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Complexes of Vitamin B_6.
Part VIII. Kinetics and Mechanism of Binary and Ternary Complex Formations Involved in the Iron(III)–Pyridoxamine–Pyridoxal System

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The kinetics of the interactions of either pyridoxal (PL) or pyridoxamine (PM) with Fe(III) have been done in acidic aqueous solutions. The rates of interactions are pH-dependent. The rates decrease as pH decreases in the Fe(III)–PM system and vice versa *with respect to the Fe(III)–PL system. The observed rate constant, at a given pH, for both systems are:*

$$k_{obs,PM} = C_1 + C_2T_{PM}$$

$$k_{obs,PL} = C_3 + C_4T_{PL}$$

The interaction of PL with PM in the presence of Fe(III) has been also studied in acidic aqueous solutions, at pH = 3.0. Two kinetic rate reactions were observed with the fast step rate 3–4 magnitudes greater than the slower one. The observed rate constant of the fast step is dependent on T_{PL} and independent of T_{PM}, i.e.

$$k_{obs,PL,PM} = C_5 + C_6T_{PL}$$

On the other hand, the slow step is dependent on both of T_{PL} and T_{PM}, i.e.

$$k_{obs,sch} = C_7 + (C_8 + C_9T_{PL})T_{PM}$$

The mechanism of complex formation involved in the binary systems were discussed in the light of the experimental results. It has been concluded that protonated complex species are formed in solution. The findings were compared with those of the Fe(III)–pyridoxol system, previously reported. The mechanism of complex formation involved in the ternary system were also discussed. It has been postulated that a ternary complex (Fe–PM–PL^+) is formed fast prior to the slow formation of iron(III) Schiff's base complex in addition of ternary complex of iron(III) Schiff's base with PL.

Introduction

Metzler and Snell [1] reported that pyridoxamine (PM) was oxidized to pyridoxal (PL) when it was heated for 30 minutes at 100 °C in presence of copper and iron salts. El-Ezaby and El-Shatti [2] have reported recently that Cu(II) ions enhanced the interaction of pyridoxal with pyridoxamine in quite acidic medium in the temperature range of 30–50 °C. A ternary complex was predicted to form prior a possible formation of a Schiff base complex.

Due to the importance of the model studies, using nonenzyme metal ions–PL systems, in the elucidation of the mechanism of the PL-dependent enzymatic reaction, we also report the effect of iron(III) on the interaction of pyridoxal with pyridoxamine.

Experimental

Materials

Pyridoxal hydrochloride (PL·HCl) and pyridoxaminedihydrochloride ($PM·(HCl)_2$) were analytically pure chemicals and were used without purification. Stock solutions of 0.1 *M* of the ligands were kept in the dark at 0 °C. Stock solution of iron(III) chloride (0.1 *M*) was prepared in 1.0 *M* HCl. The concentration of iron(III) was checked by gravimetric methods.

Measurements

pH measurements were carried out using Radiometer pH-meter type 63 equipped with combined glass electrode (GK 2301 C). Calibration of the pH-meter was done as previously reported [3]. Kinetic measurements were done using a Durrum stopped-flow apparatus. The optical path length is 20.0 mm. The mixing syringes and cuvette were thermostated at 25 °C. The observed rate constants were taken for 75% of the reaction completion. Spectrophotometric measurements were carried out on Carry 17 spectrophotometer.

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*Part VII: Ternary Complexes of Co(II), Ni(II) and Cu(II) involving some Amino Acids and Pyridoxamine, *J. Inorg. Biochem.*, in press.

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In all measurements, ionic strength was kept constant at 0.50 *M* KCl. The concentration ranges of PM, PL and Fe(III), used in this work, were (0.4–4.0)10^{-3} *M*, (0.5–3.0)10^{-3} *M* and (1.0–4.0)10^{-4} *M*, respectively.

Results and Discussion

a) *The Reaction of Fe(III) with Pyridoxamine*

A reddish solution is produced when iron(III) chloride is added to pyridoxamine solution in the pH range 2.0–4.0. Slight precipitation occurred when the pH of the solution is increased, (above pH 4.0) specially when the concentration ratios of pyridoxamine to iron(III) are less than 10. This situation is not different from that encountered in case of Fe(III)–Pyridoxol (P) system reported previously [4]. The spectra of Fe(III)–PM system are similar to that of Fe(III)–P system, except that the average molar absorptivity, at a given wavelength and pH, is lower. The absorption maximum shifts to lower wavelength as pH increases.

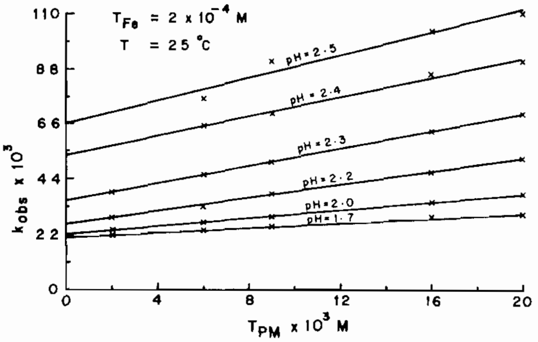

Fig. 1. The observed rate constant, k_{obs} as a function of T_{PM} at various pH values and T_{Fe} = 2 × 10^{-4} *M* for Fe(III)–PM system.

