Kinetics of Interaction of $Pd(en)Cl_2$ with Inosine in Chloride containing Aqueous Solutions

W. KADIMA and M. ZADOR

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

Received September 3, 1982

The kinetics of interaction of $Pd(en)Cl_2$ with inosine were studied by stopped flow spectrophotometry at 25 °C. At pH < 5 the reaction implies the N7 site of inosine. It is less than first order with respect to inosine and the rate is markedly decreased in the presence of added Cl^- ions. The mechanism implies a significant contribution of the solvent path. The reactive species are $Pd(en)Cl_2$ and the aquo species $Pd(en)(H_2O)Cl^+$ and $Pd(en)(H_2O)_2^{2+}$ with a corresponding ratio of reactivities of 1:25:150. The values of the rate constants are compared with similar Pd(II) and Pt(II) systems and implications of the mechanism in physiological conditions are discussed.

Introduction

Interaction of *cis*-dichloro Pt(II) amines with DNA constituents has been thoroughly investigated in recent years due to antitumor activity and the use in cancer chemotherapy of these complexes. Corresponding Pd(II) complexes have received less attention. However, due to the similarity of their reactions and of the mechanisms involved, their study is highly desirable and offers experimental advantages owing to their higher reaction rates.

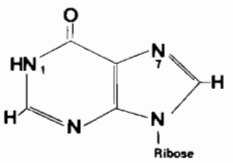
The present paper reports the results of a kinetic study of the interaction of $Pd(en)Cl_2$ (en = ethylenediamine) with inosine (In) in chloride containing aqueous solution, aimed to elucidate their behavior, and the behavior of similar complexes, in physiological environments.

Experimental

Materials

The $Pd(en)Cl_2$ complex was prepared by a slightly modified version of the method described in the literature [1], using $PdCl_2$ (Fisher Sci.) and ethylenediamine (J. T. Baker Reagent) as starting materials.

The aqueous solution of $Pd(en)(H_2O)_2^{2+}$ was obtained by precipitating chloride ions of the dichloro complex using two equivalents of $AgClO_4$. Inosine (Eastman) was used without purification. Its formula and active sites are shown below:



Kinetics

The kinetics were studied by stopped-flow spectrophotometry using a Dionex D-130 instrument. The reaction was studied at concentrations of Pd(II) of 2×10^{-4} *M* to 1×10^{-3} *M* and of inosine between 2.5×10^{-3} *M* and 2×10^{-2} *M*. The ionic strength was maintained at $\mu = 0.2$ *M* by means of $NaClO_4$. In order to minimize changes in the Pd(II)–Cl^- system upon mixing, identical Cl^- concentrations and ionic strengths were used in both syringes.

Changes in transmittance between 300 and 400 nm were monitored by a data acquisition system of our construction [2] and pseudo first-order rate constants were obtained by least-squares methods from the equation $k_{obs}\, t = \ln[(A_0 - A_\infty)/(A - A_\infty)]$, where A_0, A and A_∞ represent absorbances at 0, t and infinite time respectively.

Results

As shown by a preliminary spectroscopic study, the UV spectra both of the reactant Pd(II) complex and of the product change with concentration of Cl^- ions and with pH between 300 and 400 nm.

According to previous studies on the nature of the binding site [3–8], at pH < 5 complex formation involves the N7 site of inosine. This 1:1 complex is represented by (en)XPd-N7, where the fourth

0020-1693/83/0000-0000/$03.00

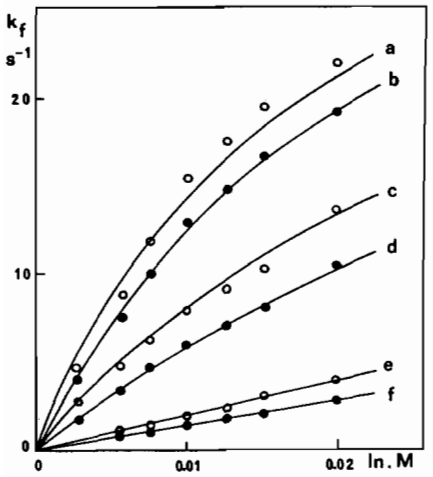


Fig. 1. Influence of inosine concentration on k_f at various Cl^- concentrations. T = 25 °C; $[H^+]$ = 0.1 *M*; μ = 0.2 *M*. a) [NaCl] = 0; b) [NaCl] = 1.0×10^{-3} *M*; c) [NaCl] = 5.0×10^{-3} *M*; d) [NaCl] = 1.0×10^{-2} *M*; e) [NaCl] = 5.0×10^{-2} *M*; f) [NaCl] = 0.10 *M*.

ligand X can be Cl^- or H_2O, depending on chloride concentration.

