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Complexes of Vitamin B_6.
XIII*. Kinetics and Mechanism of the Complex Formation of Pd(II) with Vitamin B_6 Compounds

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The stopped-flow method has been used to study the kinetics of the reaction between Pd(II) and the vitamin B_6 (VB_6) compounds, pyridoxol (P), pyridoxal (PL) and pyridoxamine (Pm) at 37 °C and ionic strength 0.15 M (NaCl) in acidic solutions. The observed dependence of the rate constants, k_{obs}, on the total concentration of the ligands, T_L, at a given pH is as follows for each system.

$k_{obs}(P) = A + BT_P + CT_P^2$

$k_{obs}(PL) = D + ET_{PL}$, *and*

$k_{obs}(Pm) = F + GT_{Pm}$

The parameters A, B, C, D, E, F and G are pH dependent. The dependence is mostly of the quadratic form. In order to account for the isolated complexes of the form $Pd(H\text{-}VB_6)_2Cl_2$, it has been suggested that in the case of Pd(II)–PL and Pd(II)–Pm systems, prior fast formation of $Pd(H\cdot VB_6)Cl_3$ complex species has been assumed.

Introduction

Some Pt(II) complexes have been shown in the last decade to have anti-carcinogenic properties [1]. These properties were attributed to several characteristics of these complexes; among which, their relative chemical inertness. On the other hand, some Pd(II) analogous of active Pt(II) have been similarly examined to decipher their possible anti-carcinogenic effects but no significant activity was observed [2,3]. Although they are bacteriostatic at low concentrations and somewhat active against some carcinogenic cells, they are not comparable to their Pt(II) analogues [1]. The speculation offered was that Pd(II) complexes are too reactive *in vivo* and are unlikely to be effective unless strong deactivating ligands are present.

Quite recently we have synthesised two Pd(II) complexes of the vitamin B_6 compounds, pyridoxol (P) and pyridoxal (PL). These complexes were shown to have inhibitory effects on some cell divisions [4]. It has also been shown that they are non-toxic. These findings encouraged us to study the kinetic factor involved in their formation, together with a new complex of Pd(II) with pyridoxamine (Pm).

Experimental

Materials

Pyridoxol hydrochloride (P·HCl), pyridoxal hydrochloride (PL·HCl) and pyridoxamine dihydrochloride (Pm·2HCl) were analytically pure chemicals and were used without further purification. Stock solutions of 0.1 *M* of the ligands were kept in the dark at 4 °C. Stock solution of Pd(II) chloride (0.098 *M*) was prepared in 1.0 *M* HCl. The concentration of Pd(II) was checked by gravimetric method as Pd(dimethylglyoxime)$_2$ [5].

Measurements

pH measurements were carried out employing Radiometer pH-meter type 62 equipped with a combined glass electrode (GK2 301 C). Calibration of the pH-meter was done successively by two Radiometer buffers at 4.01 and 7.00. Spectrophotometric measurements were carried out on Pye Unicam SP8-100 spectrophotometer. Kinetic measurements were done using a Durrum stopped flow apparatus. The optical path length was 20.0 mm. The mixing syringe and cuvette were thermostatted at 37 °C. The observed pseudo-first order rate constants were calculated for data to 80% of the reaction completion.

In all measurements the ionic strength was kept constant at 0.15 *M* NaCl. The concentration ranges of ligands used were $(0.3–4.0) \times 10^{-2}$ *M*. The Pd(II) concentration was kept constant at 4.91×10^{-4} *M*.

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The complexes of Pd(II) with P, PL and Pm were synthesised at pH ~ 4.0 as previously described [4]. The compositions of different complexes are compatible with the following formulas $Pd(HP)_2Cl_2$, $Pd(HPL)_2Cl_2$ and $Pd(H_2{\cdot}Pm)_2Cl_2$ as well as $(PdCl_4)(H_3Pm)(H_2O)$.

Results and Discussion

The Reaction of Pd(II) with Pyridoxol

A yellowish-orange solution was obtained when dilute solutions of $PdCl_2$ (~5×10^{-4} *M*) were added to pyridoxol solutions in the pH-range 3.0–6.0. Increasing the concentration of $PdCl_2$, keeping that of P in excess, usually leads after a while to the precipitation of orange-yellow crystals which have been identified as $Pd(HP)_2Cl_2$ [4]. The spectra of the system is pH-dependent. At a given pH, the spectrum consists mainly of a shoulder to the P absorption band. This is in the wavelength range of 300–400 nm. $PdCl_2$ itself exhibits a band at λ_{455} nm, which loses its identity in the presence of the ligand in acidic solutions. It shifts to shorter wavelengths as pH increases.

The kinetic runs were carried out at λ_{450} nm in the pH range 2.5–5.0. The interaction of palladium(II) species with pyridoxol species resulted in the formation of two rate steps, one of which is faster with rate constants 1–2 orders higher than the slower. Figure 1 shows the dependence of the observed pseudo-first order rate constants of the faster step, $k_{obs}(P)$ (sec^{-1}) on both pH and the total ligand concentration of P, T_P. The observed rate constants are not linear with respect to pH and T_P. Figure 2 shows the dependence of k_{obs} on T_P at various pH's. This dependence may be expressed by the following equation,

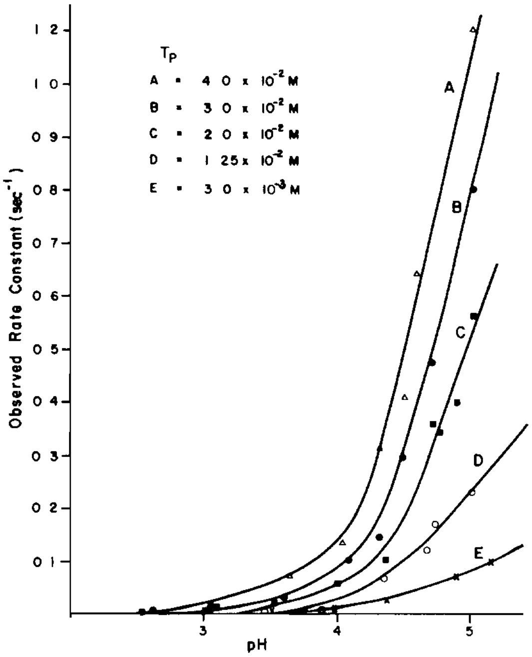

Fig. 1. $k_{obs}(P)$ in their dependence on pH and T_p.

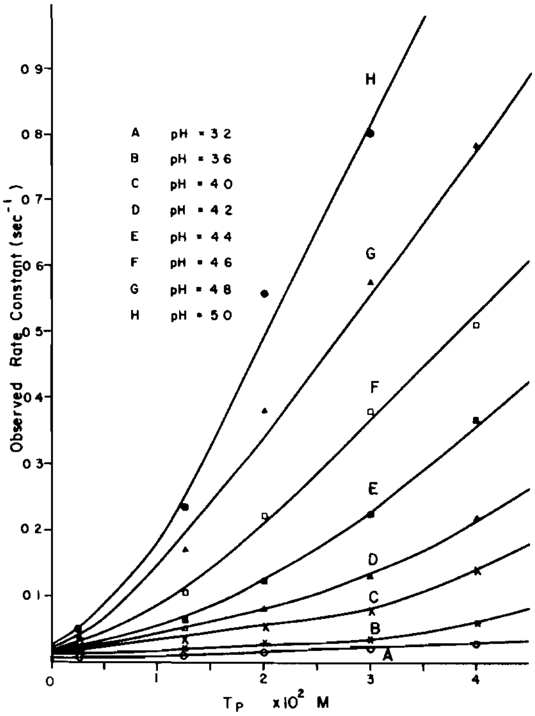

Fig. 2. $k_{obs}(P)$ as function of T_p at various pH's.