The kinetic study was made at λ = 470 nm in the pH range ~1.7–2.5. Figure 1 shows the dependence of observed pseudo first order rate constant, k_{obs}, on both pH and total ligand concentration of pyridoxamine, T_{PM}. At a given pH the dependence of k_{obs} on T_{PM} is linear and conform to the following equation:

$$k_{obs} = A + BT_{PM} \qquad (1)$$

Table I depicts the values of A and B at different pH's. It is clear that both A and B are pH dependent. The parameter B is linearly dependent on the reciprocal of hydrogen ion concentration, Fig. 2, *i.e.*

$$B = b + b'(H^+)^{-1} \qquad (2)$$

The values of b and b' are (0.14 ± 0.09) sec^{-1} and (7.12 ± 0.47)10^{-3} mol^{-1} l sec^{-1}, respectively, (R = 0.991). On the other hand A is quadratically dependent on $(H^+)^{-1}$, Fig. 3, *i.e.*

TABLE I. Variation of A and B with pH of the Fe(III)–PM System[a] at 25 °C and *I* = 0.5 *M* KCl.

pH	A (sec^{-1}) × 10^2	B (mol^{-1} l sec^{-1})	R[c]
1.70	2.11 ± 0.03	0.46 ± 0.03	0.995
2.00	2.22 ± 0.02	0.79 ± 0.02	0.999
2.20[b]	2.62 ± 0.04	1.31 ± 0.03	0.999
2.30	3.56 ± 0.00$_7$	1.72 ± 0.06	0.999
2.40[b]	5.32 ± 0.19	1.94 ± 0.14	0.995
2.50	6.61 ± 0.53	2.26 ± 0.38	0.972

[a] T_{PM} = (2–20)10^{-3} *M*; T_{Fe} = 2 × 10^{-4} *M*. [b] Data were interpolated. [c] R = correlation coefficient.

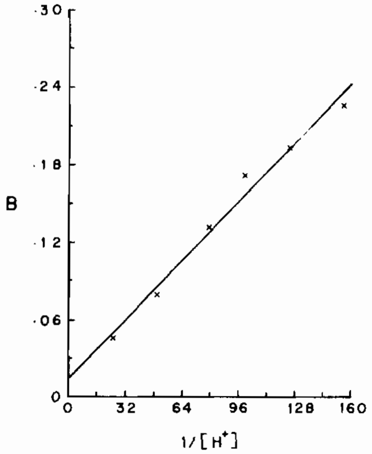

Fig. 2. The parameter B as function of $(H^+)^{-1}$.

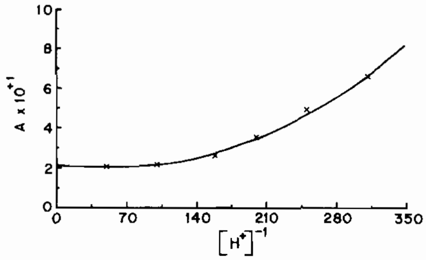

Fig. 3. The parameter A as function of $(H^+)^{-1}$.

$$A = a + a'(H^+)^{-2} \qquad (3)$$

with a and a' equal to (2.11 ± 0.03)10^{-2} sec^{-1} and (4.10 ± 0.20)10^{-7} mol^{-2} l^2 sec^{-1}, (R = 0.991). These findings are somehow different from that obtained from Fe^{3+}–P system previously reported [4] in the aspect of the hydrogen ion concentration dependence of the parameters A and B. In Fe^{3+}–P system these parameters are linearly dependent on the concentration of hydrogen ions. Different complex formation schemes were tested to account for these experimental evidences. It has been concluded that the following Scheme could explain the situation under consideration:

$$\text{a) } Fe^{3+} + H_3PM^{2+} \rightleftharpoons FeH_2PM^{4+} + H^+ \qquad k_1, k_{-1}$$

$$\Updownarrow K_1$$

$$\text{b) } Fe^{3+} + H_2PM \rightleftharpoons FeH_2PM^{4+} \qquad k_2, k_{-2}$$

$$\Updownarrow K_{11}$$

$$\text{c) } FeOH^{2+} + H_3PM^{2+} \rightleftharpoons FeH_2PM^{4+} + H_2O \qquad k_3, k_{-3}$$

$$\Updownarrow K_1$$

$$\text{d) } FeOH^{2+} + H_2PM^{+} \rightleftharpoons FeH_2PM^{4+} + OH^- \qquad k_4, k_{-4}$$

$$\text{e) } Fe(OH)_2^+ + H_3PM^{2+} \rightleftharpoons FeH_2PM^{4+} + OH^- + H_2O \qquad k_5, k_{-5}$$

$$\Updownarrow K_1$$

$$\text{f) } Fe(OH)_2^+ + H_2PM^{2+} \rightleftharpoons FeH_2PM^{4+} + 2OH^- \qquad k_6, k_{-6}$$

Scheme 1

The species HPM and PM^- of pyridoxamine have been neglected owing to their values of dissociation constants K_2 and K_3 (pK_{2PM} = 8 05, pK_{3PM} = 10 40, [2]) Dimer formation reactions and other reactions leading to the formation of hydrolytic species were ignored under the experimental conditions used in this work (pH <2 5 – $T_{Fe} = 2 \times 10^{-4}$ *M* – $T_{PM} \gg T_{Fe}$ – 0 5 *M* Cl^-)

The rate equation describing the above mechanism of complex formation may be derived,

$$\frac{d(FeH_2PM^{4+})}{dt} \qquad (4)$$

$$= (H_2PM^+)T_{Fe}[\{(H^+)/K_1\}(U) + (V)(X)^{-1}] - (FeH_2PM^{4+})(H_2PM^+)(X)^{-1}[[\{(H^+)/K_1\}(U) + (V)] + (W)]$$

where

$T_{Fe} = (FeH_2PM^{4+}) + (Fe^{3+})(X)$,
$(X) = 1 + K_{11}/(H^+) + K_{12}/(H^+)^2$,
$K_{11} = (FeOH^{2+})(H^+)/(Fe^{3+})$,
$K_{12} = (Fe(OH)_2^+)(H^+)^2/(Fe^{3+})$,
$(U) = k_1 + k_3K_{11}/(H^+) + k_5K_{12}/(H^+)^2$
$(V) = k_2 + k_4K_{11}/(H^+) + k_6K_{12}/(H^+)^2$ and
$(W) = k_{-1}(H^+) + k_{-2} + k_{-3} + (OH^-)(k_{-4} + k_{-5}) + k_{-6}(OH^-)^2$

