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INTERACTION OF (dien)Pd(II) WITH CYTIDINE AND CYTIDINE 5′-MONOPHOSPHATE

INFLUENCE OF THE PHOSPHATE GROUP ON THE KINETICS AND MECHANISM

Robert MÉNARD, Monique LACHAPELLE and Miklos ZADOR *

Département de Chimie, Université de Montréal, C.P. 6210, Succ.A, Montreal, Quebec H3C 3V1, Canada

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The kinetics and the equilibrium of (dien)$PdCl^+$ interaction with cytidine (C) and cytidine 5′-monophosphate (CMP) were studied by spectrophotometry and by stopped-flow methods. In both cases, the mechanism implies a (dien)$Pd(H_2O)^{2+}$ intermediate with a significant contribution of the solvent path at low chloride concentrations. With CMP, the rate is affected due to the addition of a mechanistic path via an intermediate formed between (dien)Pd(II) and the phosphate group of CMP. The kinetic and thermodynamic parameters have been determined and reflect the favorable electrostatic interactions due to the presence of the phosphate group of CMP. Furthermore, these parameters are in agreement with a transient (dien)Pd(II)-phosphate complex of CMP leading to the formation of the thermodynamically favored (dien)Pd(II)-N3 complex as final product.

1. Introduction

The interaction between metal ions and nucleosides or nucleotides has been widely studied [1–5]. Among the metals, platinum and, more recently, palladium, received great attention since the discovery by Rosenberg [6,7] of the antitumor activity of platinum complexes. Most of the studies are aimed at elucidating the structure of the complexes formed and at determining the sites and equilibrium constants for the binding of the metal to the nucleosides or nucleotides.

However, transient species that are not the thermodynamically preferred products can play an important role in these systems. An example is the formation of the Pt–N7 bond with purines, even though the Pt–N1 bond is the thermodynamically favored one [8]. Therefore, the knowledge of the mechanism of interaction can prove to be very useful.

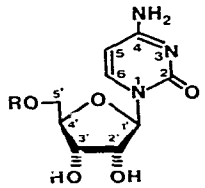
This paper reports a quantitative kinetic as well as thermodynamic investigation of the reaction of [(dien)PdCl]Cl with cytidine (C) and its 5′-monophosphate derivative (CMP). (dien)$PdCl^+$ is a square planar complex with only one labile group (Cl^-) which eliminates complexities encountered with en$PdCl_2$, which can form several reaction intermediates and products. It is well known, from several studies with Pd(II) and analogous Pt(II) complexes, that the binding site for both cytidine and CMP is at N3 [9–12].

2. Experimental

2.1. Materials

Cytidine (free base), CMP (disodium salt) and D-glucose 6-phosphate were high-purity products from Sigma Chemical Co. and used without further purification.

* To whom correspondence should be addressed.



Scheme 1. R = –H (cytidine); R = $-PO_3^{2-}$ (cytidine 5'-monophosphate).

The complex [(dien)PdCl]Cl (dien: diethylenetriamine) was prepared by a slightly modified version of the method previously described [13] using $PdCl_2$ (Fisher Scientific Co.) and diethylenetriamine (J.T. Baker Chemical Co.) and purified by recrystallization from a water/ethanol mixture. Stock solutions of the aquo derivative $[(dien)Pd(H_2O)](ClO_4)_2$ were prepared by adding 2 equiv. of $AgClO_4$ to a [(dien)PdCl]Cl solution and filtering off the precipitated AgCl.

For all the solutions, the ionic strength was maintained at 0.2 M by adding $NaClO_4$. The pH was adjusted to 7.0 (or as otherwise specified) by means of $HClO_4$ and NaOH. No buffer was used in our study in order to avoid possible interactions with the Pd(II) complexes.

2.2. Methods

Absorbance measurements were made on a Perkin-Elmer model 552 spectrophotometer. The kinetics were studied by stopped-flow spectrophotometry using a Dionex model D-130 instrument and a home-built data acquisition system that can accumulate 256 data points at a maximum rate of 1 point per μs [14]. The maximum rate of sampling used was, however, about 1 point per 0.1 ms due to the mixing time in the stopped-flow apparatus.

