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Complexes of Vitamin B_6. XIV*. Kinetics and Reaction Mechanism of the Interaction of Pyridoxal-5′-Phosphate with Cu(II)–Pyridoxamine Complexes

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Pyridoxamine (pm) reacted with pyridoxal-5′-phosphate (PLP) in presence of Cu(II) in acidic medium. It has been concluded that a ternary complex of the form $Cu(H_nPm)(H_mPLP)$ *(n = 2 or 1 and m = 2 or 1) was formed quite fast before a further slow complex formation took place. The kinetic data of the slow process in the pH range 2–4 were discussed and were found to follow the equation,*

$$k_{obs} = A' + B'T_{PLP}$$

The parameters A′ and B′ were found to be pH dependent. The formation of carbinolamine intermediate prior to the proposed formation of Schiff's base complex species was suggested to account for a fast process in the pH range 4.0–7.0. The results were compared with previously published similar data. In addition, PLP complexes with Cu(II) were studied potentiometrically and their formation constants were determined. The whole work was done at 37 °C and an ionic strength of 0.15 M.

Introduction

It has been reported recently that pyridoxal (PL) reacts with pyridoxamine (Pm) in presence of some metal ions, Cu(II) and Fe(III) metal ions [1, 2]. The reaction was facilitated in acidic media through the formation of a Schiff base metal complex via intermediate ternary metal complex formation; MPLPm. In case of Cu(II) system, the mol ratio of Cu(II):Pm.PL was 1:1:10 (or more). However, when the number of mol of Pm exceeded one, poisoning was observed which was attributed to the possible formation of two Schiff base molecules per one Cu^{2+} ions sterically hindering each other. In the case of Fe(III), however, this finding was not observed.

In this work, we report the interaction of Pm with PLP (pyridoxal-5′-phosphate) in presence of Cu(II) to decipher the effect of phosphate group on their reaction mechanism. The importance of such study in vitamin B_6-dependent enzymatic reactions cannot be ignored. Some of the vitamin B_6-dependent reactions *in vitro* involve PLP and Pm, specifically the transamination between α-amino and α-keto acids [3].

Experimental

Materials

Pyridoxal-5′-phosphate (PLP) and pyridoxamine dihydrochloride ($Pm{\cdot}(HCl)_2$) were analytically pure chemicals and were used without purification. Stock solution of 0.1 *M* concentration of the ligands were kept in dark at 4 °C. Stock solutions of $CuCl_2$ of ~0.1 *M* concentration were prepared. EDTA compleximetric titration was used to check its concentration. Ionic strength was kept constant by adding the appropriate amount of $NaNO_3$ (2 *M*) solution, at 0.15 *M*.

Measurements

The pH-titration was performed as previously reported [4], utilizing Orion Research Ionalyzer type 901 in the pH mode, equipped with radiometer combined glass electrode (GK 2301 C). Kinetic measurements were done using a Durrum stopped flow apparatus. The optical path length was 20 mm. The mixing syringes and cuvette were thermostatted at 37 °C. The observed rate constants were calculated for kinetic data up to 80% of the reaction

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*Part XIII. Kinetics and Mechanism of the Complex Formation of Pd(II) with Vitamin B_6 Compounds, *Inorg Chim Acta, 67,* 121 (1982).

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TABLE I. Summary of the Reported Protonation Constants of Pm and PL and the Formation Constants of their Cu(II) Complexes.

System	l p q s*	log β	I	T (°C)	Ref.
PmH_3^{2+}	1 0 0 1	10.407	0.15	37	4
	1 0 0 2	18.562			
	1 0 0 3	22.063			
Pm–Cu	1 0 1 0	10.805	0.15	37	4
	1 0 1 1	17.225			
	1 0 1 2	21.337			
	2 0 1 0	17.471			
	2 0 1 1	25.458			
	2 0 1 2	32.535			
	2 0 1 3	38.914			
PLH_2^+	0 1 0 1	8.54	0.50	25	8
	0 1 0 2	12.79			
PL–Cu	0 1 1 0	3.51	0.50	25	8
	0 2 1 0	7.00			

*l, p, q, and s are the stoichiometric coefficients for Pm, PL or PLP, Cu(II) and H^+.

completion. The absorption spectra were carried out on a Cary 17 spectrophotometer.

Determination of the protonation constants of PLP and the formation constants of Cu(II)–PLP complexes were done by utilizing the MINIQUAD-75 programme [5]. The procedures used to select the correct equilibrium model were as previously described [6].

Results and Discussion

Equilibrium Study

Binary systems involving Pm and Cu(II) have recently been thoroughly investigated in our lab [4].