$$k_{obs}(P) = A + BT_P + CT_P^2 \qquad (1)$$

TABLE I. The Magnitude of the Values of the Parameters A, B and C at various pH's.

pH[a]	A[b]	B	C	R[c]
3.4	0.004	0.44 ± 0.03	13.16 ± 1.05	0.991
3.8	0.007	1.02 ± 0.02	29.00 ± 0.96	0.998
4.0	0.008	1.24 ± 0.02	51.85 ± 0.92	0.999
4.1	0.010	1.40 ± 0.01	65.02 ± 0.26	0.999
4.2	0.011	1.44 ± 0.04	99.36 ± 1.74	0.999
4.3	0.012	1.77 ± 0.05	124.70 ± 1.98	0.999
4.4	0.013	2.21 ± 0.03	167.16 ± 1.07	0.999
4.5	0.014	3.29 ± 0.03	183.53 ± 1.32	0.999
4.6	0.015	5.14 ± 0.03	227.28 ± 1.20	0.999
4.7	0.016	6.78 ± 0.05	263.26 ± 1.93	0.999
4.8	0.017	8.54 ± 0.05	307.00 ± 2.07	0.999
4.9	0.018	11.53 ± 0.04	331.14 ± 1.83	0.999
5.0	0.020	13.50 ± 0.06	414.12 ± 2.24	0.999

[a] Interpolated values [b] Obtained from Fig. 2. [c] Correlation coefficients.

However, to get the magnitude of the coefficients is not straightforward by regression analysis of the data, since the intercept may be in error. The magnitude of A values were obtained graphically from Fig. 2 (listed in Table I) and plots of $(k_{obs} - A)/T_P$ *Vs.* T_P were made, Fig. 3. Table I depicts the values of slope (C) and intercept (B) of the linear plots, shown in Fig. 3 as a function of pH.

In the presence of excess concentrations of Cl^- (0.15 *M* 4.9×10^{-4} *M* Pd(II)) and in the pH range used, several species coexist. The predominant species of Pd(II) are $PdCl_4^{2-}$ and $PdCl_3OH^{2-}$ and those of P are H_2P^+ and HP, Table (II). The reaction mechanism which may account for the experimental findings expressed by eqn. 1 is shown in Scheme 1:

a) $PdCl_4^{2-} + H_2P^+ \rightleftharpoons PdHPCl_3^- + H^+ + Cl^-$; k_1, k_{-1}

$\updownarrow K_{IP}$

b) $PdCl_4^{2-} + HP \rightleftharpoons PdHPCl_3^- + Cl^-$; k_2, k_{-2}

$\updownarrow K_{CI}$

c) $PdCl_4^{2-} + 2H_2P^+ \rightleftharpoons Pd(HP)_2Cl_2 + 2H^+ + 2Cl^-$; k_3, k_{-3}

d) $PdCl_4^{2-} + 2HP \rightleftharpoons Pd(HP)_2Cl_2 + 2Cl^-$; k_4, k_{-4}

$\updownarrow K_{OH}$

e) $PdCl_3OH^{2-} + H_2P^+ \rightleftharpoons Pd(HP)Cl_3^- + H_2O$; k_5, k_{-5}

f) $PdCl_3OH^{2-} + HP \rightleftharpoons Pd(HP)Cl_3^- + OH^-$; k_6, k_{-6}

g) $PdCl_3OH^{2-} + 2H_2P^+ \rightleftharpoons Pd(HP)_2Cl_2 + H^+ + Cl^- + H_2O$; k_7, k_{-7}

h) $PdCl_3OH^{2-} + 2HP \rightleftharpoons Pd(HP)_2Cl_2 + Cl^- + OH^-$; k_8, k_{-8}

Scheme I.

where k_i's and k_{-i}'s are the forward and backward rate constants, K_{OH} the hydrolysis constant of Pd(II), and K_{CI} the first stepwise formation constant of $Pd(HP)_2Cl_2$. The rate equation describing the reaction mechanism shown in Scheme 1 can be written as follows:

$$\frac{d(C_{IP} + C_{IIP})}{dt} = Q_1 \frac{d(C_{IIP})}{dt}$$

$$= \frac{T_{Pd} - C_{IIP}Q_1}{Q_2}(Q_3 + Q_5) - C_{IIP}\left(\frac{(Cl^-)Q_4}{(HP)K_{CI}} + Q_6\right) \quad (2)$$

where C_{IP} and C_{IIP} are the ternary complexes $Pd(HP)Cl_3$ and $Pd(HP)_2Cl_2$, respectively, and

$Q_1 = 1 + (Cl^-)/K_{CI}(HP)$,

$K_{CI} = [C_{IIP}][Cl^-]/[C_{IP}][HP]$,

$Q_2 = 1 + K_{OH}(OH^-)(Cl^-)^{-1}$,

$K_{OH} = [PdCl_3OH^{2-}][Cl^-]/[PdCl_4^{2-}][OH^-]$,

$Q_3 = k_1(H_2P) + k_2(HP) + k_3K_{OH}(H_2P)(OH^-)/(Cl^-) + k_6[(HP)K_{OH}(OH^-)/(Cl^-)$

$Q_4 = k_{-1}(H^+)/(Cl^-) + k_{-2}(Cl^-) + k_{-5} + k_{-6}(OH^-)$

$Q_5 = k_3(H_2P)^2 + k_4(HP)^2 + k_7(H_2P)(OH^-)/(Cl^-) + k_8K_{OH}(HP)^2(OH^-)/(Cl^-)$

and

$Q_6 = k_{-3}(H^+)^2(Cl^-)^2 + k_{-4}(Cl^-)^2 + k_{-7}(H^+)(Cl^-) + k_{-8}(OH^-)(Cl^-)$.

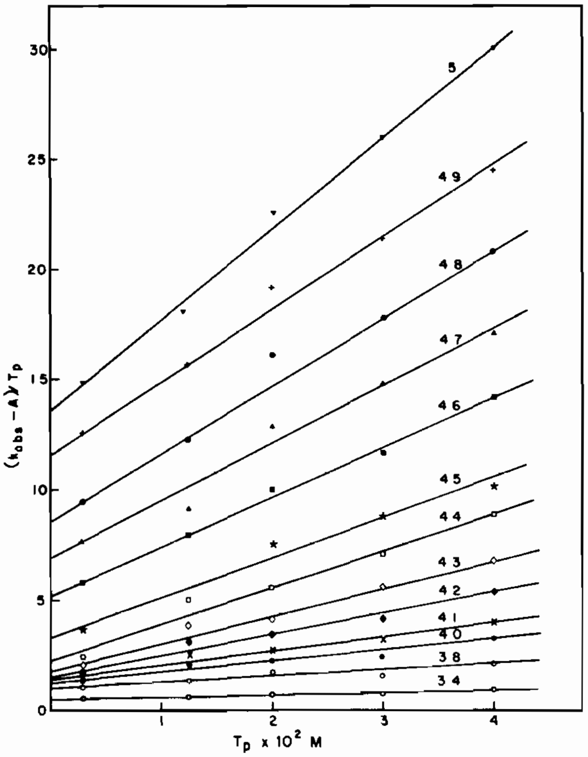

Fig. 3. $(k_{obs}(P) - A)/T_P$ as function of T_P.

TABLE II. The Equilibrium Constants of the Species Mentioned in This Work.