If we assume that pH is not altered during the reaction progress ($T_{PM} \gg T_{Fe}$, pH range (1–2 5), the following integrated expression for eqn (4) may be obtained

$$\ln \frac{C_\infty}{C_\infty - C_t} = k_{obs}t$$

where C_∞ and C_t are the concentrations of FeH_2PM^{4+} at equilibrium and at time t and,

$$k_{obs} = [(H^+)\{K_{1PM}(U)\}^{-1} + (V)]\,\alpha_{1PM}T_{PM}(X)^{-1} + (W) \qquad (5)$$

where

$$\alpha_{1PM} = (H_2PM^+)/T_{PM} = (H^+)^2K_{1PM}/\{(H^+)^3 + (H^+)^2K_{1PM} + (H^+)K_{1PM} + K_{1PM}K_{2PM}K_{3PM}\}$$

From eqn (5), one may conclude that the parameters A and B obtained from eqn (1) may be correlated with (W) and $((H^+)(K_{1PM}(U))^{-1} + (V))(X)^{-1}\cdot\alpha_{1PM}$, respectively The dependence of A and B on pH seems to be complicated However, since $A = a + a'(H^+)^{-2}$, one may expect that a and a′ are directly correlated to the terms $(k_{-2} + k_{-3})$ and $k_{-6}K_w^2$ (pK_w = 13 75 [4]), respectively On the other hand, B shows quite complicated dependence on hydrogen ion concentration, *i e*

$$B = \alpha_{1PM}(X)^{-1}\{(H^+)k_1K_{1PM}^{-1} + k_3K_{11}K_{1PM}^{-1} + k_5K_{12}(H^+)^{-1}K_{1PM} + k_2 + k_4K_{11}(H^+)^{-1} + k_6K_{12}(H^+)^{-2}\} \qquad (6)$$

Upon analyzing eqn (6), α_{1PM} and X may be simplified first to $\sim K_{1PM}(H^+)$ and ~1, respectively, in the pH range used Under these conditions,

$$B = k_1 + (k_3K_{11} + k_2K_{1PM})(H^+)^{-1} + (k_5K_{12} + k_4K_{11}K_{1PM})(H^+)^{-2} + k_6K_{12}K_{1PM}(H^+)^{-3} \qquad (7)$$

Since B is linearly dependent on $(H^+)^{-1}$, terms containing $(H^+)^{-2}$ and $(H^+)^{-3}$ should drop out In such case, the intercept (k_1) and slope ($k_3K_{11} + k_2K_{1PM}$) correspond to b and b′ in eqn 2, respectively

The above findings indicated that reactions (a), (b), (c) and (f) have considerable contribution to the formation of Fe(III) complex species If it is assumed that k_2K_1 and k_{-2} are greater than k_3K_{11} and k_{-3}, respectively, one may calculate the stability constant of reaction (b), *i e* $K_{b2} = k_2/k_{-2}$ = 6 22 and if the opposite is correct, the stability constant of reaction c may be calculated, *i e* $K_{c3} = k_3/k_{-3}$ = 103 Furthermore, $FeOH^{2+}$ species has considerable effect on the rate, since its concentration increases as pH increases However this depends to a great extent on the type of ligand species which complex the metal ion species Pyridoxamine may form readily chelate complexes with Fe(III) rather than a non-chelate, opposite to what have been encountered in Fe(III)–pyridoxol system [4] In a recent work on the interaction of Fe(III) with PM [5], it has been concluded that 1 1 and 1 2 complex species involving the nonprotonated ligand species, PM^-, were formed in quite acidic medium (in the pH range of ~3 5–4 5)

b) *The Reaction of Fe(III) with Pyridoxal*

Similar to the Fe(III)–PM system, adding Fe(III) chloride solution to the solution of PL produced reddish colored solution The red color was intensified by increasing pH of the solution (in the range of 1–2 5) The spectra, in the wavelength range 300–600 nm, are similar to that of Fe(III)–PM and Fe(III)–P systems with the maximum wavelength of absorption shifting to lower wavelength as pH increases The kinetic runs were made at λ = 470 nm in the pH range ~1 7–2 5 Figure 4 shows the dependence of observed pseudo first order rate constant, k'_{obs}, on both pH and total ligand concentration of pyridoxal, T_{PL} At a given pH, in the range studied, k'_{obs} is linearly dependent on T_{PL}, *i e*

$$k'_{obs} = C + DT_{PL} \tag{8}$$

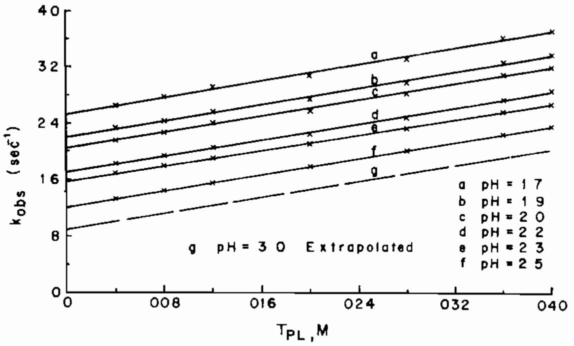

Fig 4 The observed rate constant, k'_{obs}, as function T_{PL} at various pH values and T_{Fe} = 2 × 10^{-4} *M* for Fe(III)–PL system

TABLE II Variations of C and D with pH in the Fe(III)–PL System[a] at 25 °C and *I* = 0 5 *M* KCl

pH	C (sec^{-1})	D (mol^{-1} l sec^{-1})	R[c]
1 7	25 26 ± 0 25	295 19 ± 10 19	0 997
1 9[b]	22 03 ± 0 20	290 27 ± 7 86	0 998
2 0	20 44 ± 0 18	285 75 ± 7 13	0 998
2 2[b]	17 08 ± 0 15	285 68 ± 5 88	0 999
2 3	15 67 ± 0 10	277 29 ± 4 18	0 999
2 5	12 06 ± 0 30	288 33 ± 1 20	0 999

[a] T_{PL} = (0 4–4 0)10^{-2} *M*, T_{Fe} = 4 × 10^{-4} *M* [b] Data were interpolated [c] R = correlation coefficient

Table II depicts the values of C and D at various pH's A plot of C against (H^+) is linear at pH's <2 with significant slope and intercept of 470 2 ± 41 0 (mol^{-1} l sec^{-1}) and 15 9 ± 0 6 sec^{-1}, (R = 0 996), respectively (Fig 5) On the other hand a plot of C against (H^+) at pH's >2 is also linear, but with different slope and intercept of (1 64 ± 0 26)10^3 (mol^{-1} l sec^{-1}) and (6 99 ± 1 28) sec^{-1}, R = 0 988) (Fig 5) respectively These findings may indicate different mechanism at these two pH ranges On the other hand D is almost constant at various pH values with a value of ~191 mol^{-1} l sec^{-1}