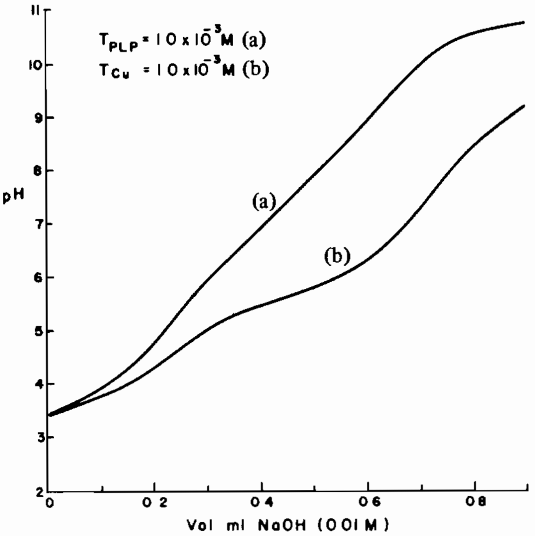


Fig. 1. Typical titration curves of PLP and PLP–Cu(II) solutions $T_{PLP} = T_{Cu} = 1 \times 10^{-3}$ *M.*

Table I depicts the possible equilibria which may occur in aqueous solutions and their formation constants. On the other hand binary complexes involving PLP and Cu(II) were not well studied. Only one report has described the preparation of Cu(II) complexes with PLP [7]. In the meantime, complexes of pyridoxal (PL) were studied in solution Table I [8].

Figure 1 shows typical pH-metric titration curves of PLP and a Cu(II)–PLP system. Precipitation usually occurs above pH ~ 8.0 in the Cu(II) system. Different equilibrium models were tried to account for the titration data using MINIQUAD-75 programme. Mononuclear as well as polynuclear complex species have been invoked in these models. The results are recorded in Table II in addition to the protonation equilibria of PLP. It has been proved that polynuclear species cannot exist in the studied pH

TABLE II. Protonation Constants of PLP and its Cu(II) Complexes at I = 0.15 and T = 37 °C, n = no. of titration data, χ_i^2 = chi square, R = reliability factor; S = sum of squared residuals; σ = standard deviation

System	l p q s	log β (±σ)	pH range	n	χ_i^2	R	S × 10^7	Reported I, T, Ref.*
PLP–H	0 1 0 1	9.242(0.029)	5.5–9.8	98	75	0.036	10.3	8.14, 0.5, 25°
	0 1 0 2	15.653(0.042)						14.12
	0 1 0 3	19.229(0.019)	3.1–5.1	66	23	0.009	1.5	17.75
	0 1 0 4							
PLP–Cu(II)	0 1 1 0	7.005(0.052)	3.0–5.8	287	582	0.016	22.7	19.35
	0 1 1 1	12.489(0.064)						
	0 1 1 2	16.750(0.346)						

*W. L. Felty, C. G. Ekstorm and D. L. Leussing, *J Am. Chem. Soc., 92,* 3006 (1970).

range in contradiction to what has been reported concerning the isolation of polymeric Cu(II)–PLP complexes [7]. In addition, 1:2:sH formation constants of Cu(II)–PLP complexes cannot be determined. Besides the 1:1:0 complex species is more stable than is that obtained from Cu(II)–PL system (Table I). This finding may be rationalized as being due to the availability of the carbonyl group of the aldehyde moiety of PLP as well as the meta oxy group for metal ligations. This situation is not allowed in PL, probably due to the internal formation of a hemiacetal grouping. This may also explain why PLP forms only 1:1 complexes with Cu(II). The PLP phosphate ligation to Cu(II) is less likely to occur since its ordinary 1:1 phosphate complexes are weak [8].

Kinetic Study

The addition of Pm solution to that of Cu(II) usually alters significantly the visible spectrum of the latter. The small broad absorption peak of aqua Cu(II) ion at 850 nm increased in height and decreased in width, accompanied by hypsochromic shift as the pH of solution increased. A slight change, however, was observed when PLP solution was added to that of Cu(II). This observation was not different from what was found in the Cu(II)–PL system [1]. On the other hand, addition of PLP to Cu(II)–Pm solution exhibits further hypsochromic shift in the spectral bands of the latter system, similar to what has been reported in the Cu(II)–Pm–PL system [1]. Usually precipitation occured at pH > 4.3 when $T_{PLP} \gtrsim 1.0 \times 10^{-2}$ *M*.

The kinetic study was carried out at the wavelength 650 nm. Two rate steps were observed: a fast one was only followed in the pH range 4.0–7.0 when the composition ratio of T_{Cu}:T_{Pm}:T_{PLP} was 1.1:10, and a slow step was only pursued in the pH range 2.0–4.0 for the composition ratios 1:1:≥10. Both slow and fast steps cannot be obtained for $T_{PLP} > 10^{-2}$ *M*, due to precipitation difficulties at pH > 4. The fast step cannot be obtained at pH < 4 due to insignificant change in absorbance with respect to time. Figure 2 shows the observed rate constants for the slow step, k_{obs}, in their dependence on pH and total concentration of PLP, T_{PLP}, at constant concentrations of Cu(II) (T_{Cu}) and Pm (T_{Pm}) ($T_{Cu} = T_{Pm} = 1 \times 10^{-3}$ *M*). Figure 3 shows the dependence of k_{obs} as function of T_{PLP} at various interpolated pH's.