The collected data points can be visualized on a Tektronix 535A oscilloscope or sent for treatment to an Apple II Plus computer via a Cyborg model 91A interface. The rate constants are obtained by least-squares treatment adapted to different integrated rate laws.

Reactions were studied in the presence of excess cytidine or CMP, therefore, pseudo-first-order rate constants, k_{obs}, were obtained. All kinetic and thermodynamic measurements were carried out at 25.0 ± 0.1°C.

3. Results

3.1. Equilibrium measurements

3.1.1. Cytidine

The absorption spectra of $(dien)PdCl^+$ show a maximum at 333 nm. As shown in fig. 1, the addition of increasing concentrations of cytidine causes a gradual decrease of the absorbance of the palladium complex and a shift of the maximum to a lower wavelength. This blue shift is characteristic of the transformation of a chloro complex to an ammine complex [15]. The presence of a well defined isosbestic point at 322 nm indicates the presence of only two absorbing species at pH 7: $(dien)PdCl^+$ and the Pd-N3 complex. Spectrophotometric titration of $(dien)PdCl^+$ by cytidine at fixed wavelength was used to determine the equilibrium constant of binding, $K_C = [(dien)PdC^{2+}][Cl^-]/[(dien)PdCl^+][C]$. A value of $K_C = 300 \pm 25$ was obtained by a computer-assisted curve-fitting technique.

Fig. 2 illustrates the changes in absorbance of the Pd(II)-cytidine system at 350 nm as a function of pH. As the pH decreases from 7 to 1, an

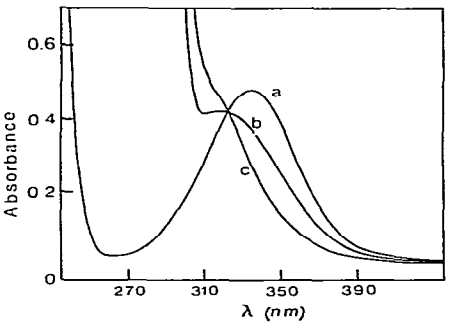


Fig. 1. Absorption spectra of $(dien)PdCl^+$ in the presence of various cytidine concentrations. Conditions: 1.00 mM [(dien)PdCl]Cl, 0.2 M ionic strength (0.1 M NaCl, 0.1 M $NaClO_4$), pH 7. Cytidine concentration: (a) 0, (b) 0.80 mM, (c) 4.00 mM.

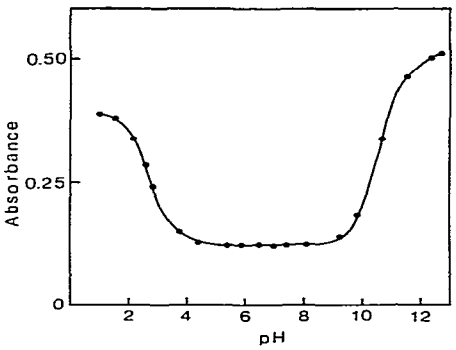


Fig. 2. Effect of pH on the absorbance at 350 nm of a (dien)$PdCl^+$-cytidine-containing solution. Conditions: 1.00 mM [(dien)PdCl]Cl, 10.0 mM cytidine, 0.2 M ionic strength (0.1 M NaCl, 0.1 M $NaClO_4$).

increase in absorbance is observed starting in the vicinity of pH 4.5, due to protonation of cytidine at N3 leading progressively to the dissociation of (dien)PdC^{2+}. At a sufficiently low pH (pH ≈ 1) the spectra correspond to those of (dien)$PdCl^+$. As compared to free cytidine ($pK_a = 4.1$, in agreement with reported values [2,16]) the protonation in the presence of (dien)$PdCl^+$ occurs at lower pH, corresponding to an apparent pK_a of 2.85, due to the presence of Pd(II) at N3. Another increase in absorbance is present at pH ≥ 8.5. This secondary reaction will be described in a later work. As seen in fig. 2, however, the Pd-N3 complex is predominant between pH 4.5 and 8.5 and for studies at pH 7, the other phenomena can be neglected.