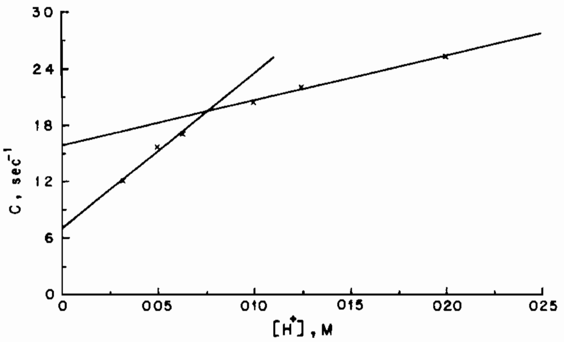

Fig 5 The parameter C as function of (H^+)

i) Kinetics of the interaction of Fe^{3+} with PL at pH's <2

In this pH range one can assume that the model described by Scheme 2 is quite reasonable

$$\text{a) } Fe^{3+} + H_2PL{\cdot}H_2O^+ \rightleftharpoons FeHPL{\cdot}H_2O^{3+} + H^+ \qquad k'_1, k'_{-1}$$

$$\text{b) } Fe^{3+} + HPL{\cdot}H_2O \rightleftharpoons FeHPL{\cdot}H_2O^{3+} \qquad k'_2, k'_{-2}$$

$$\Big\Updownarrow K_{11}$$

$$\text{c) } FeOH^{2+} + H_2PL{\cdot}H_2O^+ \rightleftharpoons FeHPL{\cdot}H_2O^{3+} + H_2O \qquad k'_3, k'_{-3}$$

$$\Big\Updownarrow K_1$$

$$\text{d) } FeOH^{2+} + HPL{\cdot}H_2O \rightleftharpoons FeHPL{\cdot}H_2O^{3+} + OH^- \qquad k'_4, k'_{-4}$$

Scheme 2

This mechanism is comparable to that obtained previously in case of Fe(III)–P system [4] In other words, the observed rate constant, k'_{obs} may take the following form,

$$k'_{obs} = (Y) + (Z_1)(X)^{-1}\alpha_{1PL}T_{PL} \tag{9}$$

where

$$(Y) = C = k'_{-1}(H^+) + (k'_{-2} + k'_{-3}) + k'_{-4}K_W(H^+)^{-1},$$

and

$$(X)^{-1}(Z_1)\alpha_{1PL} = D = (X)^{-1}\alpha_{1PL}\{k'_1(H^+)K_{1PL}^{-1} + \\ + (k'_2 + k'_3K_{11}K_{1PL}^{-1}) + k'_4K_{11}(H^+)^{-1}\}$$

and

$$\alpha_{1PL} = (HPL{\cdot}H_2O)T_{PL}^{-1} = K_{1PL}(H^+)^2\{(H^+)^3 + \\ + K_{1PL}(H^+)^2 + K_{1PL}K_{2PL}(H^+) + \\ + K_{1PL}K_{2PL}K_{3PL}\}^{-1}$$

Since (Y) is linear function of (H^+), one expects that k'_{-1} and $(k'_{-2} + k'_{-3})$ should be equal to 470 2 ± 41 0 mol^{-1} l sec^{-1} and 15 9 ± 0 6 sec^{-1}, respectively On the other hand, plotting of $D(X)\alpha_{PL}^{-1}$ *vs* (H^+) indicated a linear dependence with slope $(k'_1K_{1PL}^{-1})$ equal to (5 21 ± 0 18)10^6 sec^{-1} and zero intercept, $(k'_2 + k'_3K_{11}K_{1PL}^{-1})$ (Fig 6)

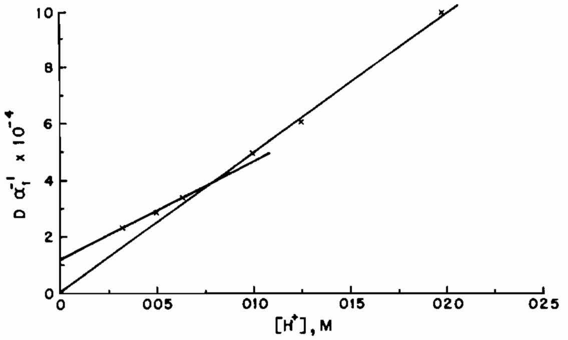

Fig 6 The plot of D^{-1} as function of (H^+)

ii) Kinetics of the interaction of Fe(III) with PL at pH's >2

If it is assumed that other reactions may also occur in addition to those mentioned in Scheme 2, it is possible to describe the mechanism in this pH range We have assumed that other complex species may also be formed as described in Scheme 3

$$\text{e)}\ Fe^{3+} + H_2PL{\cdot}H_2O \rightleftharpoons FePL{\cdot}H_2O + 2H^+ \qquad k'_5, k'_{-5}$$

$$\text{f)}\ Fe^{3+} + HPL{\cdot}H_2O \rightleftharpoons FePL{\cdot}H_2O^{2+} + H^+ \qquad k'_6, k'_{-6}$$

$$\Big\updownarrow K_{11}$$

$$\text{g)}\ FeOH^{2+} + H_2PL{\cdot}H_2O^+ \rightleftharpoons FePL{\cdot}H_2O^{2+} + H^+ \qquad k'_7, k'_{-7}$$

$$\Big\updownarrow K_{1PL}$$

$$\text{h)}\ FeOH^{2+} + HPL{\cdot}H_2O \rightleftharpoons FePL{\cdot}H_2O^{2+} + H^+ \qquad k'_8, k'_{-8}$$