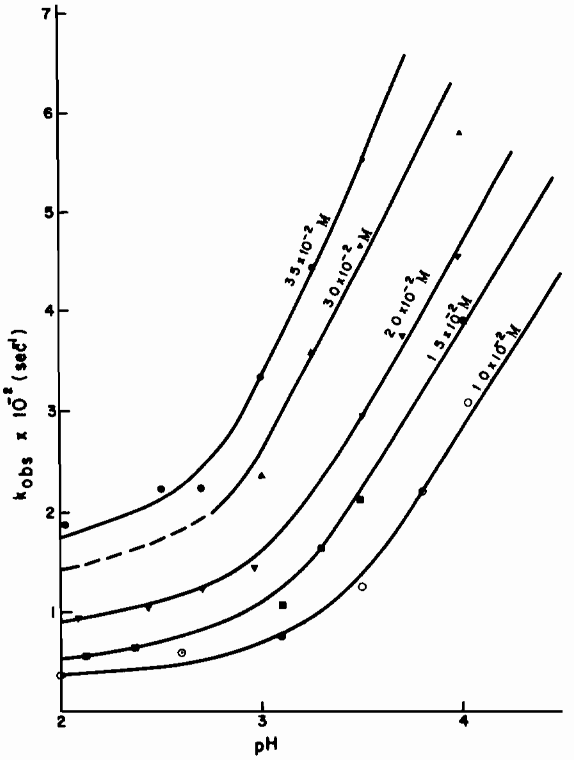


Fig. 2. The observed rate constants of the slow process as a function of pH at different T_{PLP}.

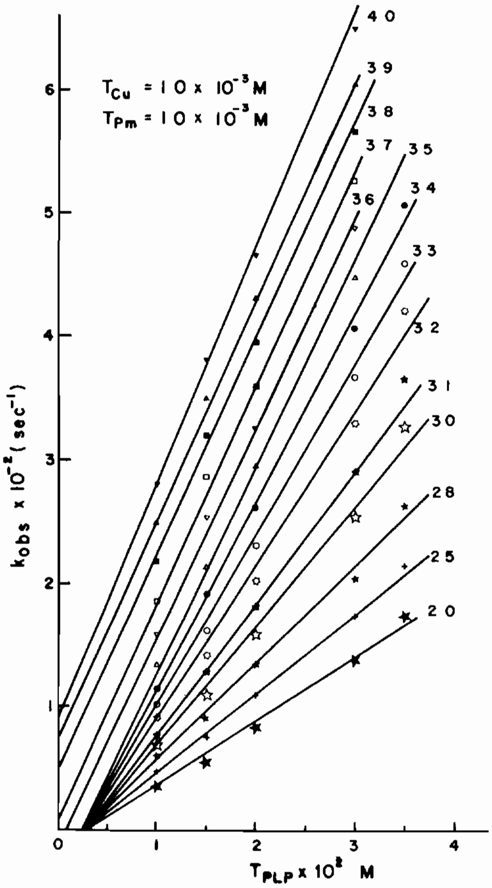


Fig. 3. Constructed curves of the observed rate constants of the slow process as a function of T_{PLP} at interpolated values of pH s using Fig. 2.

Two sets of curves were observed which generally conform to the equation:

$$k_{obs} = A + BT_{PLP}\ (sec^{-1}) \qquad (1)$$

The first set has negative values for the intercept A and is for pH values less than ~3.6. The second set has positive values for the intercept A and is for pH values greater than 3.6. These findings may mirror the mechanism of the reaction of PLP with Cu(II)–Pm complexes. At pH's lower than 3.6, there is probably a very fast ternary complex formation at PLP concentrations lower than $\sim 2.5 \times 10^{-3}$ *M*, prior to any further slow interaction between PLP and Pm in the presence of Cu(II) which was actually followed kinetically. Although this behavior was similar to what has been reported in case of Cu(II)–Pm–PL (1:1:≥10) system (1), this occurred at pH values lower than was found in the latter system. In addition, the observed rate constants are approximately 100 times greater (for $T_{PLP} \gtrsim 2.5 \times 10^{-3}$ *M*). At pH's greater than 3.6, interaction of PLP with Pm in presence of Cu(II) is highly enhanced at any concentration of the former PLP.