3.1.2. Cytidine 5'-monophosphate

The absorption spectra of solutions containing (dien)$PdCl^+$ and CMP and its variation with pH are nearly identical to those presented in figs. 1 and 2 for cytidine. The equilibrium constant for the formation of the Pd–N3 bond with CMP is slightly higher than that obtained with cytidine and was estimated at $K_{CMP} = 500 \pm 70$. The presence of the phosphate group thus increases the strength of the complex formed between (dien)$PdCl^+$ and CMP. Electrostatic factors as well as possible hydrogen bonding between a hydrogen of the dien and the phosphate oxygen could be responsible for the increased stability.

3.2. Kinetic measurements

3.2.1. Cytidine

Substitution reactions of square planar complexes of the type MA_3X have been widely studied and a two-path mechanism has been proposed to explain the rate laws obtained [17,18]. In this mechanism, the direct nucleophilic displacement of X by the ligand N (S_N2 path) is in competition with the attack of MA_3X by solvent molecule, S, to form an MA_3S intermediate which, in turn, reacts with the nucleophile to give the MA_3N product (solvent path). For the reaction of (dien)$PdCl^+$ with cytidine, the above mechanism is represented by scheme 2.

$$C + (dien)PdCl^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (dien)PdC^{2+} + Cl^- \quad (1) \Big\} \; S_N2 \text{ path}$$

$$(dien)PdCl^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (dien)Pd(H_2O)^{2+}$$

$$C + (dien)Pd(H_2O)^{2+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \begin{cases} + Cl^- & (2) \\ (dien)PdC^{2+} & \\ + H_2O & (3) \end{cases} \Big\} \text{ solvent path}$$

Scheme 2.

The kinetics of the reaction between (dien)$PdCl^+$ and cytidine were studied at pH 7 by following the changes in absorbance with time at 350 nm. A preliminary study has shown a decrease in the rate of reaction when Cl^- is added to the solution and a less than first-order reaction in cytidine, particularly at low Cl^- concentrations. Both these features are characteristic of a mechanism implying a (dien)$Pd(H_2O)^{2+}$ intermediate.

As shown by the value of the equilibrium constant K_C, the reaction is kinetically reversible under certain conditions. Therefore, the reverse reaction contributes to the observed pseudo-first-order rate constant, k_{obs}:

$$k_{obs} = k_f + k_r \quad (4)$$

The rate of complex formation, R_f (rate forward),

by the mechanism of scheme 2 is given by eq. 5.

$$R_f = k_1[(\text{dien})\text{PdCl}^+][\text{C}] + k_3\left[(\text{dien})\text{Pd}(\text{H}_2\text{O})^{2+}\right][\text{C}] \quad (5)$$

From the assumption of steady state for $(\text{dien})\text{Pd}(\text{H}_2\text{O})^{2+}$ along with the application of the mass conservation law for the Pd(II) species, one obtains the concentrations of $(\text{dien})\text{PdCl}^+$ and $(\text{dien})\text{Pd}(\text{H}_2\text{O})^{2+}$ and also that of Cl^-. The equation for the observed rate constant for forward reaction is given by eq. 6.

$$k_f = \left\{\frac{k_1k_{-2}[\text{Cl}] + k_1k_3[\text{C}] + k_2k_3}{k_{-2}[\text{Cl}] + k_3[\text{C}] + k_2}\right\}[\text{C}] \quad (6)$$

Calculating k_r and taking into account eq. 4, an expression for k_{obs} was deduced and is given by eq. 7.

$$k_{obs} = \left\{\frac{k_1k_{-2}[\text{Cl}] + k_1k_3[\text{C}] + k_2k_3}{k_{-2}[\text{Cl}] + k_3[\text{C}] + k_2}\right\}[\text{C}] + \left\{\frac{k_{-1}k_{-2}[\text{Cl}] + k_{-1}k_3[\text{C}] + k_{-2}k_{-3}}{k_{-2}[\text{Cl}] + k_3[\text{C}] + k_2}\right\}[\text{Cl}] \quad (7)$$

In order to validate this mechanism and to obtain significant values of the rate constants in eq. 7, the rate of reaction has to be studied for a wide range of concentrations of both cytidine and Cl^-. The results are illustrated in fig. 3.

