Inorganica Chimica Acta, *109* (1985) 59–63

Complexes of Vitamin B_6. XVI*. Kinetics and Reaction Mechanism of Iron(III) Complexes with Pyridoxal-5′-Phosphate

MOHAMED A. EL-DESSOUKY**, MOHAMED S. EL-EZABY† and HUSAM ABU SOUD

Department of Chemistry, Faculty of Science, University of Kuwait, Kuwait

Received June 7, 1984

Abstract

The stopped flow technique has been used to study the kinetics of complex formation of iron(III) with pyridoxal-5-phosphate (PLP) in the pH range 1.00–2.50, and in the temperature range 18 °C–30 °C, at an ionic strength of 0.50 M (NaCl). From the initial concentration dependence of PLP (T_{PLP}) of the reaction rate it can be shown that two kinetic steps can be represented as:

$k_{obs}' = m_i + m_i' T_{PLP}$

where m_i and m_i' are pH-dependent parameters. The calculated activation data are $\Delta E^* = 23.2 \pm 1.8$ kcal mol^{-1} and 10.98 ± 0.53 kcal mol^{-1} for the first and second kinetic steps, respectively and ΔS^* are -20.50 ± 5.96 e.u. and 24.62 ± 1.81 e.u., respectively.

Introduction

Pyridoxal-5′-phosphate (PLP), a vitamin B_6 compound, interacts with many amino compounds to form Schiff bases. The interaction is usually carried out in the presence of metal ions. The resulting metal complexes of Schiff bases have been the subject of several reports due to their importance as model systems for PLP-catalyzed enzymatic reactions such as transamination and deamination [1]. PLP itself has considerable complexing ability with some metal ions but only limited investigations have been reported [2–4]. A study of binary metal complex formation is essential before any further study of Schiff base complex formation involving PLP. In this work we report the kinetics of the interaction of PLP with Fe(III) in aqueous solution. Iron(III) binary complexes with other vitamin B_6 compounds have been reported quite recently and their mechanisms of complex formation were elucidated in acidic medium [5, 6].

Experimental

Materials

Pyridoxal-5′-phosphate (PLP) was an analytically pure chemical (E-Merck) used without further purification. The stock solution of PLP (0.10 M) was kept in the dark at 4 °C and was taken by weight. The stock solution of $FeCl_3$ was checked for concentration by complexometric methods [5]. Ionic strength was kept constant at 0.50 M NaCl.

Measurements

The pH measurements were made using Radiometer pH meter model 62 equipped with a combined glass electrode (GK 2301C). Calibration was done by Radiometer buffers at pH 2.0 and 4.0. Kinetic measurements were made using a Durrum Stopped Flow Apparatus. The optical path length was 20 mm. The mixing syringes and cuvette were thermostatted using a Grant circulating thermostat. The observed rate constants were calculated for kinetic data up to 80% of the reaction completion. The absorption spectra were carried out in a Pye-Unicam 8-100 in the 500–200 nm range. The concentration of the iron(III) chloride was kept constant at 3.0×10^{-4} M while that of PLP was in the range $(3.0–8.0) \times 10^{-3}$ M. The kinetic data were taken in the temperature range 18–30 °C.

Results and Discussion

The addition of PLP to a solution of Fe(III) readily produced a reddish color which intensified when the pH of the medium was raised. The spectrum of the PLP–Fe(III) system is not much different from that of PLP alone in the wavelength range

---

*Part XV: Quaternary Complexes Involving Pyridoxamine Glycine and Ethylenediamine with Co(II), Ni(II), Cu(II) and Zn(II), *Polyhedron, 8,* 775 (1983).

**Present Address: Department of Chemistry, Faculty of Science, Alexandria University, Egypt.

†Author to whom all correspondence should be addressed.