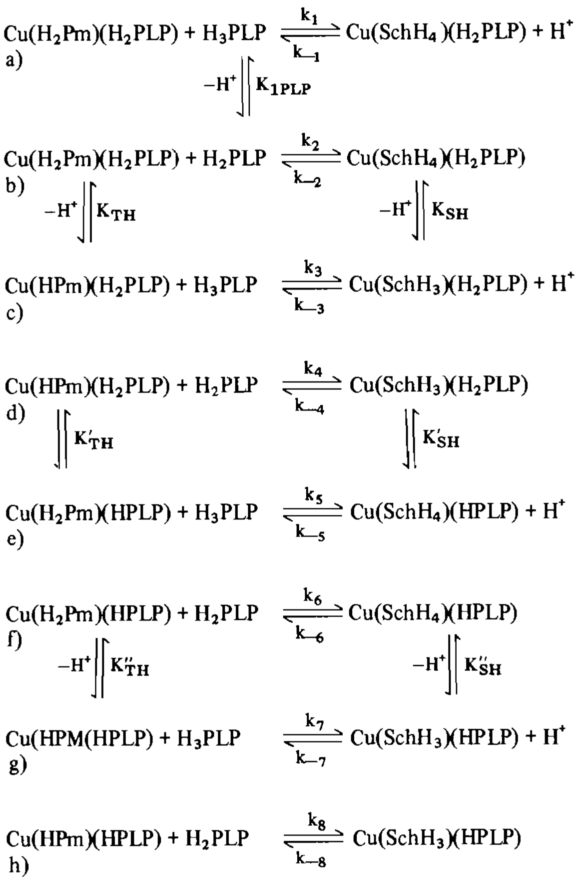
There may be another explanation to the kinetic behavior seen at pH > 3.6, which suggest the absence of prior ternary complex formation in favor of the idea that Cu(II) complexes with Pm are positively charged in this pH range and nucleophilic attack (at low concentration of PLP, $\sim 2.5 \times 10^{-3}$ *M*) is prohibited since PLP species themselves are positively charged. However, this suggestion is not entirely correct since it may be applied equally at high concentration of PLP as well as at low concentration. There is also a possibility that PLP is ligating only to the free Cu^{2+} ions, Table II. If PLP is interacting with Cu–Pm complexes and/or free Cu(II) ions, one should expect that this process is considerably faster than was measured in this work. However, one may think also of the possibility of the absence of any reactions at PLP concentrations lower than 2.5×10^{-3} *M*, especially at pH lower than 3.0. In such cases, the intercept should be zero rather than negative. If the solution of Cu(II)–Pm (1:1) system is examined it should be expected, upon consulting Table I, that several species may coexist in the pH range used. The species are mainly Cu^{2+}, H_3Pm^{2+}, H_2PM^+, $Cu(H_2Pm)^{3+}$ and $Cu(HPm)^{2+}$. In the Cu(II) complexes, H_2Pm^+ acts as a monodentate ligand utilizing the meta-oxy group as the ligating atom and HPm acts as a bidentate ligand using both the meta-oxy as well as the amino group of the para-aminomethyl group as the coordinating sites. On the other hand, PLP solutions consist of H_4PLP^+, H_3PLP and H_2PLP^- species. Of course, the relative concentration of all species is pH-dependent.

The interaction of Cu^{2+} with PLP species is too fast to be accounted for by the obtained kinetic data. It is plausible to suggest that the interaction took place mainly between Pm and PLP in presence of Cu^{2+} to form a Schiff base metal complex. The interaction between both species in absence of Cu^{2+} cannot be measured under the experimental conditions of this work. The mechanism which describe the kinetic data is shown in Scheme 1

Fast ternary complex formation:

$$Cu(H_nPm) + H_mPLP \rightleftharpoons Cu(H_{n-s}Pm)(H_{m-1}PLP) + (s+1)\,H^+$$

Slow Schiff base complex formation



a) $Cu(H_2Pm)(H_2PLP) + H_3PLP \xrightleftharpoons[k_{-1}]{k_1} Cu(SchH_4)(H_2PLP) + H^+$

$-H^+ \Updownarrow K_{1PLP}$

b) $Cu(H_2Pm)(H_2PLP) + H_2PLP \xrightleftharpoons[k_{-2}]{k_2} Cu(SchH_4)(H_2PLP)$

$-H^+ \Updownarrow K_{TH}$ $\qquad$ $-H^+ \Updownarrow K_{SH}$

c) $Cu(HPm)(H_2PLP) + H_3PLP \xrightleftharpoons[k_{-3}]{k_3} Cu(SchH_3)(H_2PLP) + H^+$

d) $Cu(HPm)(H_2PLP) + H_2PLP \xrightleftharpoons[k_{-4}]{k_4} Cu(SchH_3)(H_2PLP)$

$\Updownarrow K'_{TH}$ $\qquad$ $\Updownarrow K'_{SH}$

e) $Cu(H_2Pm)(HPLP) + H_3PLP \xrightleftharpoons[k_{-5}]{k_5} Cu(SchH_4)(HPLP) + H^+$

f) $Cu(H_2Pm)(HPLP) + H_2PLP \xrightleftharpoons[k_{-6}]{k_6} Cu(SchH_4)(HPLP)$

$-H^+ \Updownarrow K''_{TH}$ $\qquad$ $-H^+ \Updownarrow K''_{SH}$

g) $Cu(HPM(HPLP) + H_3PLP \xrightleftharpoons[k_{-7}]{k_7} Cu(SchH_3)(HPLP) + H^+$