Scheme 3

The observed rate constant can be derived from Scheme 2 and Scheme 3 in a similar way to that reported before [4], *i e*

$$k'_{obs} = (L)^{-1}\,[(Y)(H^+)/\alpha'_{1PL} + (J) + {} + \alpha_{1PL}T_{PL}/(X)\{(Z_1) + (Z_2)\}] \qquad (10)$$

where the intercept

$$C = (L)^{-1}\,\{(Y)(H^+)/\alpha'_{1PL} + (J)\},$$

and the slope

$$D = (L)^{-1}\,\{(Z_1) + (Z_2)\}\alpha_{1PL}/(X)$$

where

$$(J) = k'_{-5}(H^+)^2 + (k_{-6} + k_{-7})(H^+) + k'_{-8},$$

$$\beta'_{1PL} = (FePLH_2O)^{2+}(H^+)/(FeHPL{\cdot}H_2O)^{3+},$$

$$(L) = 1 + (H^+)/\beta'_{1PL} \text{ and}$$

$$Z_2 = k'_5(H^+)/K_{1PL} + (k'_6 + k'_7K_{11}/K_{1PL}) + {} + k'_8K_{11}(H^+)^{-1}$$

The slope and intercept obtained from eqn 10 are hydrogen-dependent in a complicated way

Plots of C and D (after rearrangement) against (H^+) are linear and are shown in Figs 5 and 6 In such case,

$$C(L) \cong C = (k'_{-4}K_W/\beta'_{1PL} + k'_{-8}) + (H^+)(k'_{-2} + {} + k'_{-3}/\beta'_{1PL} + k'_{-6} + k'_{-7}) \qquad (11)$$

and

$$D\alpha_{1PL}^{-1}(X) = (k'_2 + k'_3K_{11}/K_{1PL} + k'_6 + k'_7K_{11}/K_{1PL}) + {} + (H^+) \times (k'_1/K_{1PL} + k'_5/K_{1PL}) \qquad (12)$$

Since C (and not C(L)) is linearly dependent on (H^+), one expects that (L) must be approximately equal to one In such case, β'_{1PL} must be greater than (H^+) to render the term $\{(H^+)/\beta'_{1PL}\}$ negligible with respect to one (in the pH range used) Now, it is simple to correlate $(k'_{-4}K_W/\beta'_{1PL} + k'_{-8})$ and $(k'_{-2} + k'_{-3}/\beta'_{1PL} + k'_{-6} + k'_{-7})$ to the empirical values obtained previously (*i e* equal to (6 99 ± 1 28) mol^{-1} l sec^{-1} and (1 64 ± 0 26)10^3 sec^{-1}, respectively) The term $(k'_{-2} + k'_{-3})$ obtained previously from the kinetic data at pH's below 2 is smaller than that of $(k'_{-2} + k'_{-3}/K' + k'_{-6} + k'_{-7})$ indicating that $(k'_{-6} + k'_{-7})$ must be greater than $(k'_{-2} + k'_{-3})$

From eqn 12, one may correlate the intercept $(k'_2 + k'_3K_{11}/K_{1PL} + k'_6 + k'_7K_{11}/K_{1PL})$ and slope $(k'_1/K_{1PL} + k'_5/K_{1PL})$ to the values obtained from the plot of D_{PL}^{-1} (X) against (H^+), *i e* (1 19 ± 1 7)10^4 mol^{-1} l sec^{-1} and (3 48 ± 0 34)10^6 sec^{-1}, respectively Since $(k'_2 + k'_3K_{11}K_{1PL}^{-1})$ is equal to zero, then $(k'_6 + k'_7K_{11}/K_{1PL})$ should be equal to 1 19 × 10^4 mol^{-1} l sec^{-1} On the other hand, the value of $(k'_1 + k'_5)K_{1PL}^{-1}$ is not significantly different from that obtained only for $k'_1K_{1PL}^{-1}$, which indicates that k'_5 is approximately equal to zero

We may conclude that the interaction of Fe(III) with PL is quite complicated and completely different from that of Fe(III) with PM From the knowledge of k_1 and k_{-1} (Table III) one can calculate the stability constant of reaction a in Scheme 2 The value so obtained is 3 51 It does indicate that the stability constant of the formation of $FeHPL^{3+}$ is 1 11 × 10^4 which is comparable to that obtained for $FeHP^{3+}$ (3 2 × 10^4) [4] However, this value is quite different from that for FeH_2PM^{4+}, possibly due to the electron withdrawing property of the protonated amino group in pyridoxamine

TABLE III The Dependence of H on T_{PL}

$T_{PL} \times 10^3$ *M*	H (mol^{-1} l sec^{-1})	R
5 0	3 80 ± 0 17	0 998
7 5	5 02 ± 0 05	0 999
10 0	6 21 ± 0 20	0 999
15 0	8 36 ± 0 30	0 998
20 0	10 23 ± 0 40	0 998
30 0	13 62 ± 0 74	0 997

c) *Kinetics of the Ternary Complex Formation of the System Fe(III)–PM–PL*

The study of the binary complex formation of Fe(III) with vitamin B_6 components (P and PL) becomes quite complicated as pH increases above pH 3 0 due to the possible hydrolysis and/or polymerization of the metal ion and their complexes, even if the ligand concentration is much higher than that of the metal ion However, PM forms quite stable complexes with Fe(III) in the pH range 3 5–4 5 [5] It has been mentioned that Fe(III) formed a reddish solution with PM at pH's ⩽2 5 Furthermore, it turns to yellowish color as pH increases above pH 2 5 Although the spectra of the solution showed a shift to lower wavelengths as pH increases (470 nm → 380 nm), yet they shift to higher wavelengths again when PL is added to the Fe(III)–PM solution (380 nm → 470 nm) at pH = 3 0 This finding strongly suggested that PL interacts with the complexes of Fe(III) with pyridoxamine

The kinetic runs were taken at λ = 470 nm at pH = 3 0 The interaction of PL with Fe(III)–PM system took place at two steps, one of them is faster with rate constants of a magnitude 3–4 orders higher than the slower step In the T_{PM} range (0 4 –4 0)10^{-3} *M* and $T_{Fe} = 2 \times 10^{-4}$ *M* at a given T_{PL}, the observed rate constants for the fast process, k_{obs}, do not differ in the magnitude within experimental errors Plot of these rate constants against T_{PL} was linear (Fig 7) with significant intercept, E, and slope, F, *i e*

