Kinetics of the Formation and Hydrolysis of the Schiff's Base of Pyridoxal 5'-Phosphate with Hexylamine in Water–Dioxane

Isabel M. Plaza del Pino, Juan Llor and Jose M. Sanchez-Ruiz*
Departamento de Quimica-Fisica, Facultad de Ciencias, Universidad de Granada, 18071-Granada, Spain

The rate constants of the reaction between pyridoxal 5'-phosphate and hexylamine have been studied as a function of pH, in water–dioxane mixtures, at 25 °C, and at an ionic strength of 0.01. We have found that both the solvent effect and the intramolecular acid catalysis effect on the rates of formation, can be acceptably described on the basis of a log k_1^i *versus* ($pK_{P_i} - pK_{A1}$) correlation. $k_1^i \equiv$ rate constant for the reaction between free hexylamine and the ionic species of pyridoxal 5'-phosphate with *i* bound protons (P_i); $pK_{P_i} \equiv pK$ value of the P_i species; $pK_{A1} \equiv pK$ value of the hexylammonium ion.

In this article we describe the kinetics of formation and hydrolysis of the Schiff's base formed between pyridoxal 5'-phosphate (PLP) and hexylamine in water–dioxane mixtures, at 25 °C and ionic strength $\Gamma = 0.01$. The reasons for carrying out this study should be clear from the Introduction of the preceding paper.[1]

Experimental

The materials, the preparation of the stock solutions and the pH measurements in water–dioxane have been described in the preceding paper.[1]

Kinetic Measurements.—All the reactions were carried out in a thermostatted quartz spectrophotometric cell, at 25 ± 0.1 °C. The solutions for the kinetic experiments contained PLP (concentration *ca.* 8×10^{-5} mol dm^{-3}), hexylamine, KCl and HCl or KOH. The concentrations of hexylamine were 15 to 120 times larger than that of PLP, in order to preserve pseudo-first-order conditions. HCl or KOH were added to obtain the desired pH value. The concentration of KCl was that required to reach a total ionic strength of 0.01.

The kinetics of formation of the Schiff's base were followed by measuring the time-dependence of the absorbance (A) at a suitable wavelength (415 nm, in most cases). Pseudo-first-order rate constants for Schiff's base formation (k_{obs}) were obtained by non-linear, least-squares fitting of eqn. (1) to the experimental A/t profiles, where A_0 is the absorbance at $t = 0$ and A_∞ is the absorbance at equilibrium. Each k_{obs} value was assigned to the pH measured immediately after a constant absorbance value had been reached; nevertheless, we checked that the pH value did not change significantly during a given kinetic experiment.

$$A = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}t) \qquad (1)$$

k_{obs} measurements were carried out in water–dioxane mixtures of the following dioxane volume fractions: 0, 0.2, 0.4 and 0.6.

Results and Discussion

The reaction between PLP and n-hexylamine can be represented by:

$$\text{PLP} + \text{Amine} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Schiff's base} + H_2O$$

where k_1 and k_2 are, respectively, the apparent, pH-dependent rate constants for Schiff's base formation and hydrolysis.

Calculation of the k_1/pH *and* k_2/pH *Profiles.*—The values of k_1 and k_2 at a given pH are usually obtained from the dependence of the pseudo-first-order rate constant (k_{obs}) on the total amine concentration ($[A]_T$), as indicated by eqn. (2).

$$k_{obs} = k_2 + k_1[A]_T \qquad (2)$$

It must be noted, however, that our kinetic experiments were carried out in the absence of buffer (see Experimental section). This presents the following two advantages: (1) ionic strength can be kept at a comparatively low value ($\Gamma = 0.01$), thus minimizing the possibility of ionic associations in low-polarity media; (2) the effect of the possible general acid–base catalysis by the buffer systems does not need to be checked and corrected for.

The absence of buffer does not preclude the determination of the pH value for a given kinetic experiment; it does make it difficult, however, to obtain a series of k_{obs} values at different amine concentrations and at exactly the same pH, as the direct application of eqn. (2) requires. In order to circumvent this problem, we have calculated the k_1 and k_2 values at a given pH from a single k_{obs} value, by using eqns. (3) and (4), which are easily derived from eqn. (2) and the fact that $K_{pH} = k_1/k_2$,

$$k_1 = \frac{K_{pH} \cdot k_{obs}}{1 + K_{pH} \cdot [A]_T} \qquad (3)$$

$$k_2 = \frac{k_{obs}}{1 + K_{pH} \cdot [A]_T} \qquad (4)$$

where K_{pH} is the apparent, pH-dependent equilibrium constant for Schiff's base formation at the required pH. The K_{pH} value can be obtained from the data and equations given in the preceding paper[1] [*i.e.* by using eqns. (3) and (13) with the parameters of Table 2 and the activity coefficients calculated for $\Gamma = 0.01$].