h) $Cu(HPm)(HPLP) + H_2PLP \xrightleftharpoons[k_{-8}]{k_8} Cu(SchH_3)(HPLP)$

Scheme 1

where k_i and k_{-i} are the forward and backward rate constants and K_{TH}, K'_{TH} and K''_{TH} are the deprotonation constants of various ternary complexes, $Cu(H_2Pm)(H_2PLP)$, (C_1), $Cu(HPm)(H_2PLP)$ (C_2), $Cu(H_2Pm)(HPLP)$ (C_3) and $Cu(HPm)(HPLP)$ (C_4) and K_{SH}, K'_{SH} and K''_{SH} are the deprotonation constants of various Schiff base complexes, $Cu(SchH_4)(H_2PLP)$, (S_1), $Cu(SchH_3)(H_2PLP)$ (S_2), $Cu(SchH_4)(HPLP)$ (S_3) and $Cu(SchH_3)(HPLP)$ (S_4) and K_{1PLP} is the first deprotonation constant of PLP.

The rate equation describing the reaction mechanism in Scheme 1 is as follows:

$$\frac{d(S_1 + S_2 + S_3 + S_4)}{dt} =$$

$$[k_1 C_1(H_3PLP) + k_2 C_1(H_2PLP) + k_3 C_2(H_3PLP) +$$
$$+ k_4 C_2(H_2PLP) + k_5 C_3(H_3PLP) + k_6 C_3(H_2PLP) +$$
$$+ k_7 C_4(H_3PLP) + k_8 C_4(H_2PLP)] - [k_{-1} S_1(H^+) +$$
$$+ k_{-2} S_1 + k_{-3} S_2(H^+) + k_{-4} S_2 + k_{-5} S_3(H^+) +$$
$$+ k_{-6} S_3 + k_{-7} S_4(H^+) + k_{-8} S_4] \qquad (2)$$

In the pH range used, the mass balance equations of various components at a given pH and time may be expressed as follows:

$$T_{PLP} \cong (H_4PLP) + (H_3PLP) + (H_2PLP) +$$
$$+ (Cu(H_2PLP)) + (Cu(HPLP)) \qquad (3a)$$

$$T_{Pm} \cong (H_3Pm) + (H_2Pm) + (Cu(H_2Pm)) +$$
$$+ Cu(HPm)) + \Sigma_i^4 C_i + \Sigma_j^4 S_j \qquad (3b)$$

$$T_{Cu} \cong (Cu) + (Cu(H_2Pm)) + (Cu(HPm)) +$$
$$+ \Sigma_i^4 C_i + \Sigma_j^4 S_j \qquad (3c)$$

(charges are omitted for simplicity)

Ligand species and their Cu(II) complexes other than these mentioned in the above equations were not considered as a result of the experimental conditions used in this work. In order that eqn. 2 can be integrated a simplification is required. It is assumed that the concentration of free Cu(II), $Cu(H_2Pm)$, Cu(HPm), $Cu(H_2PLP)$, Cu(HPLP), H_3Pm and H_2Pm are insignificant with respect to other species in solution. Under these conditions equations 3(a, b and c) may be rewritten as

$$T_{PLP} \cong (H_4PLP) + (H_3PLP) + (H_2PLP) \qquad (4a)$$

$$T_{Pm} \cong \Sigma_i^4 C_i + \Sigma_j^4 S_j \qquad (4b)$$

and

$$T_{Cu} \cong \Sigma_i^4 C_i + \Sigma_j^4 S_j = C_4 Q_4 + S_4 Q_3 \qquad (4c)$$

where

$$Q_3 = [(H^+)^3 + (H^+)^2 K_{TH} + (H^+) K_{TH} K'_{TH} +$$
$$+ K_{TH} K'_{TH} K''_{TH}] (K_{TH} K'_{TH} K''_{TH})^{-1},$$

and

$$Q_4 = [(H^+)^3 + (H^+)^2 K_{SH} + (H^+) K_{SH} K'_{SH} +$$
$$+ K_{SH} K'_{SH} K''_{SH}] (K_{SH} K'_{SH} K''_{SH})^{-1}.$$

where $Q_3 = [((H^+)^3 + (H^+)^2 K_{TH} + (H^+) K_{TH} K'_{TH} + K_{TH} K'_{TH} K''_{TH}] . (K_{TH} K'_{TH} K''_{TH})^{-1}$, and $Q_4 = [(H^+)^3 + (H^+)^2 K_{SH} + (H^+) K_{SH} K'_{SH} + K_{SH} K'_{SH} K''_{SH}] . (K_{SH} K'_{SH} K''_{SH})^{-1}$.