At pH > 7, due to deprotonation of the N1-H site, a binuclear complex [6] and different polynuclear complexes [5, 6] have been observed by NMR. Under our reaction conditions, at higher inosine and lower Pd(II) concentrations the mononuclear (en)XPd-N1 complex can also have a significant concentration.

The equilibrium constant of the Pd(II)-N7 complex was determined from absorbance *vs.* [In] data. As the complex is quite stable, only data at high Cl^- concentration (0.2 *M*) could lead to a significant value. At 25 °C and μ = 0.2 *M*, K = [(en)Cl Pd-N7] $[Cl^-]$/[(en)$PdCl_2$] [In] = 16 ± 2 was obtained. This value implies that at ratios of $[In]/[Cl^-] \leqslant 1$, the complex formation is less than 95% complete and, therefore, the kinetics are affected by a contribution of the reverse reaction in these conditions.

The kinetics of formation of the (en)XPd-N7 complex can be studied at pH < 5. In agreement with spectral data, the kinetics also reveal the different nature of complexes found at pH < 5 and at pH > 7. In the latter case much lower rates are obtained, due presumably to dprotonation of the Nl-H site during formation of the Pd–Nl bond.

The formation of the (en)XPd-N7 complex has a much higher pH-independent rate at pH < 5. In order to minimize deprotonation and dimerization of hydrolyzed Pd(II) species in the absence of Cl^- [9] or at low Cl^- concentrations, and also to avoid the use of buffers which could interfere, the kinetics were studied at $[H^+]$ = 0.1 *M*.

Figure 1 shows the influence of the concentration of Cl^- and of inosine on k_{obs}. In the absence of added Cl^- the rate increases with increasing inosine concentration, although clearly not in a first-order manner. Similar curves are obtained at higher Cl^- concentration in the presence of added chloride, but the overall rates decrease markedly. This behavior is similar to that observed in the interaction of $Pd(dien)Br^+$ with inosine, where the aquo complex, $Pd(dien)H_2O^{2+}$, had a considerable effect on the rate of complex formation [10].

Therefore, the results were interpreted by a mechanism wherein aquo-complexes are reactive intermediates. The mechanism is shown in Scheme 1:

$$Pd(en)Cl_2 + In \xrightarrow{k_{S_N^2}} (en)XPd{-}N7 \quad (1)$$

$$Pd(en)Cl_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Pd(en)(H_2O)Cl^+ + Cl^- \quad (2)$$

$$Pd(en)(H_2O)Cl^+ + In \xrightarrow{k_2} (en)XPd{-}N7 \quad (3)$$

$$Pd(en)(H_2O)Cl^+ + H_2O \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} Pd(en)(H_2O)_2^{2+} + Cl^- \quad (4)$$

$$Pd(en)(H_2O)_2^{2+} + In \xrightarrow{k_4} (en)XPd{-}N7 \quad (5)$$

Scheme 1

The rate of complex formation is given by:

$$d[complex]/dt = [In](k_{S_N^2}[Pd(en)Cl_2] + k_2[Pd(en)(H_2O)Cl^+] + k_4[Pd(en)(H_2O)_2^{2+}]) \quad (6)$$

Concentrations of $Pd(en)Cl_2$, $Pd(en)(H_2O)Cl^+$ and $Pd(en)(H_2O)_2^{2+}$ were obtained from steady state equations corresponding to the above reaction scheme taking into account the material balance for Pd(II). They were introduced into eqn. (6) to yield rate law (7) (see below).