0020-1693/85/$3.30

300–500 nm except that a slight general increase in absorbance is observed, due to iron complexation with PLP, in the same wavelength range. Precipitation of probably a hydrolyzed species occurred at pHs > 1.9 on standing. The wavelength 470 nm was selected for taking the kinetic runs where the ligand and metal absorptions were minimum and the absorption was due mainly to the complex species. Two reaction kinetic steps were observed when pseudo-first order conditions were preserved (*i.e.* concentration of PLP is much greater than that of Fe(III)). The fast kinetic step started at lower pHs than the slower one. Figure 1 (a and b) shows the observed pseudo-first order rate constants of the two rate steps, $k_{obs}{}^{i}$, their dependence on pH and initial concentration of PLP ($T_{\mathbf{PLP}}$). Figure 2 (a and b) shows the dependence of $k_{obs}{}^{i}$ for the two rate steps on initial concentration of PLP at interpolated values of pHs. The behaviour may be interpreted in terms of the following empirical relation:

$$k_{obs}{}^{i} = \mathrm{m_i} + \mathrm{m_i}' T_{\mathbf{PLP}} \tag{1}$$

where $\mathrm{m_i}$ and $\mathrm{m_i}'$, are constants at a given pH (Table I). In order to propose a mechanism for Fe(III) interaction with PLP, a brief account of the different Fe(III) and PLP species in the pH range 1.0–2.5 should be given. In this range Fe(III) may be expected to exist primarily in the form of free aquated chloro complexes ($\mathrm{(Fe(H_2O)_{n-i}(Cl)_i)}$) in addition to the aquated chloro monohydroxy species ($\mathrm{(Fe(H_2O)_{n-i-k}(OH)_k\text{-}(Cl)_i)}$). The concentration of the dimer species and dihydroxy species may be assumed negligible in this acidic pH range. The ligand species on the other hand, in the same pH range, are likely to be $\mathrm{H_4PLP^+}$, $\mathrm{H_3PLP}$ and $\mathrm{H_2PLP^-}$. The pKs of PLP are 1.60, 3.58, 6.41 and 9.24 [4]. Since chlorocomplexes of Fe(III) are not strong enough [7] to exist in solution in appreciable amounts, it is not unreasonable to assume that free aquated Fe(III) and partly aquated monohydroxy species coexist in the pH range 1.0–2.5. Several mechanistic models were tested to account for the present kinetic data. The one which closely describes the first step is as follows:

$$\mathrm{H_4PLP^+} \underset{K_{1h}}{\overset{-\mathrm{H^+}}{\rightleftharpoons}} \mathrm{H_3PLP^0} \underset{K_{2h}}{\overset{-\mathrm{H^+}}{\rightleftharpoons}} \mathrm{H_2PLP^-}$$

$$\text{a) } \mathrm{Fe^{3+} + H_3PLP \rightleftharpoons FeH_3PLP^{3+}}; \qquad k_1, k_{-1}$$

$$\text{b) } \mathrm{FeOH^{2+} + H_3PLP \rightleftharpoons FeH_3PLP^{3+}}; \qquad k_2, k_{-2}$$

$$K_{\mathrm{COH}} \uparrow\downarrow + \mathrm{OH^-}$$

$$\text{c) } \mathrm{FeOH^{2+} + H_3PLP \rightleftharpoons FeOHH_3PLP^{2+}}; \qquad k_3, k_{-3}$$

$$\text{d) } \mathrm{Fe(OH)_2{}^{+*} + H_3PLP \rightleftharpoons FeH_3PLP^{3+} + 2OH^-}; \qquad k_4, k_{-4}$$

$$K_{\mathrm{COH}} \uparrow\downarrow + \mathrm{OH^-}$$

$$\text{e) } \mathrm{Fe(OH)_2{}^{+} + H_3PLP \rightleftharpoons FeOHH_3PLP^{2+} + OH^-}; \qquad k_5, k_{-5}$$

Scheme 1

where k_i and k_{-i} are the forward and backward reaction constants, K_{COH} the formation constant of the hydroxy complex species ($\mathrm{FeOHH_3PLP^{2+}}$), and K_{1h} is the first deprotonation constant of PLP. At constant pH and under the pseudo-first order reaction kinetic ($T_{\mathbf{PLP}} \gg T_{\mathbf{Fe}}$) the rate equation describing the above mechanism is:

$$\frac{\mathrm{d}C_2}{\mathrm{d}t} = \frac{\alpha_1 T_{\mathbf{PLP}}(T_{\mathbf{Fe}} - C_2 Q_2) Q_3}{Q_1 Q_2} - C_2 \frac{Q_4}{Q_2} \tag{2}$$

where C_1 and C_2 are the concentrations of $\mathrm{FeH_3\text{-}PLP^{3+}}$ and $\mathrm{FeOHH_3PLP^{2+}}$,