Reaction	log K	Ref.
$H_2P^+ \rightleftharpoons HP + H^+$	−4.85	7
$HP \rightleftharpoons P^- + H^+$	−8.92	7
$H_2PL^+ \rightleftharpoons HPL + H^+$	−4.15	7
$HPL \rightleftharpoons PL^- + H^+$	−8.67	7
$H_3Pm^{2+} \rightleftharpoons H_2Pm^+ + H^+$	−3.62	7
$H_2Pm^+ \rightleftharpoons HPm + H^+$	−8.19	7
$HPm \rightleftharpoons Pm^- + H^+$	−10.49	7
$PdCl_4^{2-} + OH^- \rightleftharpoons PdCl_3OH^{2-} + Cl^-$	5.70	6

The rate eqn. 2 can be integrated if pH and T_P are constants, which is actually the case (pH is constant due to the buffering effect of H_2P^+ and HP and $T_P > T_{Pd}$), and if it is assumed that $dC_I/dt \cong 0$. The integrated form is,

$$\ln \frac{(C_{IIP})_{eq}}{(C_{IIP})_{eq} - (C_{IIP})_t} = \left\{ \frac{Q_3 + Q_5}{Q_2} + \frac{Q_6}{Q_1} + \frac{Q_4(Cl^-)}{Q_1(HP)K_{CI}} \right\} t \qquad (3)$$

where $(C_{IIP})_{eq}$ and $(C_{IIP})_t$ are the concentration of $Pd(HP)_2Cl_2$ at equilibrium and time, t, respectively. If eqn. 3 is analyzed carefully it can be found that $Q_2 = 1.0$ under the experimental conditions used in this work. The equation takes the following form if the absorbances at equilibrium and time, t, (*i.e.* As_{eq} and As_t) are directly proportional to $(C_{IIP})_{eq}$ and $(C_{IIP})_t$.

$$\ln \frac{(As)_{eq}}{(As)_{eq} - (As)_t} = \left\{ \frac{Q_6}{Q_1} + Q_3 + Q_5 + \frac{Q_4(Cl^-)}{Q_1(HP)K_{CI}} \right\} t \qquad (4)$$

The observed rate constant may be correlated to $\frac{Q_6}{Q_1} + \frac{Q_4(Cl^-)}{Q_1(HP)K_{CI}} + Q_3 + Q_5$, *i.e.*

$$\begin{aligned} k_{obs} = {} & \frac{T_P K_{1P} K_{CI}}{T_P K_{IP} K_{CI} + (Cl^-)((H^+) + K_{IP})} (k_{-3}(H^+)^2(Cl^-)^2 + \\ & + k_{-4}(Cl^-)^2 + k_{-7}(H^+)Cl^- + k_{-8}(OH^-)(Cl^-) + \\ & + \frac{(Cl^-((H^+) + K_{IP})}{T_P K_{IP} K_{CI} + (Cl^-)((H^+) + K_{IP})} (k_{-1}(H^+)(Cl^-) + \\ & + k_{-2}(Cl^-) + k_{-5} + k_{-6}(OH^-) + \\ & + \frac{T_P}{(H^+) + K_{IP}} \{k_1(H^+) + k_5 K_{OH} K_w (Cl^-)^{-1} + \\ & + k_2 K_{IP} + k_6 K_{OH} K_{IP} (OH^-)(Cl^-)^{-1}\} + \\ & + \frac{T_P^2}{(H^+) + K_{IP}} \{k_3(H^+)^2 + k_7 K_{OH} K_W (H^+)(Cl)^{-1} + \\ & + k_4 K_{IP}^2 + k_7 K_{IP}^2 K_{OH} (OH^-)(Cl^-)^{-1}\} \end{aligned} \qquad (4)$$

where K_{IP} is the 1st dissociation constant of H_2P^+ (Table II).

The coefficients A, B and C can now take the following forms if $T_P K_{IP} K_{CI} \gg (Cl^-)((H^+) + K_{IP})$;

$$\begin{aligned} A = {} & k_{-3}(H^+)^2(Cl^-)^2 + k_{-4}(Cl^-)^2 + k_{-7}(H^+)(Cl^-) + \\ & + k_{-8}(OH^-)(Cl^-) \end{aligned} \qquad (5a)$$

(k_{obs}(P) is not function of $1/T_P$).

$$\begin{aligned} B = {} & (k_1(H^+) + k_5 K_{OH} K_W (Cl^-)^{-1} + k_2 K_{IP} + \\ & + k_6 K_{OH} K_{IP} (OH^-)(Cl^-)^{-1})((H^+) + K_{IP})^{-1} \end{aligned} \qquad (5b)$$

$$\begin{aligned} C = {} & (k_3(H^+)^2 + k_7 K_{OH} K_W (H^+)(Cl^-)^{-1} + k_4 K_{IP}^2 + \\ & + k_8 K_{OH} K_{IP}^2 (OH^-)(Cl^-)^{-1})((H^+) + K_{IP})^{-2} \end{aligned} \qquad (5c)$$

The plot of $A(H^+)^{-1}$ *vs* $(H^+)^{-1}$ (Fig. 4a) follows the equation:

$$A(H^+)^{-1} = a_0 + a_1(H^+)^{-1} + a_2(H^+)^{-2} \qquad (6)$$

The values of a_0, a_1 and a_2 are: 0, $(12.72 \pm 0.42)10^{-3}$ and $(7.59 \pm 0.42)10^{-8}$, respectively, (R = 0.999). Equation 6 implies that,

$a_0 = k_{-7}(Cl^-) = 0$

$a_1 = k_{-4}(Cl^-)^2 = 12.7 \times 10^{-3}$ $(k_{-4} = 0.56)$

$a_2 = k_{-8}K_W(Cl^-) = 7.59 \times 10^{-8}$ $(k_{-8} = 2.84 \times 10^7)$

The coefficients B and C are also pH-dependent, in a complicated way. The plot of $B((H^+) + K_{IP})(H^+)^{-1}$ *vs.* $(H^+)^{-1}$ is quadratic and is shown in Fig. 4b. The relation obeys the following equation:

$$B((H^+) + K_{IP})(H^+)^{-1} = b_0 + b_1(H^+)^{-1} + b_2(H^+)^{-2} \qquad (7)$$

where, $b_0 \cong 0$, $b_1 = (1.19 \pm 0.30)10^{-4}$ and $b_2 = (2.28 \pm 0.30)10^{-9}$ respectively, (R = 0.997).

From eqn. 7 one may conclude that

$b_0 = k_1 \cong 0$

$b_1 = k_5 K_{OH} K_W (Cl^-)^{-1} + k_2 K_{IP} = 1.19 \times 10^{-4}$

and

$b_2 = k_6 K_{OH} K_{IP} K_W (Cl^-)^{-1}$ $(k_6 = 2.72 \times 10^3)$

If $b_1 = k_5 K_{OH} K_W (Cl^-)^{-1}$ then $k_5 = 1.41 \times 10^8$, however, if $b_1 = k_2 K_{IP}$, then $k_2 = 8.42 \times 10^8$.

On the other hand, the plot of $C((H^+) + K_{IP})^2 (H^+)^{-2}$ *vs.* $(H^+)^{-1}$ (Fig. 4c) is also of the quadratic type and has the following form:

$$C((H^+) + K_{IP})^2(H^+)^{-2} = C_0 + C_1(H^+)^{-1} + C_2(H^+)^{-2} \qquad (8)$$

where $C_0 \cong 0$, $C_1 = (6.26 \pm 1.53)10^{-3}$ and C_2 (1.87 ± 0.15) 10^{-7}, respectively, (R = 0.999). Again these constants may be correlated to:

$C_0 = k_3 = 0,$

$C_1 = k_7 K_{OH} K_W{}^*(Cl^-)^{-1}$ $(k_7 = 1.05 \times 10^8)$

and

$C_2 = k_4 K_{IP}^2$ $(k_4 = 9.37 \times 10^2)$

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*$pK_W = 13.75$ at 25 °C (Ref. 8).