$$\text{Rate} = \frac{[Pd]_{tot}[In]\left\{k_2 + \frac{k_3k_4}{k_{-3}[Cl] + k_4[In]} + \frac{k_{S_N^2}}{k_1}\left(k_{-1}[Cl^-] + k_2[In] + \frac{k_3k_4[In]}{k_{-3}[Cl] + k_4[In]}\right)\right\}}{\frac{k_{-1}[Cl^-] + k_2[In] + k_1}{k_1} + \frac{k_3k_4[In] + k_1k_3}{k_1(k_{-3}[Cl^-] + k_4[In])}} \quad (7)$$

TABLE I. Observed and Calculated Rate Constants at Various Cl^- and Inosine Concentrations. T = 25 °C; $[H^+]$ = 0.1 *M*; μ = 0.2 *M*.

[NaCl] *M*	$10^3 \times$ [In] *M*	k_f s^{-1}	k_{calc} s^{-1}	[NaCl] *M*	$10^3 \times$ [In] *M*	k_f s^{-1}	k_{calc} s^{-1}
0	2.5	4.5	5.79	1.0×10^{-2}	2.5	1.8	1.55
0	5	8.7	9.4	1.0×10^{-2}	5	3.3	3.01
0	7.5	11.9	12.2	1.0×10^{-2}	7.5	4.7	4.37
0	10	15.4	14.6	1.0×10^{-2}	10	6.1	5.67
0	12.5	17.5	16.6	1.0×10^{-2}	12.5	7.1	6.89
0	15	19.7	18.4	1.0×10^{-2}	15	8.2	8.06
0	20	22	21.4	1.0×10^{-2}	20	10.5	10.24
1.0×10^{-3}	2.5	4.0	4.5	5.0×10^{-2}	2.5	0.3	0.52
1.0×10^{-3}	5	7.5	7.8	5.0×10^{-2}	5	0.8	1.04
1.0×10^{-3}	7.5	10.1	10.5	5.0×10^{-2}	7.5	1.3	1.55
1.0×10^{-3}	10	13.0	12.7	5.0×10^{-2}	10	1.9	2.06
1.0×10^{-3}	12.5	14.9	14.7	5.0×10^{-2}	12.5	2.3	2.57
1.0×10^{-3}	15	16.7	16.5	5.0×10^{-2}	15	2.9	3.07
1.0×10^{-3}	20	19.1	19.5	5.0×10^{-2}	20	3.9	4.05
5.0×10^{-3}	2.5	2.7	2.42	0.1	2.5	0.27	0.36
5.0×10^{-3}	5	4.7	4.54	0.1	5	0.7	0.73
5.0×10^{-3}	7.5	6.2	6.40	0.1	7.5	1.1	1.09
5.0×10^{-3}	10	7.8	8.19	0.1	10	1.5	1.45
5.0×10^{-3}	12.5	9.1	9.78	0.1	12.5	1.8	1.81
5.0×10^{-3}	15	10.1	11.2	0.1	15	2.1	2.17
5.0×10^{-3}	20	14.1	13.9	0.1	20	2.9	2.89

This rate law is too complex to be resolved directly or to be treated by the usual least-squares methods to yield values of the individual rate constants. Instead, in order to verify its validity, a computer simulation method was adopted, adjusting values of various parameters of eqn. 7 until good agreement with the whole set of experimental data was achieved. The initial set of rate constants was based on literature values obtained in reactions of $Pd(dien)Br^+$ and $Pd(dien)(H_2O)^{2+}$ with inosine and with Br^- [10]. These rate parameters were adjusted in a wide range above or below the literature values. Rate constants k_{-3} and k_4 were obtained in independent experiments by reacting the aquo complex, $Pd(en)(H_2O)_2^{2+}$ with Cl^- and with inosine respectively. The steady-state concentration of Cl^- was approximated from its equilibrium concentration taking into account eqns. 2 and 4 and using as equilibrium constants $K_1 = k_1/k_{-1}$ and $K_3 = k_3/k_{-3}$.

As the contribution of the reverse rate becomes significant at high Cl^- concentrations, the experimental rate constant, k_{obs}, was corrected to yield the forward rate constant, k_f, using the formula $k_f = k_{obs}/(1 + [Cl^-]/K[In])$ where K = 16, as determined spectrophotometrically.