TABLE I. The Values of $\mathrm{m_I}$ and $\mathrm{m_I}'$ Obtained from Fig. 2 (a and b) at Various Interpolated pHs at $T_{\mathrm{Fe}} = 3.0 \times 10^{-4}$ M and T = 25 °C.

pH	$\mathrm{m_I}$	$\mathrm{m_I}' \times 10^{-2}$	$(\mathrm{m_I}' \times Q_1 \times (\mathrm{H^+})^2/\alpha_1)$	$\mathrm{m_{II}}$	$\mathrm{m_{II}}' \times 10^{-2}$	$\mathrm{m_{II}}' \times 10^{-2}/\alpha_1$
1.2	0.13	1.38	1.95			
1.3	0.18	1.81	1.38			
1.4	0.30	2.38	1.01			
1.5	0.30	3.38	0.79			
1.6	0.35	5.13	0.66			
1.7	0.45	6.75	0.51	0.18	0.62	1.12
1.8	0.75	7.88	0.35	0.23	0.72	1.19
1.9	1.40	8.41	0.23	0.30	0.81	1.23
2.0	2.20	8.42	0.13	0.37	0.91	1.28
2.1	2.90	8.42	0.079	0.48	1.02	1.35
2.2	3.50	8.33	0.048	0.56	1.22	1.53

---

*Although the concentration of $\mathrm{Fe(OH)_2{}^+}$ is assumed negligible, it may play a role in the reaction mechanism.

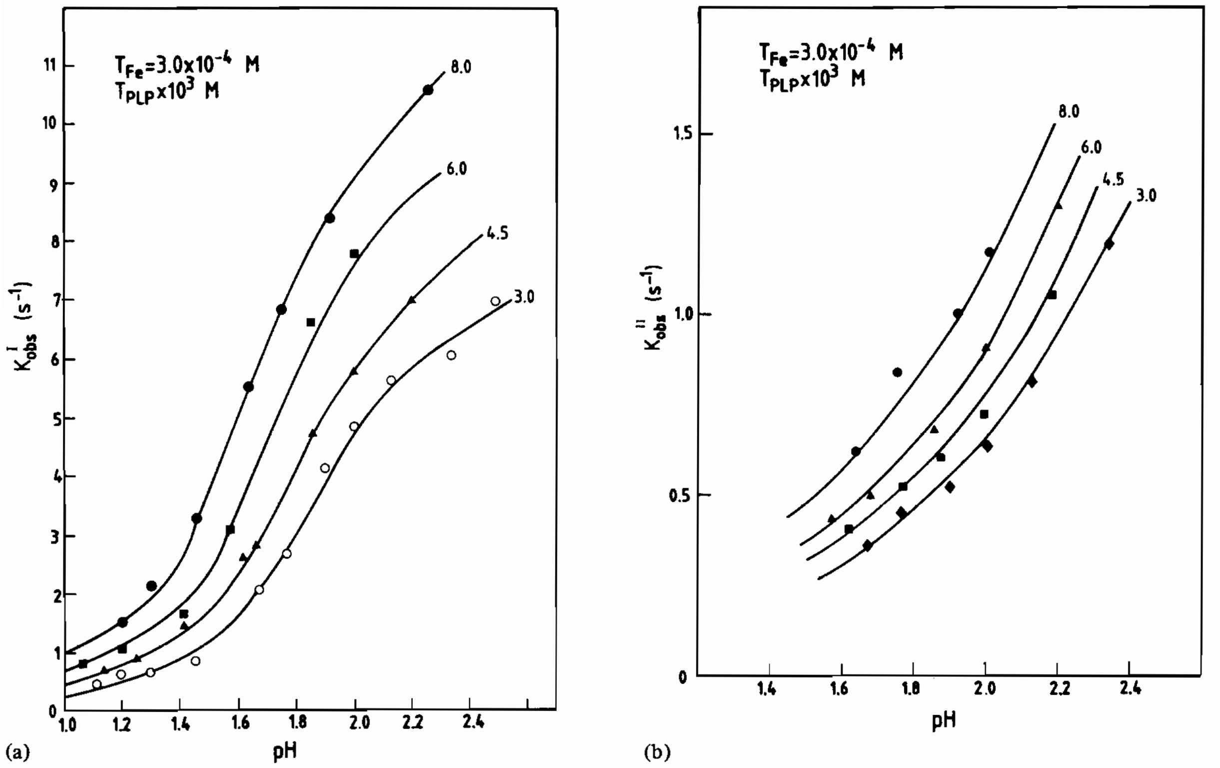

Fig. 1. (a) The dependence of k_{obs}^{I} on the initial concentration of PLP and pH at 25 °C. (b) The dependence of k_{obs}^{II} on the initial concentration of T_{PLP} and pH at 25 °C.