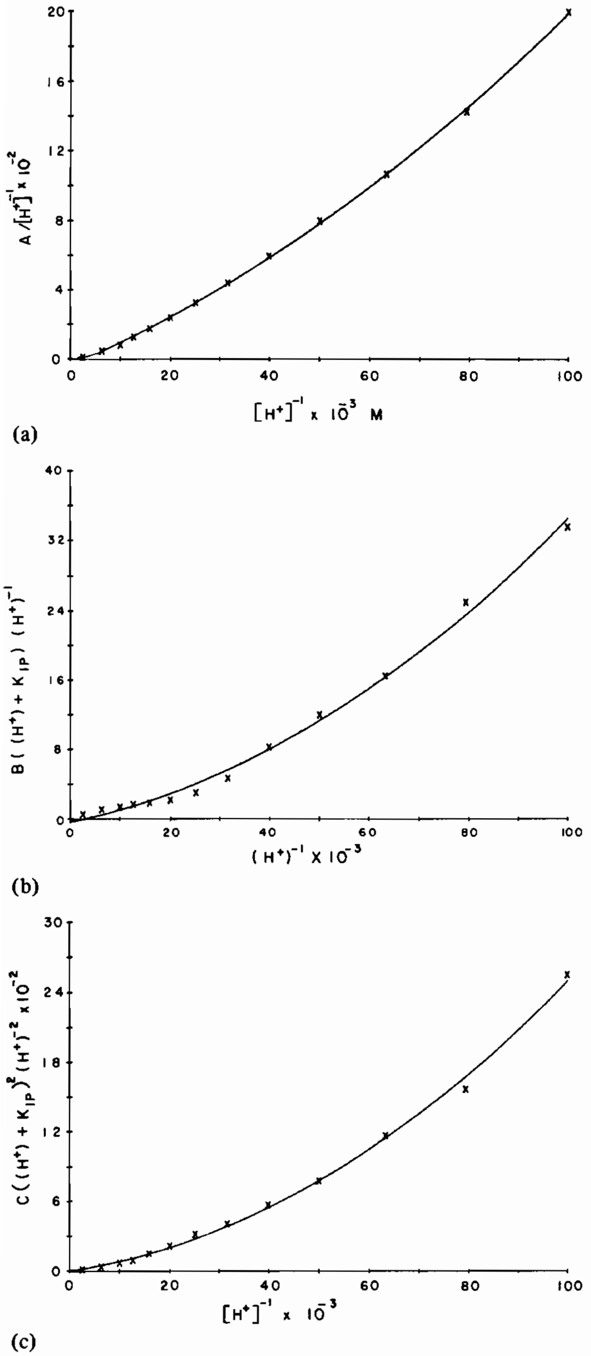


Fig 4. a) The plot of $A(H^+)^{-1}$ as function of $(H^+)^{-1}$ b) The plot of $B((H^+) + K_{IP})^{-1}$ vs. $(H^+)^{-1}$. c) The plot of $C((H^+) + K_{IP})^2(H^+)^{-2}$ vs. $(H^+)^{-1}$.

Since $C((H^+) + K_{IP})^2(H^+)^{-2}$ is not a function of $(H^+)^{-3}$, the term $k_8K_{OH}K_{IP}^2(OH^-)(Cl^-)^{-1}$ is insignificant, in other words $k_8 \ll {\sim}10^{15}$.

From the above findings, one may expect that the reactions a, c and probably f do not contribute much in the model mechanism shown in Scheme 1.

The slower step starts usually at pH's greater than 4.4, which may imply slow hydrolysis of the $Pd(HP)_2Cl_2$ complex. It has been proved by photoacoustic spectral studies that these complexes react readily with DNA at pH ~ 7.4 (4). However, we have not reached this pH in this work. A good speculation is that the complex which reacted with DNA is probably of the composition $Pd(HP)_2(OH)_2$. No attempt has been made to interpret the kinetic data of the slow process.

The Reaction of Pd(II) with Pyridoxal

A yellowish solution was obtained when a dilute solution of $PdCl_2$ (~5 × 10^{-4} *M*) was added to pyridoxal solution in the pH range 3.0–6.0. A yellow precipitate is obtained when Pd(II) concentration is increased. The precipitate was identified as $Pd(HPL)_2Cl_2$ [4]. The spectra of the system is similar to that of Pd(II)–P system: they are both pH-dependent.

The kinetic runs were carried out at λ_{470} nm in the pH-range 3.6–5.6. Figures 5 and 6 show the dependence of the pseudo first order observed rate constants $k_{obs}(PL)$ (sec^{-1}) on pH and total concentration of pyridoxal, T_{PL}. The dependence of $k_{obs}(PL)$ (sec^{-1}) on pH, on the other hand, is linear which is quite

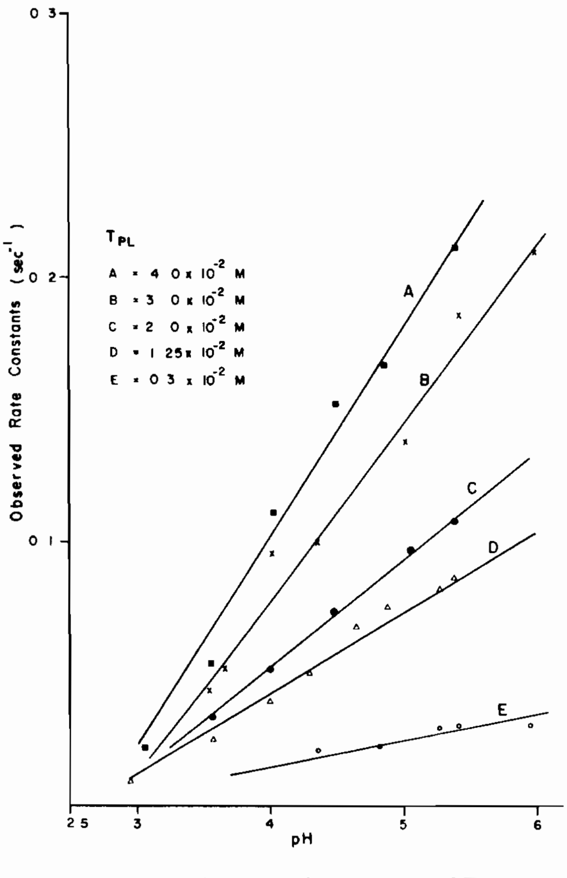


Fig 5. $k_{obs}(PL)$ in their dependence on pH and T_{PL}.

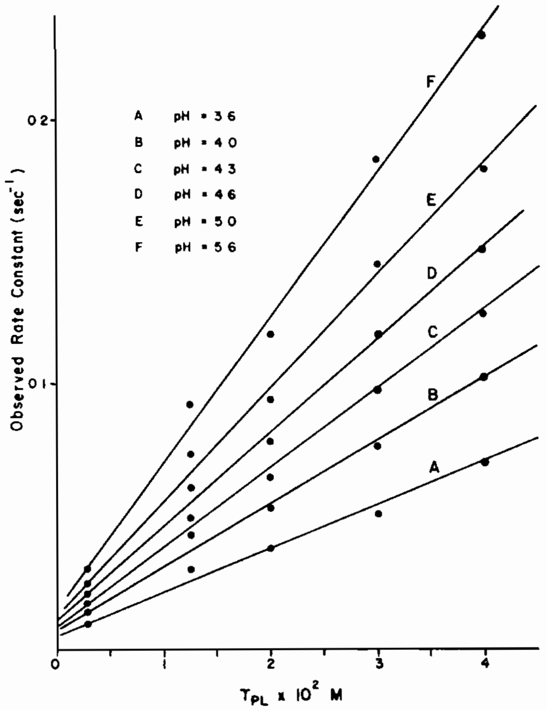


Fig. 6. k_{obs}(PL) as function of T_{PL} at various pH's.