Since eq. 7 is too complex to be resolved directly or by a least-squares method, a computer curve-fitting method was used to obtain the individual rate constants. For the reaction of $(\text{dien})\text{PdCl}^+$ with cytidine at high Cl^- concentration, only eq. 1 of scheme 2 contributes to the observed rate constant at first approximation. Values of k_1 and k_{-1} can thus be calculated from the results at $[\text{Cl}^-] = 0.1$ M, using $K_C = k_1/k_{-1}$. Values for k_2 and k_{-2} were obtained directly from the reaction of $(\text{dien})\text{Pd}(\text{H}_2\text{O})^{2+}$ with Cl^- at various Cl^- concentrations, while the reaction of $(\text{dien})\text{Pd}(\text{H}_2\text{O})^{2+}$ with cytidine at different cytidine concentrations leads to k_3 and k_{-3}. These approximate values of the individual rate constants are then introduced into eq. 7 to yield calculated values of k_{obs}. The rate constants are adjusted until agreement between the calculated curves and the experimental results is reached. The best fit is illustrated in fig. 3 and the values of the corresponding rate constants are listed in table 1.

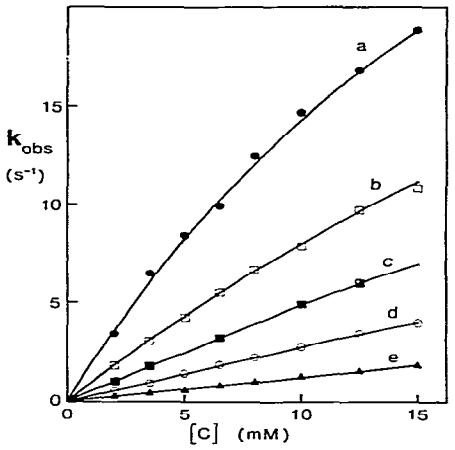


Fig. 3. Variation of k_{obs} with cytidine concentration for the reaction between $(\text{dien})\text{PdCl}^+$ and cytidine. The points represent the experimental results and the continuous lines the calculated values of k_{obs} by eq. 7. Conditions: 0.50 mM [(dien)PdCl]Cl, 0.2 M ionic strength (adjusted with $NaClO_4$), pH 7, $\lambda = 350$ nm. Reactions were carried out at various added Cl^- concentrations: (a) 0, (b) 3.0 mM, (c) 8.0 mM, (d) 20.0 mM, (e) 100 mM.

Table 1

Rate constants for the reaction of $(\text{dien})\text{PdCl}^+$ with cytidine and CMP

	Cytidine	CMP
k_1 ($M^{-1}s^{-1}$)	75	90
k_{-1} ($M^{-1}s^{-1}$)	0.25	0.18
k_2 (s^{-1})	46	46
k_{-2} ($M^{-1}s^{-1}$)	29000	29000
k_3 ($M^{-1}s^{-1}$)	2900	4000
k_{-3} (s^{-1})	0.015	0.013
k_4 ($M^{-1}s^{-1}$)	–	9200
k_{-4} (s^{-1})	–	21.6
k_5 ($M^{-1}s^{-1}$)	–	250
k_{-5} ($M^{-1}s^{-1}$)	–	0.34