If the equations 4(a, b and c) are substituted in eqn. 2, the following equation is obtained:

$$Q_3 \frac{dS_4}{dt} = \frac{(T_{Cu} - S_4 Q_3) T_{PLP}}{Q_4} \left\{ \frac{(H^+)^3}{K_{TH} K'_{TH} K''_{TH}} \times \right.$$
$$\times \left(\frac{k_5}{Q_1} + \frac{k_2}{Q_2} \right) + \frac{(H^+)^2}{K'_{TH} K''_{TH}} \left(\frac{k_3}{Q_1} + \frac{k_4}{Q_2} \right) + \frac{(H^+)}{K''_{TH}} \times$$
$$\left. \times \left(\frac{k_5}{Q_1} + \frac{k_6}{Q_2} \right) + \frac{k_7}{Q_1} + \frac{k_8}{Q_2} \right\} - S_4 \left\{ \frac{(H^+)^3}{K_{SH} K'_{SH} K''_{SH}} \times \right.$$
$$\times (k_{-1}(H^+) + k_{-2}) + \frac{(H^+)^2}{K'_{SH} K''_{SH}} (k_{-3}(H^+) + k_{-4}) +$$
$$\left. + \frac{(H^+)}{K''_{SH}} (k_{-5}(H^+) + k_{-6}) + k_{-7}(H) + k_{-8} \right\} \qquad (5)$$

where $Q_1 = ((H^+)^2 + (H^+) K_1 + K_1 K_2)((H^+) K_1)^{-1}$, and $Q_2 = ((H^+)^2 + (H^+) K_1 + K_1 K_2)(K_1 K_2)^{-1}$ and K_1 and K_2 are the first and second deprotonation constants of H_4PLP, Table II.

At a given pH equation 5 may be integrated to yield the following equation:

$$\ln \frac{S_{4,\infty}}{S_{4,\infty} - S_{4,t}} = kt \; (T_{PLP} > T_{Cu} = T_{Pm}) \qquad (6)$$

where $S_{4,\infty}$ and $S_{4,t}$ are the concentration of S_4 at equilibrium and time t. In such case k may be correlated with k_{obs}, *i.e.*

$$k = k_{obs} = A + BT_{PLP} = \left\{ \frac{(H^+)^3}{K_{SH} K'_{SH} K''_{SH}} (k_{-1}(H^+) + \right.$$
$$+ k_{-2}) + \frac{(H^+)^2}{K'_{SH} K''_{SH}} (k_{-3}(H^+) + k_{-4}) + \frac{(H^+)}{K''_{SH}} \times$$
$$\left. \times (k_{-5}(H^+) + k_{-6}) + k_{-7}(H^+) + k_{-8} \right\} / Q_3 +$$
$$+ \left\{ \frac{(H^+)^3}{K_{TH} K'_{TH} K''_{TH}} (k_1/Q_1 + k_2/Q_2) + \frac{(H^+)^2}{K'_{TH} K''_{TH}} \times \right.$$
$$\times (k_3/Q_1 + k_4/Q_2) + \frac{(H^+)}{K''_{TH}} (k_5/Q_1 + k_6/Q_2) +$$
$$\left. + k_7/Q_1 + k_8/Q_2 \right\} (T_{PLP}/Q_4) \qquad (7)$$

If we consider the formation of ternary complex prior Schiff base complex formation then there is certain concentration of PLP where the latter is not

TABLE III. The Values of the Intercept (A′) and Slope (B′) as obtained from Equation 8(a and b) at Various Interpolated pHs.

pH	A′(σ) 10^3*	B′(σ)*
2.0		0.53 (0.02)
2.5		0.64 (0.01)
2.8		0.81 (0.04)
3.0		0.94 (0.02)
3.1		1.07 (0.01)
3.2		1.20 (0.02)
3.3		1.35 (0.03)
3.4		1.53 (0.04)
3.5		1.61 (0.07)
3.6		1.64 (0.03)
3.7	6.58 (1.09)	1.69 (0.06)
3.8	9.49 (0.98)	1.72 (0.05)
3.9	12.38 (0.07)	1.75 (0.04)
4.0	14.60 (0.05)	1.84 (0.03)

*R = 0.996–0.999.

formed. Upon substraction of this amount of T_{PLP} from all values of T_{PLP} used in this work one may get two forms of eqn. 1, the first at pH lower than 3.7 and the second at pH greater than 3.7.

$$k_{obs} = B'T'_{PLP}\ (T'_{PLP} = T_{PLP} - 0.25 \times 10^{-3}\ M) \qquad pH < 3.7 \qquad (8a)$$

$$k_{obs} = A' + B'T'_{PLP} \qquad pH \gtrsim 3.7 \qquad (8b)$$

Table III lists the values of A′ and B′ at various pH values. The parameters A′ and B′ are pH-dependent. At pH's < 3.7 B′ is quadratically dependent on (H^+) when it is multiplied by the term $((H^+)^2 + K_1(H^+) + K_1K_2)$, Fig. 4, *i.e.*