TABLE III. The Values of the Parameters D and E in Their Dependence on pH.

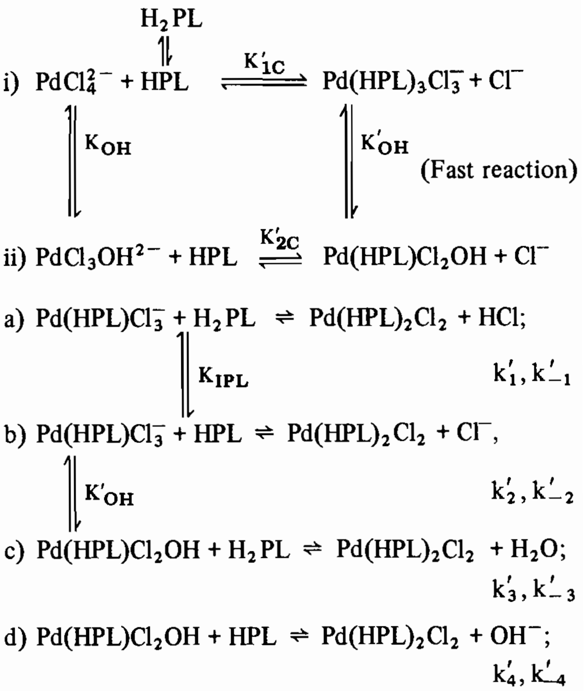
pH[a]	D × 10^3	E	R[b]
3.4	5.40 ± 2.99	1.10 ± 0.12	0.982
3.6	6.96 ± 2.79	1.47 ± 0.11	0.991
3.8	7.88 ± 2.65	1.85 ± 0.11	0.995
4.0	8.78 ± 2.64	2.25 ± 0.11	0.997
4.2	10.09 ± 2.76	2.63 ± 0.11	0.997
4.4	11.23 ± 2.65	3.01 ± 0.11	0.998
4.6	12.57 ± 3.20	3.38 ± 0.13	0.998
4.8	13.47 ± 3.75	3.78 ± 0.15	0.998
5.0	14.37 ± 3.98	4.17 ± 0.16	0.998
5.2	15.93 ± 4.44	4.54 ± 0.17	0.998
5.4	17.46 ± 5.00	4.92 ± 0.20	0.997
5.6	18.11 ± 5.24	5.33 ± 0.21	0.997
5.8	19.19 ± 6.30	5.72 ± 0.25	0.997
6.0	19.77 ± 6.59	6.15 ± 0.27	0.997

[a] Interpolated values using Fig. 6. [b] Correlation coefficients

different from that of the Pd(II)–P system, indicating different mechanisms. At a given pH, this dependence can be expressed as,

$$k_{obs}(PL) = D + ET_{PL} \qquad (9)$$

Table III depicts the values of the parameters D and E in their dependence on pH. The mechanism which may fit the experimental data is shown in Scheme II



$$\text{i) } PdCl_4^{2-} + HPL \ (\rightleftharpoons H_2PL) \xrightleftharpoons{K'_{1C}} Pd(HPL)_3Cl_3^- + Cl^-$$

$$K_{OH} \qquad K'_{OH} \qquad \text{(Fast reaction)}$$

$$\text{ii) } PdCl_3OH^{2-} + HPL \xrightleftharpoons{K'_{2C}} Pd(HPL)Cl_2OH + Cl^-$$

$$\text{a) } Pd(HPL)Cl_3^- + H_2PL \rightleftharpoons Pd(HPL)_2Cl_2 + HCl; \qquad k'_1, k'_{-1}$$

$$K_{IPL}$$

$$\text{b) } Pd(HPL)Cl_3^- + HPL \rightleftharpoons Pd(HPL)_2Cl_2 + Cl^-, \qquad k'_2, k'_{-2}$$

$$K'_{OH}$$

$$\text{c) } Pd(HPL)Cl_2OH + H_2PL \rightleftharpoons Pd(HPL)_2Cl_2 + H_2O; \qquad k'_3, k'_{-3}$$

$$\text{d) } Pd(HPL)Cl_2OH + HPL \rightleftharpoons Pd(HPL)_2Cl_2 + OH^-; \qquad k'_4, k'_{-4}$$

Scheme II

To account for the isolated complex $Pd(HPL)_2Cl_2$ it was necessary to assume the prior fast formation of $Pd(HPL)Cl_3$ and $Pd(HPL)Cl_2OH$.

The rate equation describing the above reaction mechanism, at a given pH, is

$$\frac{dC_{IIPL}}{dt} = \left(\frac{T_{Pd} - C_{IIPL}}{Q'_2}\right)\frac{T_{PL}Q_7}{((H^+) + K_{IPL})} - C_{IIPL}Q_8 \qquad (10)$$

where C_{IIPL} correspond to the concentration of Pd(HPL)Cl_2 and C_{IIPL} correspond to $Pd(HPL)_2Cl_2$ and

$$Q_7 = k'_1(H^+) + k'_2K_{IPL} + k'_3K'_{OH}(OH^-)(H^+)(Cl^-)^{-1} + $$
$$+ k'_4K_{IPL}K'_{OH}(OH^-)(Cl^-)^{-1},$$

$$Q'_2 = 1 + K'_{OH}(OH^-)(Cl^-)^{-1} \cong 1$$

$$Q_8 = k'_{-1}(H^+)(Cl^-) + k'_{-2}(Cl^-) + k'_{-3} + k'_{-4}(OH^-),$$

and

$$K'_{OH} = (Pd(HPL)Cl_2OH)(Cl^-)/(Pd(HPL)Cl_3)(OH^-)$$

and K_{IPL} is the dissociation constant of H_2PL^+, Table II.

The integrated form of eqn. 10 is obtained if T_{Pd} $(Pd(HPL)Cl_3)$ + $(Pd(HPL)Cl_2OH)$ + $(Pd(HPL)_2Cl_2)$, and $dC_{IPL}/dt = 0$

$$\frac{\ln (C_{IIPL})_{eq.}}{(C_{IIPL})_{eq} - (C_{IIPL})_t} = \{Q_8 + Q_7T_{PL}/Q_2((H^+) + K_{IPL})\}t \qquad (11)$$

Again, the observed rate constant may be correlated with $Q_8 + Q_7T_{PL}/Q_2((H^+) + K_{IPL})$, *i.e.*

$$k_{obs}(PL) =$$

$$= k'_{-1}(H^+)(Cl^-) + k'_{-2}(Cl^-) + k'_{-3} +$$
$$+ k'_{-4}(OH^-) + T_{PL}(k'_1(H^+) + k'_2K_{IPL} +$$
$$+ k'_3K'_{OH}(H^+)(OH^-)(Cl^-)^{-1}$$
$$[(1 + K'_{OH}(OH^-)(Cl^-)^{-1})((H^+) + K_{IPL})]^{-1} \quad (12)$$

Consequently, the coefficients of eqn. 9 are

$$D = k'_{-1}(H^+)(Cl^-) + k'_{-2}(Cl^-) + k'_{-3} + k'_{-4}(OH^-) \quad (13a)$$

$$E = (k'_1(H^+) + k'_2K_{IPL} + k'_3K'_{OH}K_W(Cl^-)^{-1} +$$
$$+ k'_4K_{IPL}K'_{OH}(OH^-)(Cl^-)^{-1} \times \quad (13b)$$
$$\times \{(1 + K'_{OH}(OH^-)(Cl^-)^{-1})((H^+) + K_{IPL})\}^{-1}$$

The term, $K'_{OH}(OH^-)(Cl^-)^{-1}$ is very small and may be ignored.