Therefore, instead of analysing the $[A]_T$-dependence of k_{obs} at constant pH (as is usually done), we have studied the pH-dependence of k_{obs} at constant amine concentration (see Fig. 1). Provided that eqn. (2) holds true, the k_1/pH and k_2/pH profiles can be obtained from a single k_{obs}/pH profile by using eqns. (3) and (4). However, two possible causes of deviation from eqn. (2) are: (1) general catalysis of the reaction by the amine, which would lead[2] to a quadratic dependence of k_{obs} on $[A]_T$; (2) accumulation of the carbinolamine intermediate, which would produce[3] a levelling of the k_{obs} value at high amine concentrations. To rule out these possibilities, we obtained (for each water–dioxane mixture) three k_{obs}/pH profiles, corres-

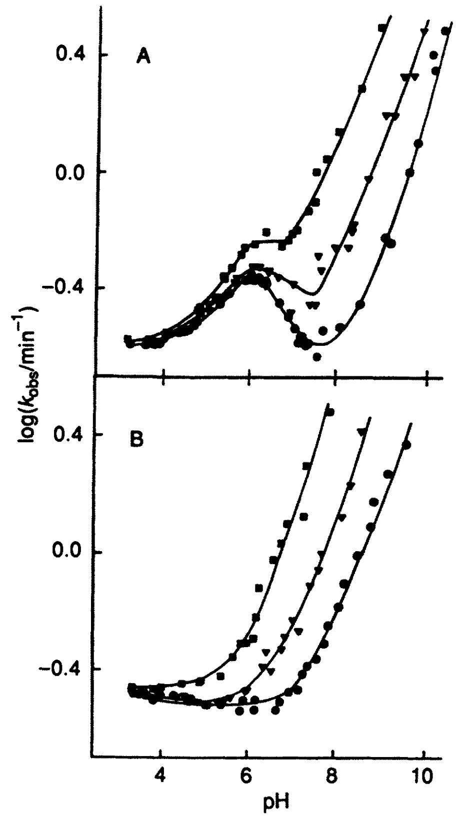


Fig. 1 log k_{obs} *versus* pH profiles in water (A) and water–dioxane, 60:40 v/v (B). The different types of symbols refer to the hexylamine concentrations: (●), 10^{-3}; (▲), 2.5×10^{-3}; (■), 9.5×10^{-3} mol dm^{-3}. The lines are shown to guide the eye and have no theoretical significance.

ponding to three different amine concentrations (10^{-3}, 2.5×10^{-3} and 9.5×10^{-3} mol dm^{-3}). An excellent agreement was found between the k_1 values (and the k_2 values) derived from the three profiles (Fig. 2), which indicates that eqn. (2) is valid in our case. The same conclusion was reached in previous kinetic studies on Schiff's base formation from PLP in aqueous solution[4,5] and water–ethanol mixtures.[6]

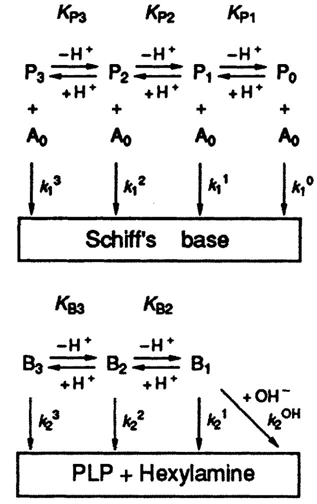
Analysis of the pH-*Dependence of* k_1 *and* k_2.—The pH-dependent rate constants of formation and hydrolysis of the Schiff's base can be analysed in terms of the rate constants for the individual ionic species,[4–7] as shown in Scheme 1. From this model eqns. (5) and (6) can be easily derived.

$$k_1 = X_{A0} \cdot \sum_{i=0}^{3} k_1^i \cdot X_{Pi} \tag{5}$$

$$k_2 = k_2^{OH} \cdot X_{B1} \cdot [OH^-] + \sum_{i=1}^{3} k_2^i \cdot X_{Bi} \tag{6}$$

The molar fractions (X_{Pi}, X_{Bi}, X_{A0}) in eqns. (5) and (6) can be easily calculated from the equations and data given in the preceding paper[1] [*i.e.* by using eqns. (3) and (4) with the parameters of Table 2 and the activity coefficients calculated for $\Gamma = 0.01$]. Thus, the individual rate constants can be derived from the least-squares fitting of eqns. (5) and (6) to the k_1/pH and k_2/pH profiles, respectively. The values obtained for log k_1^i, log k_2^i and log k_2^{OH} are shown in Table 1; standard errors (calculated according to Bevington)[8] were about 0.1 or smaller, indicating that these parameters are accurately determined from the fitting.

