMP, i.e. the

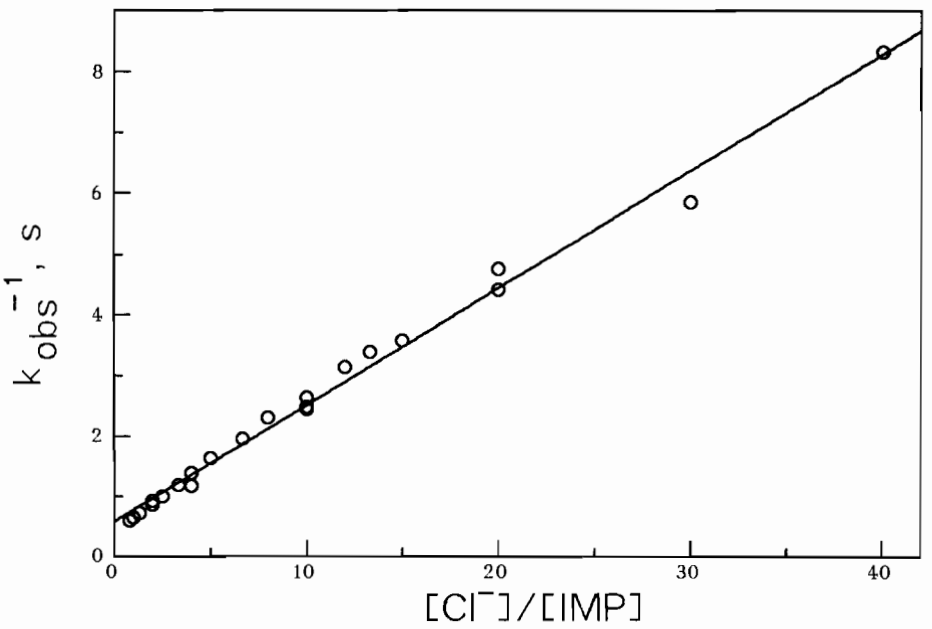

Fig. 3. Plot of k_{obs}^{-1} vs. [Cl^-]/[IMP] for the reaction with IMP according to the scheme in eqn. (3) and eqn. (5). The experimental conditions are given in Table 2, temperature = 25.5 °C.

TABLE 2. Rate and activation parameters for the reactions of $Pd(Me_4en)Cl_2$ with Ino and IMP in the presence of an excess Cl^- according to the reaction scheme in eqn. (3)[a]

Nucleophile	Temperature (°C)	k_3/k_4k_5[b] (s)	k_4[c] (s^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})	k_5[d] (M^{-1} s^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})
Ino	15.0	0.262±0.004	1.06±0.05	27±2	−151±8	288±4	54±2	−11±6
	25.4	0.208±0.004	1.54±0.07			624±12		
	34.6	0.126±0.006	2.32±0.08			1290±60		
IMP	15.4	0.255±0.004	1.04±0.06	32±1	−133±3	302±5	53±4	−12±12
	25.5	0.193±0.003	1.69±0.05			613±10		
	34.6	0.113±0.006	2.56±0.08			1300±70		

[a]Experimental conditions: [Pd(II)] = 5×10^{-4} M; [Cl^-] = 0.020–0.30 M; [Nu] = $(5.0–25)\times10^{-3}$ M; pH ≈ 4.6 (Ino), ≈ 3.5 (IMP); ionic strength = 0.6 M. [b]Slope of k_{obs}^{-1} vs. [Cl^-]/[Nu]. [c]Calculated from intercept of k_{obs}^{-1} vs. [Cl^-]/[Nu]. [d]Calculated from k_3/k_4k_5, using $k_3 = 80\pm2$, 200 ± 4 and 376 ± 4 M^{-1} s^{-1} for 15.3, 25.5 and 34.5 °C, respectively, from which $\Delta H^{\neq} = 57\pm3$ kJ mol^{-1} and $\Delta S^{\neq} = -10\pm10$ J K^{-1} mol^{-1}.