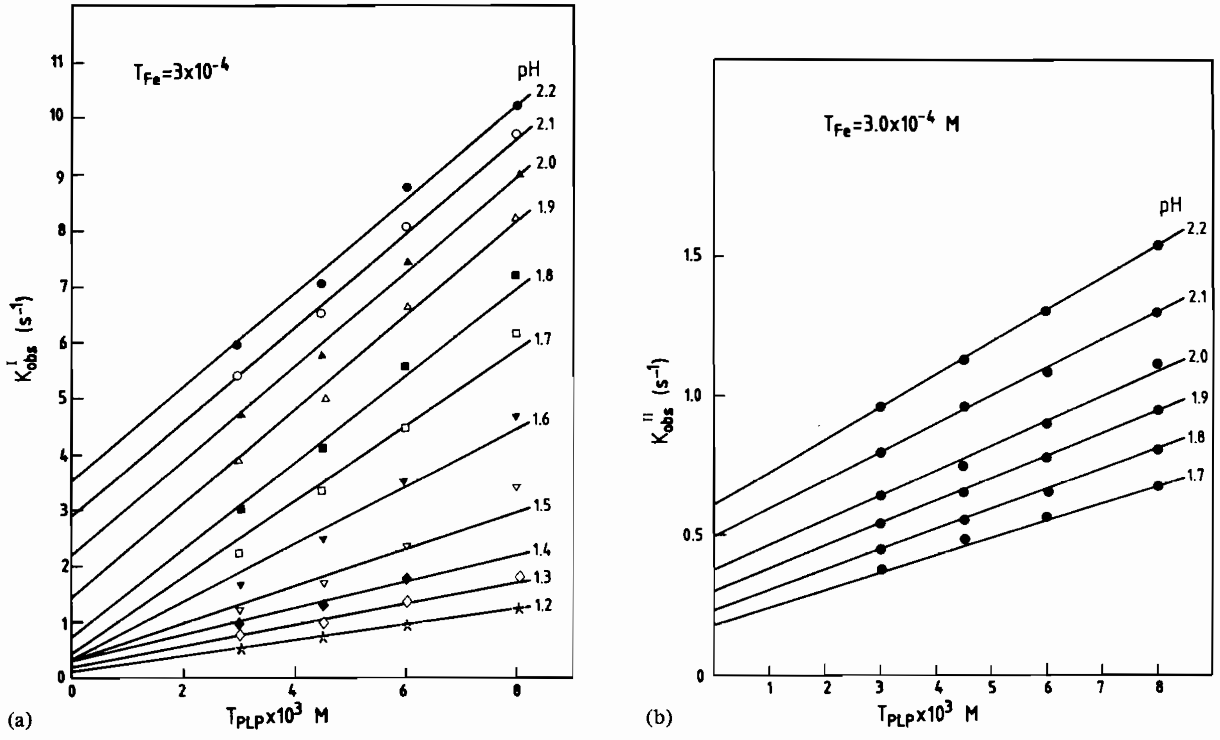

Fig. 2. (a) The dependence of k_{obs}^{I} on T_{PLP} at interpolated values of pHs. (b) The dependence of k_{obs}^{II} on T_{PLP} at interpolated values of pHs.

$$\alpha_1 = (\mathrm{H}^+)K_{1\mathrm{h}}/((\mathrm{H}^+)^2 + K_{1\mathrm{h}}(\mathrm{H}^+) + K_{1\mathrm{h}}K_{2\mathrm{h}})$$

$$Q_1 = ((\mathrm{H}^+)^2 + K_{1\mathrm{OH}}(\mathrm{H}^+) + K_{1\mathrm{OH}}K_{2\mathrm{OH}})/(\mathrm{H}^+)^2$$

$$Q_2 = (K_{\mathrm{COH}}(\mathrm{OH}^-) + 1)/K_{\mathrm{COH}}(\mathrm{OH}^-)$$

$$Q_3 = k_1 + (k_2 + k_3)K_{1\mathrm{OH}}/(\mathrm{H}^+) + (k_4 + k_5)K_{1\mathrm{OH}}K_{2\mathrm{OH}}/(\mathrm{H}^+)^2$$

$$Q_4 = (k_{-1} + k_{-2}(\mathrm{OH}^-) + k_{-4}(\mathrm{OH}^-)^2)/K_{\mathrm{COH}}(\mathrm{OH}^-) + k_{-3} + k_{-5}(\mathrm{OH}^-)$$