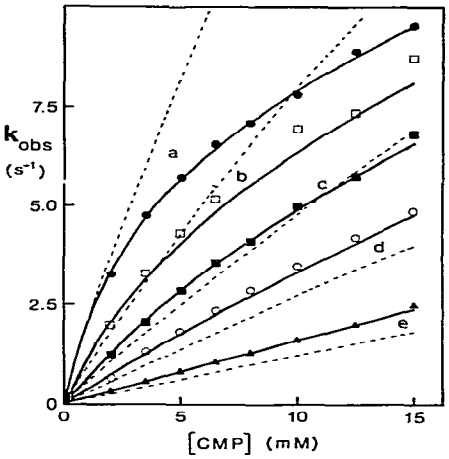


Fig. 4. Variation of k_{obs} with CMP concentration for the reaction between (dien)PdCl$^+$ and CMP. The points represent the experimental results, the dashed lines are for the calculated values of k_{obs} with the mechanism of scheme 2 and eq. 7 (using the rate constants obtained for cytidine) and the continuous lines represent the calculated values of k_{obs} using the mechanism of scheme 3 and eq. 13. Conditions: 0.50 mM [(dien)PdCl]Cl, 0.2 M ionic strength (adjusted with $NaClO_4$), pH 7, $\lambda \approx 350$ nm. Reactions were carried out at various added Cl^- concentrations: (a) 0, (b) 3.0 mM, (c) 8.0 mM, (d) 20.0 mM, (e) 100 mM.

3.2.2. *Cytidine 5'-monophosphate*

The kinetics of the (dien)PdCl$^+$-CMP system were investigated under the same conditions as those for cytidine. The rate of the reaction was determined for various CMP concentrations and at different Cl^- concentrations. The results are shown in fig. 4. In addition to experimental points for CMP, calculated curves for cytidine with the model of scheme 2 are also presented in fig. 4 (dashed lines) which allows direct comparison between the two systems.

It is clear that the model described in scheme 2 cannot account for the experimental results with CMP. Even if values of individual rate constants are changed, good agreement between theoretical curves and experimental points cannot be achieved. In particular, the pronounced curvature at low Cl^- concentration cannot be reproduced with any set of reasonable rate constants, indicating that the mechanism of the reaction between (dien)PdCl$^+$ and CMP is different from that described in scheme 2. This is attributed to an interaction between (dien)Pd(II) and the phosphate moiety of CMP leading to the mechanism depicted by scheme 3.

In the model proposed, there are three different mechanistic paths for the formation of the Pd-N3 product of CMP. In addition to the usual nucleophilic substitution path and the solvent path via a (dien)Pd$(H_2O)^{2+}$ intermediate, there is a third possibility where (dien)Pd$(H_2O)^{2+}$ forms a new intermediate, CMP-Pd(dien) (eq. 11). This phosphato intermediate is formed by substituting the water molecule of (dien)Pd$(H_2O)^{2+}$ by the phosphate group of CMP (which is doubly deprotonated at pH 7 having a $pK_a = 6.2$ [2,19]). Eq. 12

$$\text{CMP}^{2-} + \text{(dien)PdCl}^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{(dien)PdCMP} + \text{Cl}^- \quad (8) \Big\} \text{S}_\text{N}2 \text{ path}$$

$$\text{(dien)PdCl}^+ + \text{H}_2\text{O} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{(dien)Pd(H}_2\text{O)}^{2+} + \text{Cl}^- \quad (9) \Big\} \text{solvent path}$$

$$\text{CMP}^{2-} + \text{(dien)Pd(H}_2\text{O)}^{2+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{(dien)PdCMP} + \text{H}_2\text{O} \quad (10) \Big\} \text{solvent path}$$

$$\text{(dien)Pd(H}_2\text{O)}^{2+} + \text{CMP}^{2-} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{CMP-Pd(dien)} + \text{H}_2\text{O} \quad (11) \Big\} \text{phosphate path}$$

$$\text{CMP}^{2-} + \text{CMP-Pd(dien)} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \text{(dien)PdCMP} + \text{CMP}^{2-} \quad (12) \Big\} \text{phosphate path}$$

Scheme 3. (dien)PdCMP, Pd-N3 complex; CMP-Pd(dien), phosphato intermediate.

represents the reaction of this intermediate with another CMP molecule to give the thermodynamically favored Pd-N3 complex.