The best fit achieved between experimental data and eqn. 7 is shown in Table I and in Fig. 1, while the corresponding rate constants are listed in Table II. Due to the complexity of the mechanism and to the number of rate parameters involved, it is necessary to justify the results and to discuss the errors involved.

It is significant to note that deleting any one of the steps from the mechanism depicted in Scheme 1,

TABLE II. Rate Parameters of Equation 7 at 25 °C and 0.2 *M* Ionic Strength.

$k_{S_N^2}$	= 80	M^{-1} s^{-1}
k_1	= 40	s^{-1}
k_1	= 1.3×10^4	M^{-1} s^{-1}
k_2	= 2.0×10^3	M^{-1} s^{-1}
k_3	= 8.3	s^{-1}
k_{-3}	= 4.0×10^4	M^{-1} s^{-1}
k_4	= 1.2×10^4	M^{-1} s^{-1}

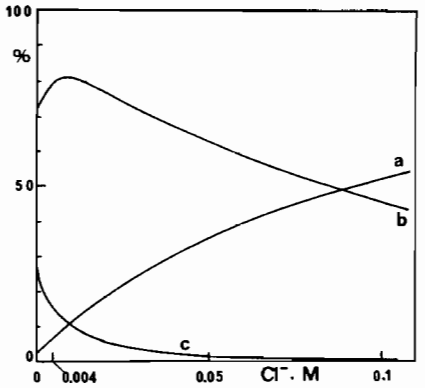


Fig. 2. Contribution (in %) of different enPd(II) species to the rate of complex formation. a) $Pd(en)Cl_2$; b) $Pd(en)(H_2O)Cl^+$; c) $Pd(en)(H_2O)_2^{2+}$.

which implies rate laws different from eqn. 7, makes it impossible to find a reasonable agreement with experimental results in the entire range of concentrations of inosine and Cl^-. The sensitivity of k_f on the values chosen for individual rate constants varies widely however. k_f is quite sensitive to values of k_{S_N2}, k_1, k_{-1}, k_2 and k_3 and corresponding errors are estimated to be between 10 and 20%. Sensitivity to values of k_{-3} and k_4 is much lower, due mainly to the smaller contribution of $Pd(en)(H_2O)_2^{2+}$ to the rate (see below). Fortunately these rate constants could be determined by direct measurements.

Discussion

The results of the present work contribute to the understanding of the mechanisms of interaction of Pd(II), and by analogy of Pt(II) complexes with nucleic acid constituents in chloride containing aqueous solutions.

The results clearly show that the mechanism implying the solvent path is important in this reaction, and, consequently, the overall rate of complex formation with inosine is greatly influenced by the concentration of the leaving ligand, Cl^-.

It follows that the reactivity of the enPd(II) complexes would increase by a factor of ≈6 when entering the cell ($[Cl^-] \cong 0.004$ *M* in the cytoplasm) from the blood ($[Cl^-] \cong 0.1$ *M*). A similar qualitative conclusion has been reached in the case of $Pt(en)Cl_2$ by Lim and Martin, based on equilibrium distribution of enPt(II) species and on rates of reaction of pyridine with dienPt(II) complexes [9].

These overall conclusions are substantiated by the detailed scrutiny of the rate constants of elementary processes in Scheme 1. Rate constants in Table II show that reactivity of inosine toward the different Pd(II) species increases markedly when chloride ions of $Pd(en)Cl_2$ are replaced successively by one and two water molecules, the ratio $k_{S_N2}:k_2:k_4$ being 1:25:150. The increase in reactivity of $Pd(en)(H_2O)Cl^+$ is similar to that observed between $Pd(dien)Br^+$ and $Pd(dien)(H_2O)^{2+}$ where a ratio of 1:28 was observed [10]. There is a further increase in reactivity for $Pd(en)(H_2O)_2^{2+}$, due presumably to more favorable electrostatic interactions and to the presence of two labile sites instead of one.

It is interesting to compare the rate of the S_N2 path, $k_{S_N2}[Pd(en)Cl_2][In]$, to that of aquation, $k_1[Pd(en)Cl_2]$. Even at the relatively high concentration of inosine of 0.01 *M*, the rate of aquation is more than 50 times greater than that of complex formation via direct S_N2 substitution. This explains the greater than usual importance of the solvent mechanism in this case.