Solvent and Intramolecular Catalysis Effects on the Rate



Scheme 1

Table 1 Optimum values of kinetic constants obtained in the fitting

	Dioxane volume fraction			
Constant	0	0.2	0.4	0.6
log k_1^0	3.75	3.12	*a*	2.96
log k_1^1	4.64	4.72	4.46	4.11
log k_1^2	5.97	5.55	5.29	5.11
log k_1^3	7.77	7.23	6.81	7.31
log k_2^1	−0.958	−0.907	−0.880	−1.49
log k_2^2	−0.242	−0.390	−0.433	−0.870
log k_2^3	−0.602	−0.587	−0.506	−0.329
log k_2^{OH}	3.26	3.15	*a*	3.85

[a] The fitting was not sensitive to the value of this parameter.

Constants of Schiff's Base Formation.—The most significant feature observed in Table 1 is the fact that, in all solvent conditions, the log k_1^i values increase (by several orders of magnitude) with the value of *i*, *i.e.* with the number of protons bound to the PLP species. This effect has also been found in previous studies[4–7] and has been attributed to the existence of intramolecular acid catalysis. In fact, Brønsted-like plots of log k_1^i *versus* pK_{Pi} (the pK of the catalyst) have been reported to be linear[4,6,7] with slopes in the range −0.5 to −0.8.

The log k_1^i *versus* pK_{Pi} plots previously reported in the literature[4,6,7] include data obtained in a single solvent mixture; by contrast, the plot shown in Fig. 3A includes all the log k_1^i values given in Table 1 (corresponding to different water–dioxane mixtures). Note that the acceptable correlation seen in Fig. 3A describes both the effect of general acid catalysis and the solvent effect on the k_1^i values.

To the best of our knowledge, only two studies dealing with solvent effects on the individual rate constants of Schiff's base formation from Vitamin B_6 substances have been previously reported in the literature: PLP *plus* hexylamine in water–ethanol mixtures, $\Gamma = 0.1$ (Garcia del Vado *et al.*)[6] and 5'-deoxypyridoxal *plus* hexylamine in water–dioxane, $\Gamma = 0.1$ (Vazquez *et al.*).[9] However, the general log k_1^i *versus* pK_{Pi} plot (including all solvent conditions) for these two systems shows poor correlations (see Figs. 3C and 3E). We have found, nevertheless, that a modified Brønsted-like plot of log k_1^i *versus* (pK_{Pi} − pK_{A1}) yields acceptable correlations (Figs. 3B, 3D and 3F) for the two systems mentioned above, as well as for our data (Table 1). Fig. 4 shows the pK values of the hexylammonium ion (pK_{A1}) employed to construct the modified plots; the fact that good correlations are observed for our data in both plots, the original (*versus* pK_{Pi}) and the modified one (*versus* pK_{Pi} − pK_{A1}), is a result of the small solvent effect on

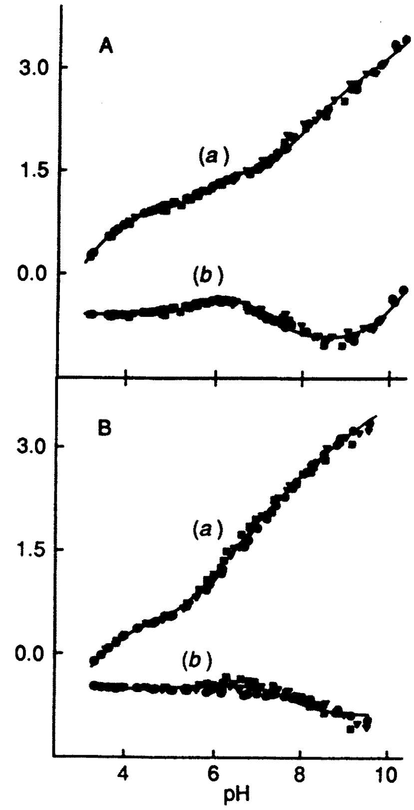
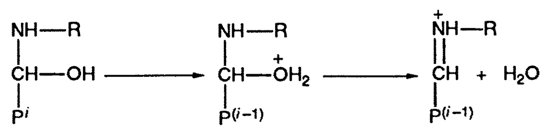


Fig. 2 $\log k_1$/pH (*a*) and $\log k_2$/pH (*b*) profiles in water (A) and water–dioxane, 60:40 v/v (B). Each rate constant was calculated from a single k_{obs} value by using eqns. (3) and (4). The different types of symbols refer to the hexylamine concentration at which the k_{obs} value was measured (see legend to Fig. 1). (——), theoretical lines calculated from eqns. (5) and (6) with the parameters given in Table 1.