A steady-state approximation for both (dien)Pd$(H_2O)^{2+}$ and CMP-Pd(dien) intermediates leads to the following equation for k_{obs}:

$$k_{obs} = \left\{ \frac{(k_1k_{-2}[Cl] + k_1k_3[CMP] + k_2k_3)(k_{-4} + k_5[CMP]) + k_1k_4k_5[CMP]^2 + k_2k_4k_5[CMP]}{(k_{-4} + k_5[CMP])(k_{-2}[Cl] + k_3[CMP] + k_2) + k_4k_5[CMP]^2 + k_2k_4[CMP]} \right\}[CMP]$$
$$+ \left\{ \frac{(k_{-1}k_{-2}[Cl] + k_{-1}k_3[CMP] + k_{-2}k_{-3})(k_{-4} + k_5[CMP]) + k_{-1}k_4k_5[CMP]^2 + k_{-2}k_{-4}k_{-5}[CMP]}{(k_{-4} + k_5[CMP])(k_{-2}[Cl] + k_3[CMP] + k_2) + k_4k_5[CMP]^2 + k_2k_4[CMP]} \right\}[Cl] \quad (13)$$

Proceeding as previously described for cytidine, the rate parameters of eq. 13 have been determined and are listed in table 1. The corresponding fit between experimental data and calculated values is shown in fig. 4 (continuous lines). D-Glucose 6-phosphate was used as a model compound for the (dien)Pd$(H_2O)^{2+}$-phosphate interaction of eq. 11, to evaluate values of k_4, k_{-4} and the corresponding equilibrium constant, $K_4 = k_4/k_{-4}$, since its phosphate group is very similar to that of CMP as reflected by its identical pK_a [20]. The values obtained for D-glucose 6-phosphate ($k_4 = 9200\ M^{-1}\ s^{-1}$, $k_{-4} = 23\ s^{-1}$, $K_4 = 400\ M^{-1}$) are very close to those giving the best fit for CMP ($k_4 = 9200\ M^{-1}\ s^{-1}$, $k_{-4} = 21.6\ s^{-1}$, $K_4 = 425\ M^{-1}$).

The influence of the phosphate group of CMP is also evidenced in the rate vs. pH profiles of its reactions with (dien)$PdCl^+$ and (dien)Pd$(H_2O)^{2+}$ as compared to those of cytidine. The results in table 2 show that the rate of reaction of (dien)$PdCl^+$ is greater with CMP than with cytidine. This can be attributed to the presence of an electrostatic interaction between the positively charged palladium complex and the negatively charged CMP which decreases the free energy of activation.

Opposite results are obtained for (dien)Pd$(H_2O)^{2+}$ even though similar favorable electrostatic effects are present. Furthermore, the ratio k_{obs}(CMP)/k_{obs}(C) decreases with increasing pH. This effect is mainly due to a decrease of k_{obs}(CMP) while k_{obs}(C) remains nearly constant. The lower rate constant for CMP is in accordance with scheme 3 and is attributed to the formation of the phosphato intermediate CMP-Pd(dien) which has a lower rate of reaction with CMP ($k_5 = 250\ M^{-1}\ s^{-1}$) than does (dien)Pd$(H_2O)^{2+}$ ($k_3 = 4000\ M^{-1}\ s^{-1}$). This effect becomes more pronounced with an increase in pH, which leads to the doubly deprotonated phosphate group interacting more strongly with (dien)Pd(II). As a matter of fact, the rate vs. pH profile for the (dien)Pd$(H_2O)^{2+}$-CMP system closely parallels the

Table 2

Observed rate constants and their ratio for cytidine and CMP at various pH values

k_{obs}(C or CMP), observed rate constant for the reaction with C or CMP; $r = k_{obs}$(CMP)/k_{obs}(C).

pH	(dien)$PdCl^+$			(dien)Pd$(H_2O)^{2+}$		
	k_{obs}(CMP) (s^{-1})	k_{obs}(C) (s^{-1})	r	k_{obs}(CMP) (s^{-1})	k_{obs}(C) (s^{-1})	r
5.0	0.62	0.49	1.27	11.5	12.1	0.95
5.5	0.71	0.54	1.31	10.6	12.5	0.85
6.0	0.75	0.56	1.34	8.4	12.6	0.67
6.5	0.76	0.57	1.33	5.6	12.6	0.44
7.0	0.77	0.58	1.33	3.1	12.0	0.26
7.5	0.77	0.57	1.35	1.5	11.5	0.13