The plot of $D(H^+)^{-1}$ *vs.* $(H^+)^{-1}$ (Fig. 7a) may be represented by the following eqn.:

$$D = d_0 + d_1(H^+)^{-1} + d_2(H^+)^{-2}$$

The values of d_0, d_1 and d_2 are 0, $(17.5 \pm 0.4)10^{-3}$ and $(2.48 \pm 0.42)10^{-9}$, respectively. (R = 0.999). In other words, $k'_{-1}(Cl^-) = 0$. $(k'_{-3} + k'_{-2}(Cl^-)) = 17.5 \times 10^{-3}$, ($k'_{-2} = 11.67 \times 10^{-2}$ if $k'_{-3} = 0$ and $k'_{-3} = 17.5 \times 10^{-3}$ if $k'_{-2} = 0$) and $k'_{-4}K_W = 2.48 \times 10^{-4}$ ($k'_{-4} = 1.39 \times 10^5$).

The variation of $E((H^+) + K_{IPL})(H^+)^{-1}$ as function of $(H^+)^{-1}$ is shown in Fig. 7(b). The regression analysis of the data indicated that

$$E = e_0 + e_1(H^+)^{-1} + e_2(H^+)^{-2} \quad (15)$$

The magnitude of the values of the coefficients e_0, e_1 and e_2 are 0.66 ± 0.05, $(3.31 \pm 0.04)10^{-4}$ and $(8.84 \pm 0.54) \times 10^{-11}$. These coefficients may correspond to $k'_1 = 0.66$, $(k'_2K_{IPL} + k'_3K'_{OH}K_W(Cl^-)^{-1}) = 3.31 \times 10^{-4}$ and $k'_4K_{IPL}K'_{OH}(Cl^-)^{-1} = 8.84 \times 10^{-11}$. However, if k'_2K_{IPL} is only equal to 3.31×10^{-4}, then $k'_2 = 4.67$ and if $k'_3K'_{OH}K_W(Cl^-)^{-1}$ takes the value of 3.31×10^{-4}, then k'_3 may be equal to 5.57×10^3 if $K'_{OH} \cong K_{OH}$. A rough value for k'_4 may be obtained if $K'_{OH} = K_{OH}$, *i.e.* $k'_4 \cong 4.7 \times 10^{-10}$.

It seems from the above discussion that the complexation reactions of Pd(II) with PL are different from that of Pd(II) with P, which implies that both complexes are not structurally identical. This was reflected on their inhibitory effects on the cell divisions of *E. coli* [4]. $Pd(HPL)_2Cl_2$ inhibits more cell divisions of *E. coli* than $Pd(HP)_2Cl_2$.

The Reaction of Pd(II) with Pyridoxamine

Similar to the Pd(II)–PL system, the addition of a solution of Pm to a solution of Pd(II) chloride yields a yellowish brown solution. However, a yellow precipitate is not readily obtained under the same con-

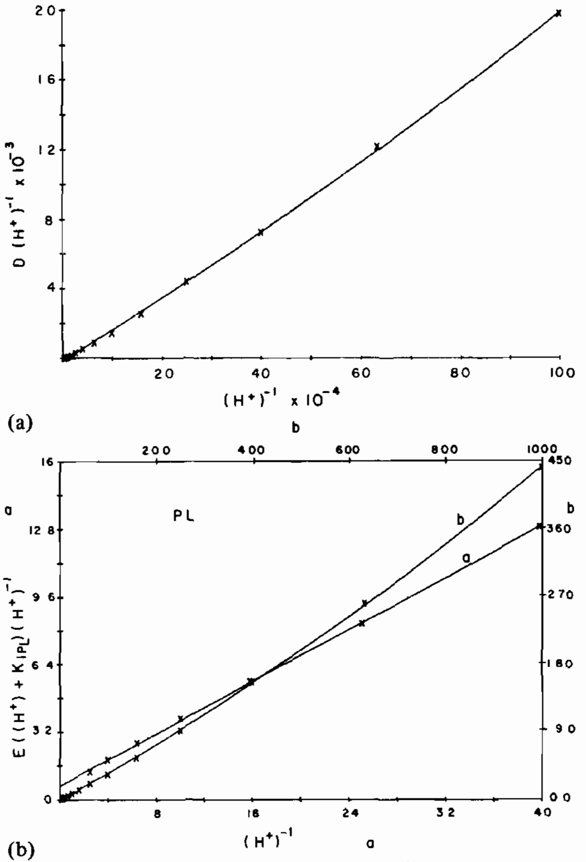


Fig 7. a) The plot of $D(H^+)^{-1}$ *vs.* $(H^+)^{-1}$. b) The variation of $E((H^+) + K_{IPL})(H^+)^{-1}$ as function of $(H^+)^{-1}$.

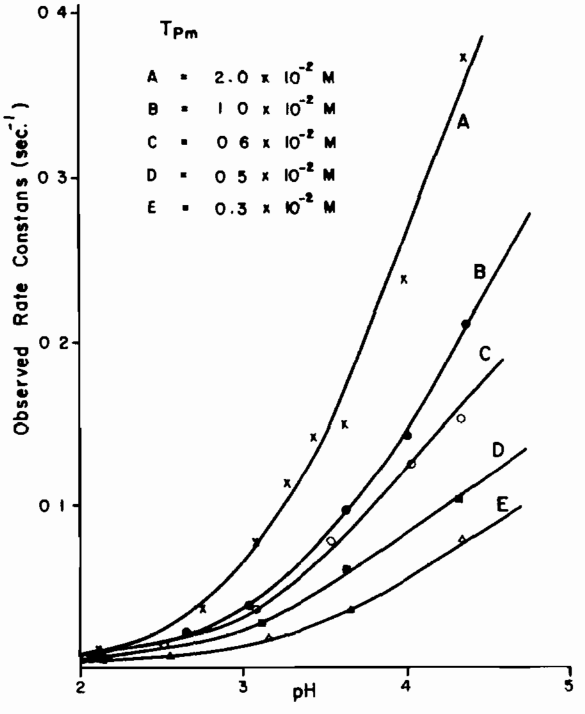


Fig. 8. The dependence of $k_{obs}(Pm)$ on pH and T_{Pm}.

ditions of precipitation in Pd(II)–P or Pd(II)–PL systems. Isolation of a brownish complex of possible composition PdH_3PmCl_4 at pH ~2.0, and a yellow one with the composition $Pd(HPm)_2Cl_2$ at pH ~4.0, was achieved on slow evaporation for at least 3 days under ordinary atmospheric conditions. The solubility of these complexes are much higher than those obtained from Pd(II)–P and Pd(II)–PL systems. The spectra of dilute solutions of Pd(II)–Pm ($T_{Pd} = 2 \times 10^{-4}$ *M* – $T_{Pm} = 2 \times 10^{-3}$ *M*) exhibit a band at λ_{385} nm at pH = 1.62 while that of Pm alone has no absorption band in the $\lambda_{370} - \lambda_{550}$ nm, and that of $PdCl_2$ has an absorption band at λ_{455} nm. The band of the complex has twice the intensity of that of $PdCl_2$. However, by increasing the pH to 5 a large increase in absorption is achieved while those of $PdCl_2$ and Pm are more or less the same.