The contribution of the three Pd(II) species to the overall rate was calculated as a function of Cl^- concentration and is shown in Fig. 2. With no chloride added, or at low chloride ion concentration similar to that prevailing in the cytoplasm (≈0.004 *M*), the main contribution to the rate is that of $Pd(en)(H_2O)Cl^+$, while that of $Pd(en)Cl_2$ is negligible. Due to its high rate constant, $Pd(en)(H_2O)_2^{2+}$ contributes significantly to the rate in these conditions, despite its low steady-state concentration.

At high chloride ion concentration (≥0.1 *M*), $Pd(en)Cl_2$ has the main contribution to the rate, but $Pd(en)(H_2O)Cl^+$ also contributes significantly. However, the overall rate is much lower in this case. This is due to the fact that rate constants of reactions of $Pd(en)(H_2O)Cl^+$ and of $Pd(en)(H_2O)_2^{2+}$ with inosine ($k_2 = 2 \times 10^3$ M^{-1} s^{-1}; $k_4 = 1.2 \times 10^4$ M^{-1} s^{-1}) are similar to those with Cl^- ($k_{-1} = 1.3 \times 10^4$ M^{-1} s^{-1}; $k_{-3} = 4 \times 10^4$ M^{-1} s^{-1}) and therefore Cl^- can effectively compete with inosine, maintaining the steady-state concentrations of these reactive species at a low level.

Both k_{-1} and k_{-3} are more than 10^5 times greater than corresponding rate constants (respectively 0.015 M^{-1} s^{-1} and 0.31 M^{-1} s^{-1}) obtained in the enPt(II)–Cl^- system by Coley and Martin [11]. This typical difference between analogous Pd(II) and Pt(II) complexes is also found in the aquation rate constants where for $Pd(en)Cl_2$ and $Pd(en)(H_2O)Cl^+$ values of 46 s^{-1} and 10 s^{-1} were obtained compared with literature values for enPt(II) of 3.4×10^{-5} s^{-1} and 4.4×10^{-5} s^{-1} [11].

It is significant that despite these differences in reactivity, aquation equilibrium constants are similar, $K_1 = 3.5 \times 10^{-3}$ *M* and $K_3 = 2.5 \times 10^{-4}$ *M* in this case compared with 2.19×10^{-3} *M* and 1.43×10^{-4} *M* in the case of $Pt(en)Cl_2$ [11].

This implies a very similar distribution of different aquo-species of Pd(II) and Pt(II) in similar chloride containing environments, such as that of living organisms treated by antitumor $Pt(en)Cl_2$.

However it is clear that while enPd(II) establishes equilibrium in a matter of seconds with H_2O, Cl^- and other ligands upon entering the blood stream, Pt(en)Cl_2 has a life-time of several hours and can migrate to all parts of the organism before actually reacting and, therefore, exerting its physiological influence.

References

1 H. D. K. Drew, F. W. Pinkard, G. H. Preston and W. Wardlaw, *J. Chem. Soc.*, 1895 (1932).
2 K. Thammavong and M. Zador, *J. Phys. E. Sci. Instrum., 9,* 1041 (1976).
3 P. C. Kong and T. Theophanides, *Bioinorg. Chem., 5,* 51 (1975).
4 D. M. I. Goodgame, I. Jeeves, F. L. Phillips and A. C. Sapski, *Biochim. Biophys. Acta, 378,* 153 (1975).
5 G. Y. H. Chu and R. S. Tobias, *J. Am. Chem. Soc., 98,* 2641 (1976).
6 I. Sovago and R. B. Martin, *Inorg. Chem., 19,* 2868 (1980).
7 G. Pneumatikakis, M. Hadjiliadis and T. Theophanides, *Inorg. Chem., 17,* 915 (1978).
8 P. C. Kong and F. D. Rochon, *Inorg. Chim. Acta, 46,* L1 (1980).
9 M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem., 38,* 1911 (1976).
10 J. Y. Séguin and M. Zador, *Inorg. Chim. Acta, 20,* 203 (1976).
11 R. F. Coley and D. S. Martin, Jr., *Inorg. Chim. Acta, 7,* 573 (1973).