The integrated form of eqn. 2 is as follows:

$$\ln \frac{(C_2)_\infty}{(C_2)_\infty - (C_2)_t} = k_{\mathrm{obs}}t \qquad (3)$$

where $(C_2)_\infty$ and $(C_2)_t$ are the concentrations of $\mathrm{FeOHH_3PLP^{2+}}$ at infinite time and time t, and

$$k_{\mathrm{obs}}{}^{\mathrm{I}} = Q_4/Q_2 + \alpha_1 Q_3 T_{\mathrm{PLP}}/Q_1 = \mathrm{m_I} + \mathrm{m_I}' T_{\mathrm{PLP}} \qquad (4)$$

where

$$\mathrm{m_I} = Q_4/Q_2 = k_{-1} + (k_{-2} + k_{-3}K_{\mathrm{COH}})(\mathrm{OH}^-) + (k_{-4} + k_{-5}K_{\mathrm{COH}})(\mathrm{OH}^-)^2 \qquad (5)$$

(if $K_{\mathrm{COH}}(\mathrm{OH}^-)$ is much less than one in the pH range used) and $\mathrm{m_I}' = \alpha_1 Q_3/Q_1$ or upon rearrangement.

$$(\mathrm{H}^+)^2\mathrm{m_I}'Q_1/\alpha_1 = (k_4 + k_5)K_{1\mathrm{OH}}K_{2\mathrm{OH}} + (k_2 + k_3)K_{1\mathrm{OH}}(\mathrm{H}^+) + k_1(\mathrm{H}^+)^2 \qquad (6)$$

The regression analysis of the intercept data *vs.* $(\mathrm{H}^+)^{-1}$ reveals the following values for the coefficients, $k_{-1} \approx 0$, $K_\mathrm{w}(k_{-2} + k_{-3}K_{\mathrm{COH}}) = (3.7 \pm 5.5)\,10^{-3}$ (K_w = ionic product of $\mathrm{H_2O} = 10^{-13.78}$ [7]) and $(k_{-4} + k_{-5}K_{\mathrm{COH}})K_\mathrm{w} = (1.63 \pm 0.40)10^{-5}$. On the other hand, the regression analysis of $\mathrm{m_I}'Q_1(\mathrm{H}^+)^2/\alpha_1$ *vs.* (H^+) yields the following values for the coefficients of eqn. 6: $(k_4 + k_5)K_{1\mathrm{OH}}K_{2\mathrm{OH}} \approx 0$, $(k_2 + k_3)K_{1\mathrm{OH}} = 21 \pm 2$ and $k_1 = 170 \pm 35$. It seems from the above findings that paths a, b, and c are effective in complex formation of iron(III) with PLP since k_{-1}, k_{-2} and k_{-3} are probably much less than k_1, k_2 and k_3. On the other hand, $k_{-4} + k_{-5}K_{\mathrm{COH}} \approx 10^9$ while the term $(k_4 + k_5)K_{1\mathrm{OH}}K_{2\mathrm{OH}}$ is much smaller indicating the importance of the hydrolysis reaction on the formation of $\mathrm{FeH_3PLP^{3+}}$ and $\mathrm{FeOHH_3PLP^{2+}}$. The second kinetic step may be described by the following mechanism:

$$\mathrm{FeH_3PLP + H_3PLP \rightleftharpoons Fe(H_3PLP)_2}; \qquad k_6, k_{-6}$$

$$K_{\mathrm{COH}} \ \uparrow\downarrow\ \mathrm{OH^-}$$

$$\mathrm{FeOHH_3PLP + H_3PLP \rightleftharpoons Fe(H_3PLP)_2 + OH^-}; \qquad k_7, k_{-7}$$