The kinetics run were taken at $\lambda = 500$ nm in the pH-range 2.2–4.2. Figure 8 shows the dependence of $k_{obs}(Pm)$ (sec^{-1}) on pH and total concentration of Pm, T_{Pm}. Figure 9 is constructed from Figure 8 to show the linear dependence of $k_{obs}(Pm)$ on T_{Pm}, which may be empirically represented as follows:

$$k_{obs}(Pm) = F + GT_{Pm} \qquad \text{(at a given pH)} \qquad (16)$$

Table IV depicts the values of F and G at different pHs.

The isolation of a solid complex of the composition $Pd(H_3Pm)Cl_4$ at low pH s (<2.0) in addition to the linear dependence of $k_{obs}(Pm)$ on T_{Pm} beside the separation of another complex at higher pH s of the composition $Pd(H_2Pm)_2Cl_2$ may suggest that the obtained kinetic data correspond to the formation of the latter complex from the former. The formation of the first complex is much faster than that of the second complex. The reaction mechanism compatible with the experimental data is shown in Scheme III.

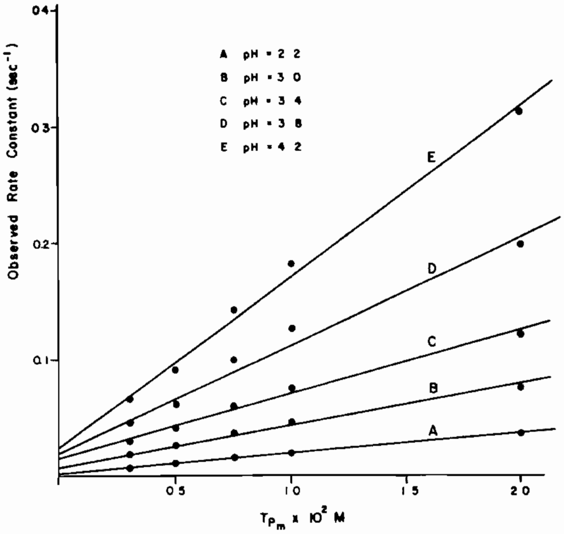


Fig. 9. The linear dependence of $k_{obs}(Pm)$ on T_{Pm}.

Again the rate equation and its integrated form describing the mechanism in Scheme III is similar to that obtained for Scheme II. The observed rate constant, $k_{obs}(Pm)$ (sec^{-1}) is

$$k_{obs}(Pm) = Q_8 + Q_9/Q_2'' = F + GT_{Pm} \qquad (17)$$

where $Q_2'' = 1 + K''_{OH}(OH^-)(Cl^{-1})^{-1}$ (which may be assumed to be equal to one),

$$K''_{OH} = \frac{(Pd(H_2Pm)Cl_2OH)(Cl^-)}{Pd(H_2Pm)(Cl_3)(OH^-)},$$

$$Q_8 = F = k''_{-1}(H^+) + k''_{-2} + k''_{-3} + k''_{-4}(OH^-)$$

$$Q_9 = G = T_{Pm}\left[\frac{(k''_1 + k''_3(OH^-)(Cl^-)^{-1})(H^+)}{(H^+) + K_{IPm}} + \frac{(k''_2 + k''_4K''_{OH}(Cl^-)^{-1}(OH^-))}{(H^+) + K_{IPm}}K_{IPm}\right]$$

At pHs < 2.0

$$\left.\begin{array}{l} PdCl_4^{2-} + H_3Pm^{2+} \rightleftharpoons PdCl_4\cdot H_3Pm \\ PdCl_3OH^{2-} + H_3Pm^{2+} \rightleftharpoons PdCl_3OH\cdot H_3Pm \end{array}\right\} \text{outer sphere complexes}$$

$$\left.\begin{array}{l} PdCl_4\cdot H_3Pm \rightleftharpoons Pd(H_2Pm)Cl_2 + HCl \\ PdCl_3OH\cdot H_3Pm \rightleftharpoons Pd(H_2Pm)Cl_2(OH) + HCl \end{array}\right\} \text{inner sphere complexes}$$

At pH > 2.0

a) $Pd(H_2Pm)Cl_3 + H_3Pm^{2+} \rightleftharpoons Pd(H_2Pm)_2Cl_2^+ + H^+ + Cl^-;$ $\quad k''_1, k''_{-1}$

$K_{IPm} \upharpoonleft\downharpoonright$

b) $Pd(H_2Pm)Cl_3 + H_2Pm^+ \rightleftharpoons Pd(H_2Pm)_2Cl_2^+ + Cl^-;$ $\quad k''_2, k''_{-2}$

$\upharpoonleft\downharpoonright K''_{OH}$

c) $Pd(H_2Pm)Cl_2OH + H_3Pm^{2+} \rightleftharpoons Pd(H_2Pm)_2Cl_2^+ + H_2O;$ $\quad k''_3, k''_{-3}$

d) $Pd(H_2Pm)Cl_2OH + H_2Pm^+ \rightleftharpoons Pd(H_2Pm)_2Cl_2^+ + OH^-;$ $\quad k''_4, k''_{-4}$

Scheme III

TABLE IV. The Values of the Parameters F and G as a function of pH.

pH[a]	F	G	R
2.5	5.23 ± 1.62	1.64 ± 0.15	0.987
2.6	6 44 ± 1.86	1.84 ± 0.17	0.987
2.7	7.50 ± 1.84	2.14 ± 0.17	0 991
2.8	7.76 ± 2 06	2.49 ± 0 19	0.991
2 9	9.51 ± 1.66	2 78 ± 0.15	0.995
3.0	9.53 ± 2.39	3 30 ± 0.22	0.993
3.1	10.06 ± 2.26	3.77 ± 0.21	0.995
3.2	10 64 ± 2.00	4.39 ± 0.18	0.997
3.4	13 33 ± 3 00	5 63 ± 0.28	0.996
3.6	15.24 ± 4.71	7.40 ± 0.43	0.995
3.8	20.25 ± 7.43	9.25 ± 0.68	0.992
4 0	24.13 ± 9.12	11.61 ± 0.84	0 992
4.2	25.65 ± 11.21	14 98 ± 1.03	0.993
4.4	28.66 ± 12.79	18 43 ± 1.18	0.994

[a] Interpolated pHs from Fig. 8.

and K_{1Pm} is the first dissociation constant of H_3Pm^{2+}, Table II. Table IV lists the values of the coefficients F and G at different pH's.