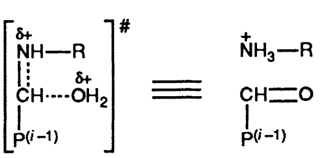
the $\mathrm{p}K_{\mathrm{A1}}$ value under our conditions (water–dioxane, $\Gamma = 0.01$).

It remains now to make some suggestions as to the origin of the $\log k_1^i$ *versus* $(\mathrm{p}K_{\mathrm{P}i} - \mathrm{p}K_{\mathrm{A1}})$ correlation. We believe that the following discussion, although speculative, deserves attention. It is based on the approach employed by Brault *et al.*[10] to explain solvent effects on the rate of hydrolysis of α,β-unsaturated Schiff's bases; that is, we shall propose a reasonable structure for the transition state and we shall describe its interaction with the solvent in terms of the interactions of the reactants in given ionization states.

Schiff's base formation takes place according to a two-step mechanism involving an intermediate carbinolamine.[11] It is generally accepted[4–7,9,12] that carbinolamine dehydration is the rate-determining step in the formation of Schiff's bases of Vitamin B_6 substances and we shall proceed under this assumption. When catalysed by the hydrogen ion, carbinolamine dehydration requires the formation of an oxonium ion as an intermediate;[11] in the case of Schiff's base formation from PLP, the proton comes from the pyridoxal phosphate moiety (intramolecular acid catalysis) and the dehydration process could be depicted as in Scheme 2 where P^i represents the pyridoxal phosphate moiety with *i* bound protons. The transition state (**1**) would then possess[11] partial positive charges on the oxonium oxygen and the imine nitrogen.* We shall assume that, in the transition state, most of the positive charge is already on the imine nitrogen. Then, from the point of view of the interactions with the solvent, it does not appear unreasonable to represent the transition state by a protonated amine molecule *plus* a PLP molecule with *i* − 1 bound protons:



Scheme 2



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The activation Gibbs energy ($\Delta G^{\ddagger}$) for the reaction $\mathrm{P}_i + \mathrm{A}_0 \longrightarrow$ Schiff's base, may be expressed as the sum of two terms [eqn. (7)] where $\Delta G_0^{\ddagger}$ is a constant, while $\Delta G_s^{\ddagger}$ is sensitive to

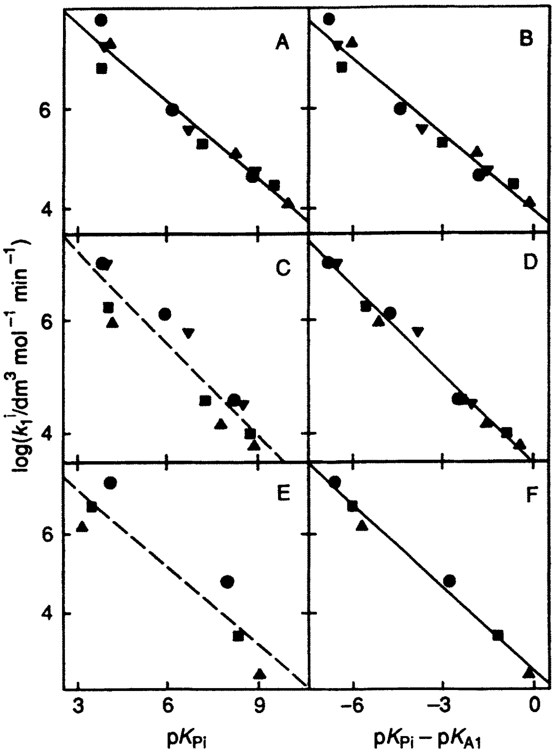

Fig. 3 Plots of $\log k_1^i$ *versus* $\mathrm{p}K_{\mathrm{P}i}$ (A, C and E) and *versus* $(\mathrm{p}K_{\mathrm{P}i} - \mathrm{p}K_{\mathrm{A1}})$ (B, D and F). The data correspond to the following systems and conditions. A and B: PLP *plus* hexylamine in water–dioxane, $\Gamma = 0.01$ (this work); the dioxane volume fractions are 0 (●), 0.2 (▼), 0.4 (■) and 0.6 (▲). C and D: PLP *plus* hexylamine in water–ethanol, $\Gamma = 0.1$ (Garcia del Vado *et al.*);[6] the ethanol volume fractions are: 0 (●), 0.3 (▼), 0.6 (■) and 0.8 (▲). E and F: 5′-deoxypyridoxal *plus* hexylamine in water–dioxane, $\Gamma = 0.1$ (Vazquez *et al.*);[9] the dioxane volume fractions are: 0 (●), 0.5 (■) and 0.7 (▲).