$$B'((H^+)^2 + K_1(H^+) + K_1K_2) - b_o + b_1(H^+) + b_2(H)^2 \qquad (9)$$

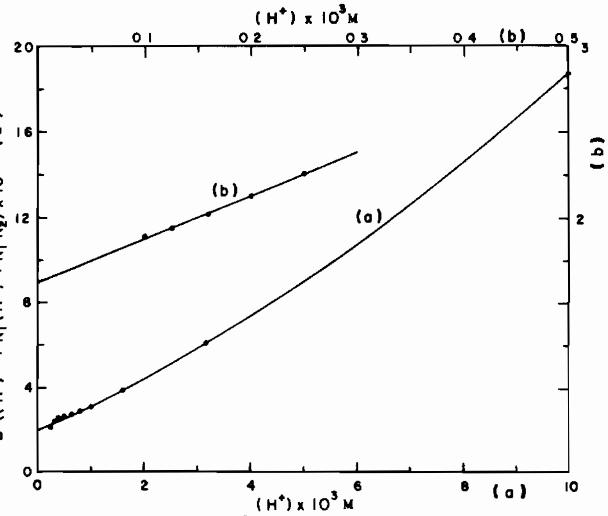


Fig. 4. The plot of $B'((H^+)^2 + K_1(H^+) + K_1K_2)$ as function of (H^+).

where b_o, b_1 and b_2 are constants equal to (1.98 ± 0.05) × 10^{-5}, (1.13 ± 0.05) × 10^{-2} and (0.56 ± 0.05), respectively. In order to correlate the coefficients b_o, b_1 and b_2 with the slope in eqn. 7 one should consider Q_4 as approximately equal to one since the left term is independent of $(H^+)^{-1}$. In such case, the coefficients take the forms $b_o \approx k_8K_1K_2 = 1.98 \times 10^{-5}$ and $k_8 \cong 3.4$, $b_1 \approx K_{TH}^{-1}(K_1K_2k_6) + K_1k_7 = 1.13 \times 10^{-2}$ and if $k_o = 0$, then $K_1k_7 = 1.13 \times 10^{-2}$ ($k_7 = 0.45$) and if $k_7 = 0$ then $K_{TH}^{-1}K_1K_2k_6 = 1.13 \times 10^{-2}$ ($k_6 \cong 0.13$) where K_{1TH} was taken to be equal to ~7.76 × 10^{-5}, (Table IV) and $b_2 \cong (K'_{TH}K''_{TH})^{-1}K_1K_2k_4 + K_{TH}^{-1}K_1k_5 = 0.56$ so if $(K'_{TH}K''_{TH})^{-1}K_1K_2k_4 = 0.56$ then $k_4 \cong 3.99 \times 10^{-3}$ (where the value of $K'_{TH}K''_{TH}$ was taken to be equal to ≅3.9 × 10^{-8}, Tables I, II, and IV, but if $K_{TH}^{-1}K_1k_5 = 0.56$, then $k_5 = 1.73 \times 10^{-3}$.

TABLE IV. Summary of the Rate Constants and Equilibrium Constants obtained in this Work.

Rate constant	
k_1, k_{-1}	0
k_2, k_{-2}	0
k_3, k_{-3}	0
k_4, k_{-4}	3.29 × 10^{-3}, 4.9 × 10^4
k_5, k_{-5}	1.73 × 10^{-5}, 0
k_6, k_{-6}	0.13–0.36
k_7, k_{-7}	0.45–1.22
k_8, k_{-8}	3.4–2.03
Estimated values of deprotonation constants	
K_{TH}	~7.76 × 10^{-5}
K'_{TH}	~5.00 × 10^{-3}
K''_{TH}	≥7.76 × 10^{-5}

At pH ≥ 3.7 the term $B'((H^+)^2 + K_1(H^+) + K_1K_2)$ is linearly dependent on (H^+), Fig. 4, *i.e.*

$$B'((H^+)^2 + K_1(H^+) + K_1K_2) = b_3 + b_4(H^+) \qquad (10)$$

where b_3 and b_4 are constants equal to (1.34 ± 0.02) × 10^{-5} and (3.07 ± 0.13) × 10^{-2}, respectively. The values of k_6, k_7 and k_8 which may be obtained from b_3 and b_4 are somewhat different (*i.e.* 0.36, 1.22 and 2.03 for k_6, k_7 and k_8) from those obtained with data at pH < 3.7. This may indicate an error in some of the above assumptions or may be due to different mechanisms.

The parameter A′ is linearly-dependent on the reciprocal of the H^+ concentration, Fig 5.

$$A' = a_o + a_1(H^+)^{-1} \qquad (11)$$

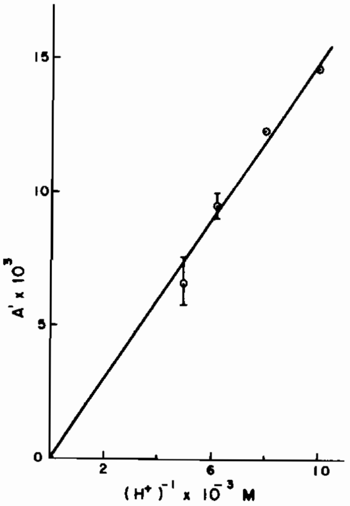


Fig. 5. The plot of A′ as a function of $(H^+)^{-1}$.