The plot of $F(H^+)^{-1}$ as a function of $(H^+)^{-1}$ follows approximately a quadratic relation, as shown in Fig. 10a. This relation is represented as follows:

$$F(H^+)^{-1} = f_0 + f_1(H^+)^{-1} + f_2(H^+)^{-2} \qquad (18a)$$

where f_0, f_1 and f_2 have the values ~0, (2.0 ± 0.1) 10^{-2}, (3.49 ± 0.44)10^{-7}, respectively. These coefficients may correspond to k''_{-1}, $(k''_{-2} + k''_{-3})$, and $k''_{-4}K_W$, *i.e.*

$$k''_{-1} \cong 0$$

$$k''_{-2} + k''_{-3} = 2.0 \times 10^{-2}$$

$$k''_{-4}K_W = 3.49 \times 10^{-7} \quad \text{and}$$

$$k''_{-4} = 1.96 \times 10^{7}$$

Similarly, the plot of $G((H^+) + K_{1Pm})(H^+)^{-1}$ *vs.* $(H^+)^{-1}$, Fig. 10b follow this equation:

$$G((H^+) + K_{1Pm})(H^+)^{-1} = g_0 + g_1(H^+)^{-1} + g_2(H^+)^{-2} \qquad (18b)$$

The coefficients g_0, g_1 and g_2 have the values (0.47 ± 0.12), (3.72 ± 0.04)10^{-3}, and (1.16 ± 0.02) 10^{-7}. Again, these coefficients may be correlated with k''_1 ($\cong$0), k''_2K_{1Pm} (therefore k''_2 = 15.51) and $(k''_3K_W(Cl^-)^{-1} + k''_4K''_{OH}K_W(Cl^-)^{-1}$ ($\cong 1.16 \times 10^{-7}$), respectively. In the case of g_2, if $k''_3K_W(Cl^-)^{-1} = 0$ then $k''_4K''_{OH}K_W(Cl^-)^{-1} = 1.16 \times 10^{-7}$ and $k''_4 \cong 2$ (if $K''_{OH} = K_{OH}$), however, if $k''_4K''_{OH}K_W(Cl^-)^{-1} = 0$, then $k''_3 = 9.8 \times 10^5$.

The aforementioned analysis of the kinetic data of the Pd(II)–Pm system indicated that the reactions a and d in Scheme III are not significant in the formation of the $Pd(H_2Pm)_2Cl_2$.

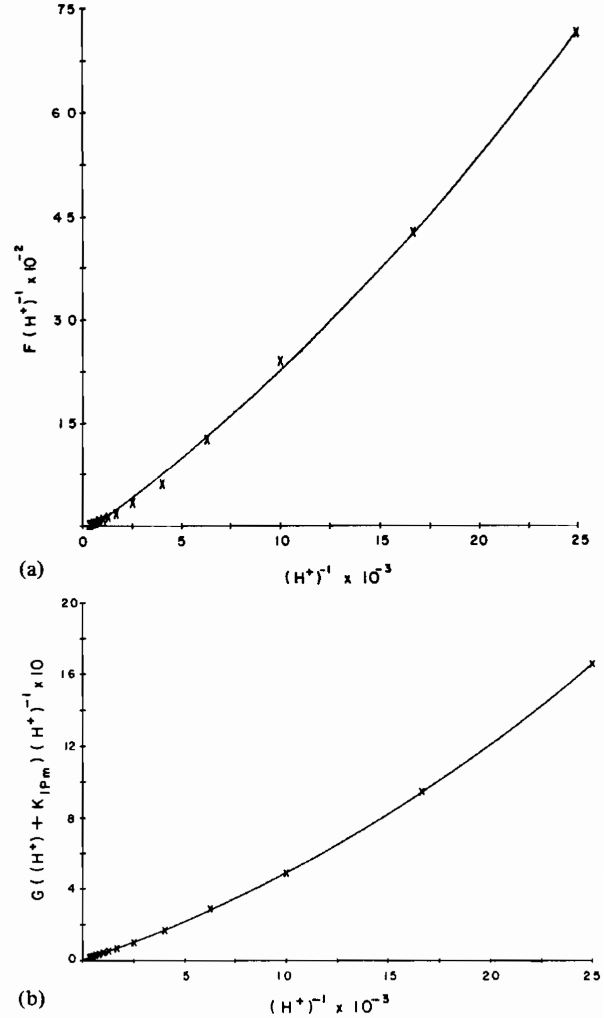


Fig. 10. a) The plot of $F(H^+)^{-1}$ as function of $(H^+)^{-1}$. b) The plot of $G((H^+) + K_{1Pm})(H^+)^{-1}$ *vs.* $(H^+)^{-1}$.

Conclusion

Table V summarizes the rate constants and the possible formation constants corresponding to different equilibria described in the aforementioned three schemes.

It is quite obvious that Pd(II) may interact differently with vitamin B_6 compounds P, PL and Pm. However, there may be a unique mechanism exhibited by all ligands which presumably involves the fast formation of an outer complex as follows.

$$PdCl_{n-l}OH_l^{2+} + 2(H_2(P\text{ or }PL))^+ \rightleftharpoons PdCl_{n-l}OH_l\cdot(H_2(P\text{ or }PL))_2^0$$

$$PdCl_{n-l}OH_l^{2+} + H_3Pm^{2+} \rightleftharpoons PdCl_{n-l}OH_l\cdot(H_3Pm)^0$$

where n = 4 and l = 0 or 1 (in acidic solutions).

TABLE V. Summary of the Rate Constants Evaluated in This Work.

Scheme	Rate Constant		Values	K
I	k_1	k_{-1}	0, 0	
	k_2	k_{-2}	8.42×10^8, 0	$>8.42 \times 10^8$
	k_3	k_{-3}	0, 0	
	k_4	k_{-4}	9.37×10^2, 0.56	1.67×10^3
	k_5	k_{-5}	1.41×10^8, 0	
	k_6	k_{-6}	2.72×10^3, 4.27×10^6	6.37×10^{-4}
	k_7	k_{-7}	1.05×10^8, 0	$>1.05 \times 10^8$
	k_8	k_{-8}	$\cong 10^{15}$, 2.84×10^{-7}	$>10^7$
II	k'_1	k'_{-1}	0.66, 0	
	k'_2	k'_{-2}	4.67, 11.67×10^{-2}	38 9
	k'_3	k'_{-3}	$5\ 57 \times 10^3$, ~ 0	$\approx 6 \times 10^3$
	k'_4	k'_{-4}	4.7×10^{-10}. $1\ 39 \times 10^5$	$\sim 3.36 \times 10^{-5}$
III	k''_1	k''_{-1}	~ 0, ~ 0	0
	k''_2	k''_{-2}	15.51, 2×10^{-2}	7.75×10^2
	k''_3	k''_{-3}	9.8×10^5, 2×10^{-2}	4.9×10^7
	k''_4	k''_{-4}	2, 1.96×10^7	$\sim 10^{-7}$

This mechanism is confirmed by the separation of only ($PdCl_4 \cdot H_3Pm$) from aqueous solutions at pH's < 2.0. The mode of interaction of P with Pd(II) species to form an inner complex is quite different from that of PL and Pm, in that the reaction rate constant is quadratically dependent on T_P. This is compatible with the isolation of $Pd(HP)_2Cl_2$ from aqueous solutions at pH ~ 4.0. The finding, however, indicated that the rates of formation of 1:1 as well as 2:1 Pd(II)–P complexes are of comparable magnitudes. On the other hand, the observed rate constants of the reactions of PL and Pm with Pd(II) species are not quadratically dependent on their respective T_{PL} and T_{Pm}. These findings were not, however, in accordance with the isolation of their 2:1 complexes; $(Pd(HPL)_2Cl_2)^0$ and $(Pd(H_2Pm)_2Cl_2)^{2+}$. However, this was explained as being due to the fast formation of 1:1 complex which slowly reacts further with another molecule of the ligands to form 2·1 complexes.

It is not unreasonable to assume that the *trans* effect played a role in the formation of $Pd(VB_6)_2Cl_2$ complexes. As soon as the first ligand displaced one of the Cl^- mainly in the Pd(II) species it labilizes the *trans* Cl^- for the attack of the second ligand. In such a case, the ligation will be through the pyridinic nitrogen since ligation through the meta-oxy group is not sterically favored. The difference in the rate behavior may be attributed to the magnitude of the electron density on the pyridinic nitrogen, where it is plausible to suggest that Pm > PL > P under the experimental conditions used. If the ligation site is the pyridinic nitrogen, the formation constants of the complexes should be reasonably high. This has been obtained for some of the reactions in the three schemes shown in Table V.

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