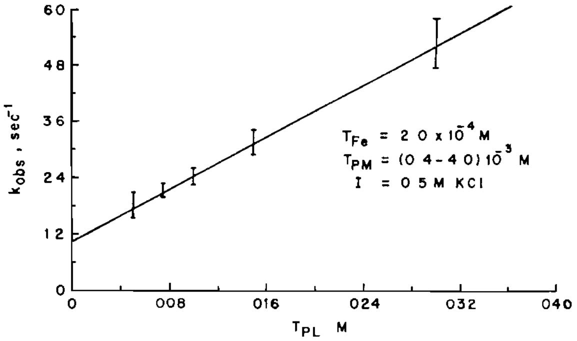

Fig 7 The observed rate constant, k''_{obs}, as function of T_{PL} at pH = 3 and $T_{Fe} = 2 \times 10^{-4}$ *M* for the fast step of Fe(III)–PM–PL system

$$k''_{obs} = E + FT_{PL} \tag{13}$$

The magnitude of E and F are (10 54 ± 0 94) sec^{-1} and (1 39 ± 0 06)10^3 mol^{-1} l sec^{-1}, respectively, (R = 0 985)

On the other hand, the observed rate constants for the slow process, k'''_{obs}, are linearly dependent on T_{PM} for a given T_{PL} (Fig 8) At constant T_{PL}, the linear dependence of K'''_{obs} on T_{PM} may be represented by the following equation,

$$k'''_{obs} = G + HT_{PM} \tag{14}$$

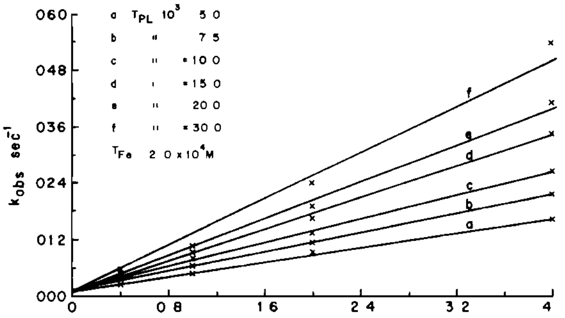

Fig 8 The observed rate constant, k''_{obs}, as function of T_{PM} at various values of T_{PL} at pH = 3 0 and $T_{Fe} = 2 \times 10^{-4}$ *M* for the Fe(III)–PM–PL system

The parameter, H, Table III, is linearly dependent on T_{PL} The linear dependence may be expressed as follows,

$$H = h + h'T_{PL} \tag{15}$$

The intercept (h) and slope (h') obtained from the plot of H *vs* T_{PL} (Fig 9) are (2 16 ± 0 25) mol^{-1} l sec^{-1} and (3 91 ± 0 15) 10^2 sec^{-1}, respectively, (R = 0 997) Although H is linearly dependent on T_{PL}, yet G is constant with a value of (1 28 ± 0 19)10^{-3} sec^{-1}

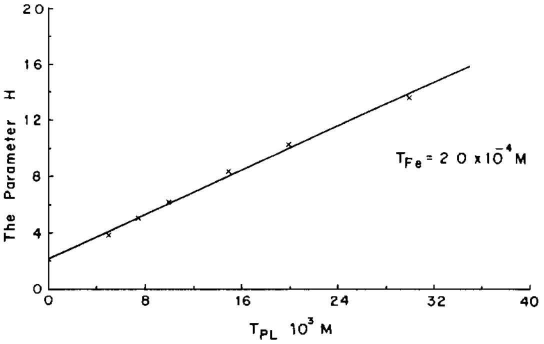

Fig 9 The parameter H as function of T_{PL} at pH = 3 and $T_{Fe} = 2 \times 10^{-4}$ *M*

i) Kinetics and mechanism involved in the fast step of the interaction of PL with Fe(III)–PM system

At pH = 3, several species coexist in equilibrium. In the Fe(III)–PM system, FeH_2PM^{4+}, $FeHPM^{3+}$, $FePM^{2+}$, $FeOH^{2+}$, $Fe(OH)_2^+$, $(FeOH)_2^{4+}$, Fe^{3+}, H_3PM^{2+} and H_2PM^+ exist at different concentration. In the PL system, the species H_2PL^+ and HPL mainly coexist.

It is clear that the concentration of PM has no effect on the observed pseudo first order rate constants which indirectly indicated that the concentration of the species of iron(III) complexes with pyridoxamine has approximately no influence on rate. Moreover, the magnitudes of the rate constants are greater than those anticipated for the interaction of PL alone with Fe(III) species at this pH. Scheme 4 may describe the mechanism of the interaction of PL with Fe(III)–PM system in the fast step, i.e.

$$FeH_2PM^{4+} \xrightleftharpoons{H_2PM} FeHPM^{3+} \xrightleftharpoons{HPM} FePM^{2+}$$

$$\text{a) } FePM^{2+} + H_2PL^+ \rightleftharpoons FePMHPL + H^+ \qquad k_9, k_{-9}$$

$$\begin{tikzcd}
\text{b) } FePM^{2+} + H_2PL^+ \arrow[r, shift left] \arrow[d, shift left, "K_{1PL}"] & FePMPL + 2H^+ \arrow[l, shift left] \arrow[d, shift left, "\beta'_T"] & k_{10}, k_{-10} \\
\text{c) } FePM^{2+} + HPL \arrow[u, shift left] \arrow[r, shift left] & FePMHPL \arrow[u, shift left] \arrow[l, shift left] & k_{11}, k_{-11}
\end{tikzcd}$$

$$\text{d) } FePM^{2+} + HPL \rightleftharpoons FePMPL + H^+ \qquad k_{12}, k_{-12}$$

Scheme 4

where FePMHPL and FePMPL are ternary complex species in which the amino group of PM is probably added to the aldehyde group of PL in presence of Fe(III) (Fig 10)