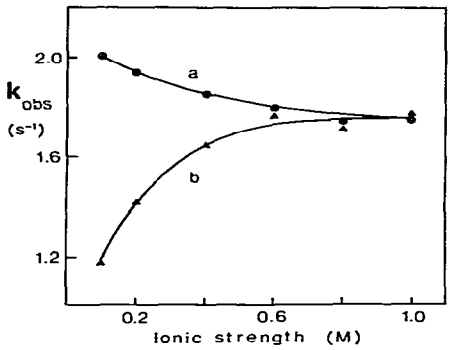


Fig. 5. Ionic strength effect on k_{obs} for the reaction of (dien)PdCl$^+$ with cytidine and CMP. Conditions: 0.50 mM [(dien)PdCl]Cl, 0.02 M NaCl, pH 7; ionic strength was adjusted with $NaClO_4$. (a) Reaction with 5.00 mM CMP. (b) reaction with 5.00 mM cytidine.

second deprotonation of the phosphate ($pK_a = 6.2$).

Inorganic phosphates have similar inhibiting effects on reactions of (dien)Pd(H_2O)$^{2+}$. Addition of 0.006 M NaH_2PO_4 decreases the rate constant for the reaction between (dien)Pd(H_2O)$^{2+}$ and cytidine from $k_{obs} = 12.5\ s^{-1}$ to a value of $2.4\ s^{-1}$ at pH 7. Similar inhibition by added phosphate was observed in reactions of antitumor *cis*-Pt(II) compounds with DNA [21]. The results show clearly that reliable rate constants cannot be obtained in the presence of phosphate buffers in systems where significant amounts of aquo complexes of Pd(II) (and probably of Pt(II)) are present.

The reactions with cytidine and CMP are also affected by the ionic strength of the solution as illustrated in fig. 5. For cytidine, the value of k_{obs} increases with ionic strength while the reverse effect is observed with CMP. These results further indicate the distinct kinetic behaviour of the two systems.

4. Discussion

The results of this study bring further support to the now widely accepted mechanism of substitution reactions of palladium(II) (and platinum(II)) complexes. However, in the case of CMP, where multiple sites are involved, further mechanistic paths can be present and complicate the interpretation of kinetic data. Therefore, the use of (dien)Pd(II) complexes, with only one labile site, was essential to the understanding and quantitative kinetic characterization of the system.

The reaction of (dien)PdCl$^+$ with cytidine involves a mechanism with three reversible steps: a direct S_N2 substitution path along with a solvent path proceeding in two steps with formation of an aquo intermediate (scheme 2). The values of the individual rate constants agree well with published results on similar systems. In particular, the rate constants k_2 and k_{-2} fall in the range of values found for $enPdCl_2$ (for the first and second aquation [22]) and for (dien)PdBr$^+$ [23].

The results also show the importance of the solvent path's contribution to the overall rate of reaction. This is due to the relatively high reactivity of the aquo intermediate ($k_3 = 2900\ M^{-1}\ s^{-1}$) and to the fact that the S_N2 path is relatively slow as compared to the aquation of (dien)PdCl$^+$. This allows the formation of (dien)Pd(H_2O)$^{2+}$ which is particularly important at low Cl^- concentration. At higher Cl^- concentrations, the less reactive (dien)PdCl$^+$ becomes predominant leading to the characteristic inhibition by halides [22,23].

When CMP is the reacting ligand, the mechanism features five reversible steps. In addition to the two paths described for cytidine, a third reaction path occurs. The aquo intermediate reacts with the phosphate moiety of CMP giving a phosphato intermediate. Since the complex at N3 is the thermodynamically favored product, this second intermediate reacts with another CMP molecule to give the Pd-N3 complex.

In this system, the presence of the phosphate moiety of CMP leads to two opposing effects: (i) an electrostatic effect between positively charged palladium complexes and negatively charged CMP leading to an increase of k_{obs} and (ii) complexation of (dien)Pd(II) by the phosphate decreasing the rate of reaction.