Scheme 2

The rate equation describing the mechanism in Scheme 2 is:

$$\frac{\mathrm{d}C_3}{\mathrm{d}t} = \alpha_1 T_{\mathrm{PLP}}\left(\frac{T_{\mathrm{Fe}} - C_3}{Q_2}\right)\left(\frac{k_6}{K_{\mathrm{COH}}(\mathrm{OH}^-)} + k_7\right) - C_3(k_{-6} + k_{-7}(\mathrm{OH}^-)) \qquad (7)$$

where C_3 is the concentration of $\mathrm{Fe(H_3PLP)_2}$ and

$$T_{\mathrm{Fe}} \simeq (\mathrm{FeOHH_3PLP}) + \mathrm{Fe(H_3PLP)_2}$$

The integrated form of eqn. 7 is

$$\ln \frac{(C_3)_\infty}{(C_3)_\infty - (C_3)_t} = k_{\mathrm{obs}}{}^{\mathrm{II}}t \qquad (8)$$

where

$$k_{\mathrm{obs}}{}^{\mathrm{II}} = (k_{-6} + k_{-7})(\mathrm{OH}^-) + \frac{\alpha_1}{Q_2}\left(\frac{k_6}{K_{\mathrm{COH}}(\mathrm{OH})} + k_7\right)T_{\mathrm{PLP}} \qquad (9)$$

Equation 9 is correlated with eqn. 1 where

$$\mathrm{m_{II}} = k_{-6} + k_{-7}(\mathrm{OH}^-) = k_{-6} + k_{-7}\frac{K_\mathrm{w}}{(\mathrm{H}^+)} \qquad (10)$$

and

$$\mathrm{m_{II}}' = \frac{\alpha_1}{Q_2}\left(\frac{k_6}{K_{\mathrm{COH}}(\mathrm{OH})} + k_7\right) \qquad (11)$$

or

$$\mathrm{m_{II}}'/\alpha_1 = k_6 + k_7K_{\mathrm{COH}}K_\mathrm{w}/(\mathrm{H}^+) \quad (\text{if } 1 > K_{\mathrm{COH}}(\mathrm{OH}^-)) \qquad (12)$$

The coefficient of eqn. 9 was evaluated from the linear plot of $\mathrm{m_{II}}$ *vs.* $(\mathrm{H}^+)^{-1}$ to be: $k_{-6} = (3.1 \pm 2.6)\,10^{-2}$ and $k_{-7}K_\mathrm{w} = (3.33 \pm 0.25)10^{-3}$ where $k_{-7} = 2.0 \times 10^{11}$.

In addition k_6 and $k_7K_{\mathrm{COH}}K_\mathrm{w}$ were determined from the linear plot of $(\mathrm{m_{II}}'/\alpha_1)$ *vs.* $(\mathrm{H}^+)^{-1}$. Their magnitudes are: $k_6 = (0.96 \pm 0.02)$ and $k_7K_{\mathrm{COH}}K_\mathrm{w} = (0.33 \pm 0.02)$ where $k_7 \gg 3.7 \times 10^2$ (if $K_{\mathrm{COH}} = K_{1\mathrm{OH}}$). It is clear that the formation of $\mathrm{Fe(H_3PLP)_2{}^{3+}}$ is opposed by the formation of $\mathrm{Fe(OH)(H_3PLP)^{2+}}$ where $k_6/k_{-6} = 31$ and $k_7/k_{-7} \geq 1.85 \times 10^{-9}$. Table II depicts the effect of T on both kinetic steps. Figures 3a and b show the plot of $\ln(\mathrm{h}k_{\mathrm{obs}}{}^{\mathrm{i}}/\mathrm{e}Tk_\mathrm{B})$ *vs.* T^{-1} for both kinetic steps. The experimental activation energy obtained from Fig. 3a and b for both steps is (23.3 ± 1.8) kcal mol^{-1} and (10.98 ± 0.53) kcal mol^{-1}, respectively. The entropy of activation ΔS^* is −20.50 ± 5.96 e.u. and 24.62 ± 1.81 e.u. respectively. These results indicate that the formation of $\mathrm{Fe(H_3PLP)_2{}^{3+}}$ is energetically more favorable from $\mathrm{Fe(H_3PLP)^{3+}}$ than $\mathrm{Fe(H_3PLP)^{3+}}$ from $\mathrm{Fe^{3+}}$, $\mathrm{Fe(OH)^{2+}}$ or $\mathrm{Fe(OH)_2{}^+}$. However, the entropy of activation is less favorable for the formation of $\mathrm{Fe(H_3PLP)_2{}^{3+}}$ than for $\mathrm{Fe(H_3PLP)^{3+}}$ and $\mathrm{Fe(OH)(H_3PLP)^{2+}}$.