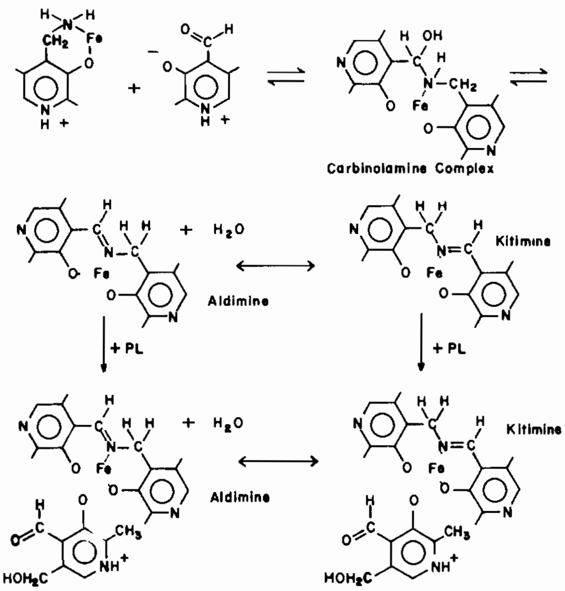

Fig 10 Reaction equilibria involved in the Fe(III)–PM–PL system

The rate equation describing the above mechanism may be written as follows

$$\left(1 + \frac{(H^+)}{\beta'_T}\right)\frac{d(FePMPL^+)}{dt}$$
$$= (FePM^{2+})(HPL)(Q_1) - (FePMPL^+)(Q_2) \qquad (16)$$

where

$$Q_1 = (k_9 + k_{10})(H^+)/K_{1PL} + k_{11} + K_{12},$$
$$Q_2 = (k_{-9}/\beta'_T + k_{-10})(H^+)^2 + (k_{-11}/\beta'_T + k_{-12})(H^+)$$

and

$$\beta'_T = (FePMPL^+)(H^+)/(FePMHPL^{2+})$$

Equation 16 may be integrated if the following conditions are taken in consideration

1) T_{Fe} (at any time) = $(Fe^{3+}) + (FeOH^{2+}) + Fe(OH)_2^+ + (FePMHPL^{2+}) + (FePMPL^+) + (FePM^{2+}) + (FeHPM^{3+}) + (FeH_2PM^{3+})$

(The concentrations of iron(III) complexes with PL were ignored at the fast step of the reaction rate)

2) Vertical protolytic reactions are assumed to be very fast

3) Hydrogen ion concentration is assumed to be constant in the reaction progress. This was fulfilled by using excess PM

4) The absorption at 470 nm is proportional to the concentration of (FePLPM)

The integrated form of eqn 16 may be written as follows

$$\ln \frac{(FePMPL)}{(FePMPL)_t - (FePMPL)} = k_T(t) \qquad (17)$$

where

$$k_T = Q_1\alpha_{1PL}T_{PL}Q_3^{-1} + Q_2\{1 + (H^+)/\beta'_T\}^{-1} \qquad (18)$$

and

$$Q_3 = \{(X)/PM^-)\beta_{PM}\} + \{1 + (H^+)/\beta_{H_2PM} + (H^+)/\beta_{H_2PM}\beta_{HPM}\}$$

k_T may be correlated with k''_{obs} (eqn 13) with the slope F and the intercept E expressed as

$$F = Q_1\alpha_{1PL}Q_3^{-1} = \alpha_{1PL}\{(k_9 + k_{10})(H^+)/K_{1PL} + k_{11} + k_{12}\} \times [(X)/(PM^-)\beta_{PM} + \{1 + (H^+)/\beta_{H_2PM} + (H^+)^2/\beta_{HPM}\beta_{H_2PM}\}]^{-1} \qquad (19)$$

and

$$E = \{(k_{-9}/\beta'_T + k_{-10})(H^+)^2 + (k_{-11}/\beta'_T + k_{-12})(H^+)\} \times \{1 + (H^+)/\beta'_T\}^{-1} \qquad (20)$$

Apart from the dependence of eqn 19 on pH it also depends on the concentration of (PM^-) It has been recognized experimentally that the fast step does not depend on T_{PM} in the concentration range $(0\ 4-4\ 0)10^{-3}$ *M* Upon careful analysis of eqn 19, the term which depends on (PM^-), $\{(X)/(PM^-)\beta_{PM}\}$ (range of values from 4 to 44), may be smaller than $\{1 + (H^+)/\beta_{H_2PM} + (H^+)^2/\beta_{HPM}\beta_{HPM}\}$ at pH = 3 and β_{HPM} and/or β_{H_2PM} should be smaller than (H^+) Since (H_2PL) is greater than (HPL) at pH = 3, it may be expected that reactions (a) and (b) are more favourable than those of (c) and (d) in Scheme 4 Since $\alpha_{1PL}(H^+)/K_{1PL} \sim 1$ then one should expect that $(k_9 + k_{10})/\{1 + (H^+)/\beta_{H_2PM} + (H^+)^2/\beta_{HPM}\beta_{H_2PM}\}$ is equal to $1\ 39 \times 10^3\ mol^{-1}\ l\ sec^{-1}$ The parameter E may be equal to $(k_9/\beta'_T + k_{-10})(H^+)^2/(1 + (H^+)\beta'_T) = 10\ 54$

ii) Kinetics and mechanism involved in the slow step of the interaction of PL with Fe(III)–PM system

In this step, one may conclude that PL interacts actively with different simple iron(III) species (Fe^{3+}, $FeOH^{2+}$, $Fe(OH)_3^+$, *etc*) However, the magnitudes of the observed pseudo first order rate constants are much smaller than those for the interaction of PL with Fe(III) alone A more reasonable conclusion may suggest that dehydration of the complex species FePMHPL and FePMPL took place in this kinetic step to form Schiff's base complexes, (Fig 10), in addition to the possible formation of another ternary complex involving the Schiff's base as shown in Scheme 5

$$\text{FePMHPL} \rightleftharpoons \text{FeSch} + H_2O \qquad k_{13}, k_{-13}$$

$$\text{FePMHPL} + H_2PL \rightleftharpoons \text{FeSchHPL} + H^+ + H_2O \qquad k_{14}, k_{-14}$$

$$\text{FePMHPL} + \text{HPL} \rightleftharpoons \text{FeSchHPL} + H_2O \qquad k_{15}, k_{-15}$$