k_3 and k_5 reaction paths in eqn. (3), are according to the results in Table 2 very similar. Most surprising are the almost identical rate and activation parameters found for Ino and IMP. A comparison with the values of k_1 and k_3 for the substitution reactions of the diaqua complexes in Table 1, indicates that Cl^- exhibits almost the same reactivity as IMP for the Me_4en complex. Furthermore, all three nucleophiles (Cl^-, Ino and IMP) exhibit a very similar reactivity towards the Et_4en complex. In the case of the unsubstituted en complex, the values of k_1 and k_3 are considerably higher than those for Ino and Cl^-. Thus the steric hindrance on the amine ligand controls the nucleophilicity order of the entering nucleophile, and most probably also the transition state stabilization effect in the case of the phosphate induced reactivity observed in some cases (see Table 1). Finally, a comparison of the $\Delta S^{\neq}$ values reported in Table 2 indicates that the solvolysis of $Pd(Me_4en)Cl_2$ (k_4 in eqn. (3)) is characterized by a significantly more negative $\Delta S^{\neq}$ value than the substitution of $Pd(Me_4en)(Cl)H_2O^+$ by Cl^-, Ino and IMP. This may be related to the fact that Cl^- is released during the solvolysis reaction, which may cause an increase in electrostriction and a stronger solvated (more structured) transition state.

A series of experiments was performed to study the pH dependence of the reactions with Ino and IMP in the presence of 0.020 and 0.30 M Cl^-. The reactions exhibited no meaningful dependence on pH in the range 3–5, but evidence for more complicated kinetic behaviour was observed at higher pH. This is presumably due to the interference of $Pd(Me_4en)(Cl)OH$ under

such conditions [6], which can slow down the overall substitution process considerably and account for the occurrence of subsequent reactions under such conditions.

Comparison with related systems

The results of this study reveal that Ino is a significantly stronger nucleophile than Ado (see Table 1). This is important since it is known that Ino closely resembles the reactivity of guanosine [2]. Thus in terms of the antitumor activity of *cis*-$Pt(NH_3)_2Cl_2$, these results indicate that binding to the nucleic base guanine will be largely favoured over the binding to adenine [1]. The $[Cl^-]$ dependence study clearly demonstrated that it will be the chloroaqua complex that will bind to the nucleic bases especially at the chloride concentration level of 4 mM in the cell. Under these conditions further aquation of the chloro complex will result in the binding of a second nucleic base to produce the 1:2 species usually found in biological studies. At higher chloride concentration, Cl^- will be an effective scavenger for the reactive aqua complexes and so prevent unwanted side reactions occurring during the transport of the reagent to the wanted site. Of the investigated nucleotides GMP is the most reactive one, which accounts for the preferred binding of such sites during the reaction of *cis*-$Pt(NH_3)_2Cl_2$ with DNA [1]. The pH at which antitumor complexes bind to DNA is significantly higher than that used in this study, and our preliminary results indicate the interference of non-labile hydroxo complexes at $pH \approx 7$. This means that relatively small differences in the acid dissociation constants of the various antitumor complexes, as well as small differences in the pH of healthy and tumor cells, may cause a significant difference in the substitution behaviour, i.e. the binding rate to DNA, of such complexes. Finally, the ability to control the reactivity of the model Pd(II) complexes via the influence of steric hindrance on the amine ligands may indicate a way to kinetically tune the reactivity of antitumor reagents for specific applications in chemotherapy.

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References

1 S. E. Sherman and S. J. Lippard, *Chem. Rev., 87* (1987) 1153; W. I. Sundquist and S. J. Lippard, *Coord. Chem. Rev., 100* (1990) 293; J. Reedijk, *Pure Appl. Chem., 59* (1987) 181; P. Umapathy, *Coord. Chem. Rev., 95* (1989) 129.
2 E. L. J. Breet and R. van Eldik, *Inorg. Chem., 26* (1987) 2517.
3 E. L. J. Breet and R. van Eldik, *Inorg. Chem., 23* (1984) 1865; M. Kotowski and R. van Eldik, *Inorg. Chem., 23* (1984) 3310; *25* (1986) 3896; J. J. Pienaar, M. Kotowski and R. van Eldik, *Inorg. Chem., 28* (1989) 373; J. Berger, M. Kotowski, R. van Eldik, U. Frey, L. Helm and A. E. Merbach, *Inorg. Chem., 28* (1989) 3759.
4 J. J. Pienaar, E. L. J. Breet and R. van Eldik, *Inorg. Chim. Acta, 155* (1989) 249.
5 H. Hohmann and R. van Eldik, *Inorg. Chim. Acta, 174* (1990) 87.
6 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chim. Acta, 188* (1991) 25.
7 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chem., 31* (1992) 345.
8 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chem., 31* (1992) 1090.
9 A. Laouni, J. Kozella and J.-C. Chottard, *Inorg.Chem., 27* (1988) 2753.
10 C. Verma, M. Green and R. M. Wing, *J. Chem. Soc., Chem. Commun.,* (1988) 884.
11 D. J. Evans, M. Green and R. van Eldik, *Inorg. Chim. Acta, 128* (1987) 27.