At high Cl^- concentration, steady-state calculations show that (dien)PdCl$^+$ is the dominant species in solution and makes the major contribu-

tion to the rate. Under these conditions, the rate of reaction is higher with CMP than with cytidine (see k_1 in table 1) due to the favorable electrostatic effect. Furthermore, no evidence of a (dien)Pd(II)-phosphate interaction was found. At low Cl^- concentration, (dien)$Pd(H_2O)^{2+}$ makes the main contribution to the rate. Electrostatic effects are also important as reflected by values of k_1 and k_3 in table 1 ($k_1(CMP) > k_1(C)$ and $k_3(CMP) > k_3(C)$). However, (dien)$Pd(H_2O)^{2+}$ does interact with the phosphate group. Due to the much lower reactivity of the phosphato complex ($k_5 \ll k_3$), this leads to a decrease in k_{obs} for CMP as compared to cytidine.

The ionic strength of the solution also has a distinct effect on the two systems (fig. 5). Increasing ionic strength favors charge separation in eq. 2 and therefore formation of the more reactive (dien)$Pd(H_2O)^{2+}$ from (dien)$PdCl^+$. This explains the increase in rate with ionic strength in the case of cytidine.

The same should be expected for CMP while experimentally the opposite is observed at pH 7 and in the presence of 0.02 M Cl^-, where (dien)$PdCl^+$ is predominant. In this case, at low ionic strength, favorable electrostatic interaction between (dien)$PdCl^+$ and CMP^{2-} leads to a rate exceeding that observed for cytidine. Increase in ionic strength leads to a decrease of this effect due to the shielding effect of the ions in solution producing a decrease in rate. The latter effect is dominating in the case of CMP. It has to be noted that at high ionic strength, where the electrostatic effect becomes negligible, cytidine and CMP react at the same rate.

Comparison between values of rate constants of different reacting species should reflect the effect of the leaving group on the rate of reaction. Since $k_3 \gg k_5 > k_1$, the results indicate a decreasing rate for changes in leaving group in the order $H_2O \gg PO_4^{2-} > Cl^-$. The position of PO_4^{2-} is uncertain in this series, due to increased steric hindrance introduced by the cytidine portion of CMP. For that reason, the value of k_5 for the reaction of the phosphato complex with CMP is probably smaller than the corresponding reaction of a less bulky phosphate complex.

Interaction of Pt(II) with phosphate groups of nucleotides has been postulated in several cases. It was based on crystal structures containing platinum–phosphate bonds and on NMR and Raman studies [10,24,25]. The corresponding interaction with Pd(II) is more obscure and few results are reported [26]. Palladium is a relatively soft acid and has stronger affinity for nitrogen than for oxygen. Therefore, the overwhelming interaction on nitrogen may mask the weaker interaction on phosphate. In thermodynamic studies involving Pd(II) complexes and nucleotides at equilibrium, only interaction at the nitrogen bases is generally observed.

The proposed mechanism takes into account an interaction with the phosphate and the values of equilibrium constants derived are in accordance with the above discussion. Namely, interaction at the phosphate leads only to a transient species with a relatively low stability ($K_4 = k_4/k_{-4} = 425$ M^{-1}), as compared to the formation of the final product, the Pd-N3 complex, with a much higher stability ($K_3 = k_3/k_{-3} = 3.1 \times 10^5$ M^{-1}). The results show that there is an interaction between the phosphate moiety of CMP and the Pd(II) complex and that the technique and experimental conditions used are critical for the observation of such an interaction.

Finally, in the cell, where Cl^- concentration is low (≈ 0.004 M), the presence of various phosphates (inorganic phosphates, nucleotides, nucleoside di- and triphosphates, polynucleotides, etc.) can lead to lower reaction rates with Pd(II) and Pt(II) complexes than those expected from in vitro studies in the absence of phosphates. It can also be predicted that in the presence of high concentration of Cl^- (e.g., 0.1 M as in blood plasma), the generally low concentration of phosphates has no inhibiting effect due to strong complexation by Cl^-, favoring the S_N2 path. As general conclusion, the kinetics of these reactions in biological fluids is strongly influenced by the medium, due to formation of a great number of reactive species of Pd(II) and Pt(II) complexes.

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