Scheme 5

The rate equation describing the mechanism shown in Scheme 5 may be written as follows

$$(Q_2)\frac{d(\text{FeSchHPL})}{dt} = (PM^-)T_{Fe}\beta_T(Q_3)/(Q_1) - (\text{FeSchHPL}) \times \{(Q_2)(Q_3)\beta_T(PM^-)/(Q_1) + (Q_4)\} \qquad (21)$$

where

$$(Q_1) = (Fe^{3+})\{(X) + \beta_{PM}(PM^-) + \beta_{HPM}(HPM) + \beta_{H_2PM}(H_2PM) + \beta_{PL}(PL^-) + \beta_{HPL}(HPL) + \beta_T(PM^-)(HPL)\},$$

$$(Q_2) = \{1/(HPL)\beta_{sch}\} + 1,$$

$$(Q_3) = k_{13} + k_{14}(H_2PL^+) + k_{15}(HPL),$$

$$(Q_4) = k_{-13}\beta_{sch}(HPL) + k_{-14}(H^+) + k_{-15},$$

$$\beta_T = (\text{FePMHPL})^{2+}/(\text{FePM})^{2+}(HPL),$$

$$\beta_{sch} = (\text{FeSchHPL})/(\text{FeSch})(HPL),$$

and

$$T_{Fe} = (Fe^{3+})(Q_1) + (\text{FeSchHPL})(Q_2)$$

Upon integrating eqn 21 one can obtain the following eqn,

$$k_{obs,T} = \alpha_{3PM}T_{PM}\alpha_{1PL}T_{PL}\beta_T(Q_3)/(Q_1) + (Q_4)/(Q_2) \qquad (22)$$

The plot of k_{obs} *vs* T_{PM} at constant T_{PL} should be linear with significant slope and intercept The values of the slopes and the intercepts may be correlated with those obtained empirically by eqn 14 In such case,

$$G = (Q_4)/(Q_2),$$

and

$$H = \alpha_{3PM}\alpha_{1PL}T_{PL}\beta_T[(Q_3)/(Q_1)] + (Q_4)/(Q_2)$$

since G is not dependent on T_{PL}, it turns out to have this form (if 1 is greater than $1/(HPL)\beta_{sch}$),

$$G = k_{-14}(H^+) + k_{-15} = 1\ 28 \times 10^{-3}\ sec^{-1} \qquad (23)$$

The finding that $k_{obs,T}$ is linearly dependent on T_{PM} at constant T_{PL}, may lead to the conclusion that

$$H = \frac{\beta_T\alpha_{3PM}\alpha_{1PL}T_{PL}\{k_{13} + k_{14}(H_2PL) + k_{15}(HPL)\}}{(X) + \beta_{PL}(PL^-) + \beta_{HPL}(HPL)}$$

Since H is found to be linearly dependent on T_{PL} alone (Fig 9), the parameter H should take the following form

$$H = \frac{\beta_T\alpha_{3PM}\{k_{13} + k_{14}(H^+)/K_{1PL} + k_{15}\}\alpha_{1PL}T_{PL}}{\beta_{PL}K_{2PL}/(H^+) + \beta_{HPL}}$$

Assuming that $\{\beta_{PL}(PL^-) + \beta_{HPL}(HPL)\} > (X)$ Now, the parameters h and h' of eqn 15 are

$$h = \beta_T\alpha_{3PM}k_{13}/\{\beta_{PL}K_{2PL}/(H^+) + \beta_{HPL}\}$$

and

$$h' = \beta_T\alpha_{3PM}\alpha_{1PL}\{k_{14}(H^+)/K_{1PL} + k_{15}\}/\{\beta_{PL}K_{2PL}/(H^+) + \beta_{HPL}\}$$

If the assumption was made that $(X) > \{\beta_{PL}(PL^-) + \beta_{HPL}(HPL)\}$ we may not be able to get h, since it will be of zero magnitude, *i e*

$$k_{obs} = \beta_T\alpha_{3PM}\alpha_{1PL}T_{PL}k_{13}/(X)$$

From the foregoing results it has been concluded that all reactions in Scheme 5 are significant in the formation of FeSch and FeSchHPL However, it is not simple to predict the values of their rate constants since the values of β_T and β_{PL} are not available Although this model is closely related to that postulated for Cu(II)–PM–PL system [2], yet we have not noticed any comparable ambiguities, when the mole ratio of Fe(III) PM PL is 1 2 10 or 1 ⩾2 10 This conclusion is in accordance with the fact that coordination number greater than 4 is more favourable than in the Cu(II) system

Conclusion

The findings encountered in this work indicate beyond doubt that PM behaves quite different from P and PL towards complex formation with Fe(III). Although this conclusion is not surprising, it shows that PM acts as a bidentate ligand in which the aminomethyl group and the *meta*-oxy atom participate in the complex formation specially as pH is increased above 2 0

It has been also concluded that the interaction between PL and PM cannot take place in absence of Fe(III) Moreover, the poisoning effect which has been observed in the kinetics of the Cu(II)–PM–PL system when 1 2 10 or 1 ⩾2 10 mole ratios were used [2], did not occur in the Fe(III)–PM–PL system This may be rationalized in terms of the relative high coordination number of Fe(III) with respect to Cu(II) In this work, however, we have reached the same conclusion postulated in the Cu(II)–PM–PL system, namely a ternary complex (FePMPL) was formed in solution at pH = 3 prior to the formation of a Schiff's base iron(III) complex The ternary complex formation of Fe(III)–PM system with PL is a quite fast process in comparison with that of Schiff's base iron(III) complex formation On the other hand, the magnitude of the rate constants of the slow process in the interaction of PL with Fe(III)–PM system are at least ten times greater than those of PL with Cu(II)–PM system [2] Fig 10 shows the possible equilibrium reactions involved in the interaction of Fe(III)–PM with PL These reactions have been postulated in the light of mechanism proposed in previous report ([7] and references therein) which was based on the extensive studies of the interaction of some amino acids with pyridoxal promoted by complexing with some metal ions The mechanism involved the intermediate formation of an aldimine which undergoes a prototropic shift to form kitimine with further complexation of the Schiff's base of Fe(III) with another PL molecule

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