where a_0 and a_1 are constants having the values ~0 and 1.48×10^{-6}, respectively. Apparently the intercept obtained from eqn. 7 is not simply correlated with this finding without significant contribution from the term Q_3. In the pH range 3.7–4, $Q_3 \cong 3.02 \times 10^{-11}/(H^+)^3$ which imply that the most important terms in the intercept obtained from eqn. 7 are: $a_o = (k_{-2}K_{TH}K'_{TH}K''_{TH} + k_{-3}K_{SH})/K_{SH}K'_{SH}K''_{SH} \cong 0$ and $a_1 = (k_{-4}K_{TH}K'_{TH}K''_{TH} + k_{-5}K'_{SH})/K'_{SH}K''_{SH} \cong 1.48 \times 10^{-6}$; ($k_{-4} \cong 4.9 \times 10^4$ if $k_{-5} = 0$).

The aforementioned discussion indicates that Schiff base complex formation is enhanced by several steps in Scheme 1, *i.e.* d, e, f, and g reactions. The first four reactions seems to have no significant contribution.

Figure 6 shows the dependence of k_{obs} for the fast step on pH. It is linear with a negative slope. In other words, the rate decreases with the decrease in H^+ concentration. This may reflect the formation of a carbinolamine intermediate which is stabilized in presence of H^+, as well as Cu^{2+}, Fig. 7. At low pH protonated complexes are more predominant and the loss of a H_2O molecule to form the respective Schiff complex is not as feasible as at higher pH. The latter process is enhanced as pH increases, possibly through the formation of a stable pentacoordinated arrangement, Fig. 7.

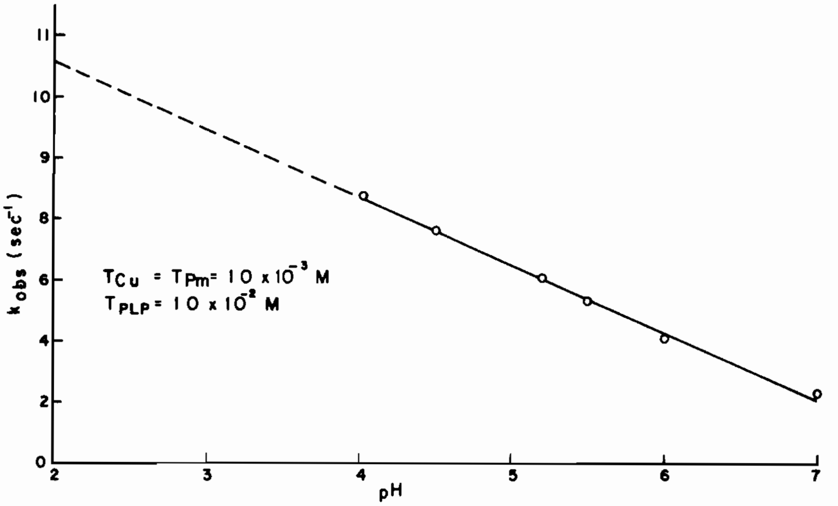


Fig. 6. The observed rate constants of the fast process as a function of pH.

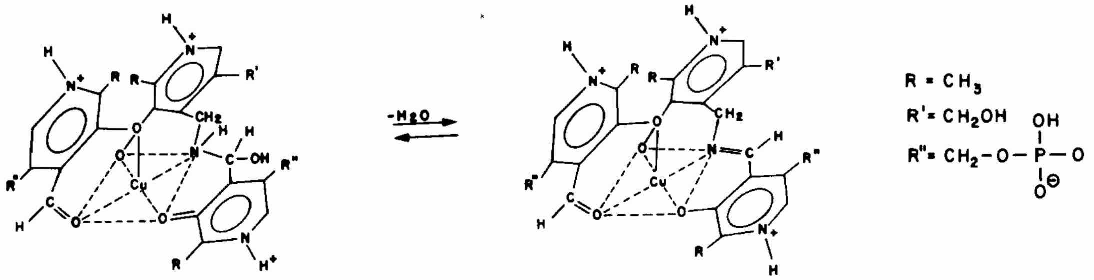


Fig. 7. Proposed structural formulas of carbinolamine and Schiff base complexes.

Conclusion

In this study we have shown that interaction of PLP with Pm cannot take place in the absence of cupric ions, especially in acidic solutions. In addition, the presence of phosphate moiety in PLP enhance this interaction more than with PL. This is not surprising since hemiacetal formation exhibited by PL is prohibited in PLP. It seems also that prior formation of a ternary complex of the form $Cu(H_mPm)(H_1PLP)$ is essential for effective Schiff base complex formation. The latter complex is formed through carbinolamine intermediate which is favorable in acidic medium. Similar to Cu–Pm–PL system, increasing the composition ratio of Cu:Pm from 1:1 to 1:≥2 usually inhibits possibly Schiff base complex formation.

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