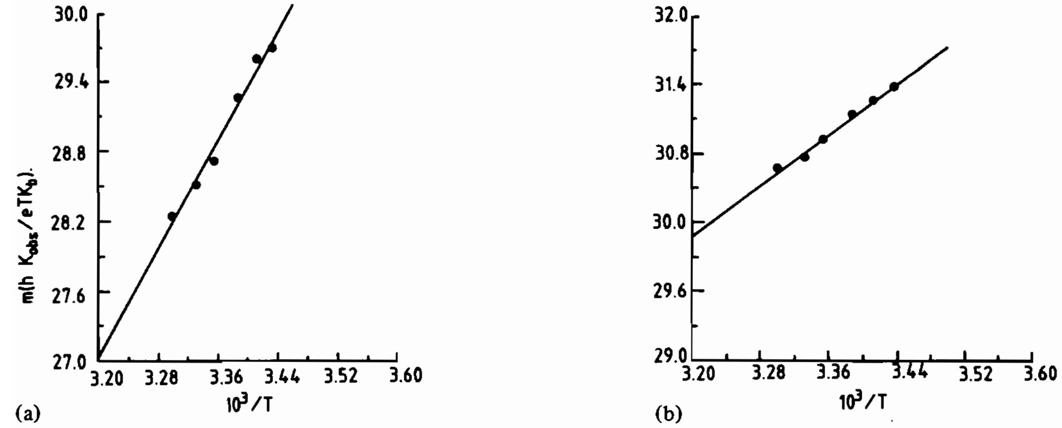

Fig. 3. (a) The plot of $\ln(h\, k_{obs}{}^{I}/e\, Tk_b)$ as a function of (T^{-1}) for the first kinetic step. (b) The plot of $\ln(h\, k_{obs}{}^{II}/e\, Tk_b)$ as a function of (T^{-1}) for the second kinetic step.

TABLE II. The Effect of Temperature Variation on the Observed Rate Constants of both Kinetic Steps at T_{PLP} = 4.5×10^{-3} M, $T_{Fe} = 3.0 \times 10^{-4}$ M and pH = 2.0.

Temp. (°C)	$K_{obs}{}^{I}(s^{-1})$	$K_{obs}{}^{II}(s^{-1})$
18	2.07	0.384
20	2.30	0.440
22	3.20	0.500
25	5.70	0.621
27	7.00	0.736
30	9.20	0.822

Conclusion

In this work H_3PLP has been selected as the ligating species with Fe(III) rather than H_2PLP^-. The choice was made because of the readily available H_3PLP species in the pH range used as well as on the compatibility of this species with the interpretation of the kinetic data. Since there are three ligating sites on PLP, the pyridinic nitrogen, the phosphate group and the phenoxy group, iron(III) may coordinate to any one of them. The interaction of two or more iron(III) species with one PLP molecule is unlikely to occur since the ligand is highly protonated in the pH range used in this work. The ligating site may reasonably be suggested to be the phosphate group since in this pH range (1.0–2.5) the first deprotonation step is due to the dissociation of one of the phosphate group protons. This conclusion is sound since the kinetic data of the Fe(III)–pyridoxal system [6] is quite different from that of the present system since in the former the ligating site is probably the phenoxy group.

References

1 E. E. Snell and D. E. Metzler, *J. Am. Chem. Soc., 77,* 2431 (1955).

2 M. E. Farago, M. M. McMillan and S. S. Sabir, *Inorg. Chim. Acta, 14,* 207 (1975).

3 T. S. Viswanathan and T. J. Swift, *Can. Chem. 57,* 1050 (1979).

4 N. Al-Awadi, M. S. El-Ezaby and H. Abu-Soud, *Inorg. Chim. Acta, 67,* 131 (1982).

5 M. S. El-Ezaby, A. I. Abu-Shady, N. Gayed and F. R. El-Eziri, *J. Inorg. Nucl. Chem., 39,* 169 (1977).

6 M. S. El-Ezaby and A.I. Abu-Shady, *Inorg. Chim. Acta, 55,* 29 (1981).

7 E. Hogfeldt (ed.), 'Stability Constants of Metal Ion Complexes. Part A: Inorganic Ligands', IUPAC Chem. Data. Series, No. 21, Pergamon, New York, 1982.