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* The transition state **1** resembles that for carbinolamine dehydration subject to specific acid catalysis,[11] the difference being that the proton does not come from the solvent, but from the pyridoxal phosphate moiety. Oxonium ions are unstable;[11] in the case of Schiff's base formation from PLP, the oxonium ion could be stabilized by the interaction with the ionized 3-hydroxy group. This would be consistent with the role for the 3-hydroxy group proposed by Auld and Bruice[13] and Sanchez-Ruiz *et al.*,[4] and with the fact that 3-*O*-methylpyridoxal phosphate reacts with amines at a slower rate.[14] Note that, if we accept this role for the 3-hydroxy group, the transition state would resemble that for carbinolamine dehydration subject to general acid catalysis (see ref. 11, page 240). This point, however, makes no difference to the derivation given in the text.

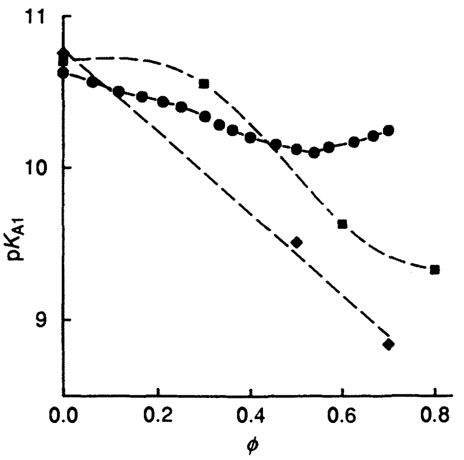

Fig. 4 Effect of the organic cosolvent volume fraction (φ) on the pK of the hexylammonium ion. (●), water–dioxane mixtures, $\Gamma = 0.01$ (Llor *et al.*);[15] (■), water–ethanol mixtures, $\Gamma = 0.1$ (Garcia del Vado *et al.*);[6] (◆), water–dioxane mixtures, $\Gamma = 0.1$ (Vazquez *et al.*).[9] The lines are shown to guide the eye and have no theoretical significance.

$$\Delta G^{\ddagger} = \Delta G_0^{\ddagger} + \Delta G_S^{\ddagger} \qquad (7)$$

both, solvent and intramolecular catalysis effects.

Given that the reactants are P_i *plus* A_0 and the transition state has been assumed to be equivalent to P_{i-1} *plus* A_1, $\Delta G_S^{\ddagger}$ may be assumed to be equal to the Gibbs energy change for the following proton transfer process: $P_i + A_0 \longrightarrow P_{i-1} + A_1$; *i.e.* $\Delta G_S^{\ddagger} = RT \times (\ln 10) \times (pK_{Pi} - pK_{A1})$. Then, using the theory of the absolute reaction rates, it is straightforward to arrive at eqn. (8) which explains the correlation observed in Figs. 3B, 3D and 3E.

$$\log k_1^i = \text{constant} - (pK_{Pi} - pK_{A1}) \qquad (8)$$

It must be noted that eqn. (8) has been derived assuming that a positive charge is fully developed on the imine nitrogen in the transition state and predicts, therefore, a value of -1 for the slope of the $\log k_1^i$ *versus* ($pK_{Pi} - pK_{A1}$) plot. Smaller values (in absolute value) are to be expected if only a partial positive charge is developed on the imine nitrogen; in fact, the experimental slopes are: -0.51 (PLP *plus* hexylamine in water–dioxane, $\Gamma = 0.01$; Fig. 3B), -0.52 (PLP *plus* hexylamine in water–ethanol, $\Gamma = 0.1$; Fig. 3D) and -0.67 (deoxypyridoxal *plus* hexylamine in water–dioxane, $\Gamma = 0.1$; Fig. 3F).

It is also interesting that the slope for the two PLP *plus* amine systems is smaller (in absolute value) than that for the deoxypyridoxal *plus* amine system. This might reflect the electrostatic interaction of the positive charge in the transition state with the additional negative charges of the 5′-phosphate group of PLP.

To summarize, we have found that solvent and intramolecular acid catalysis effects on the rates of Schiff's base formation can be acceptably described on the basis of a single $\log k_1^i$ *versus* ($pK_{Pi} - pK_{A1}$) plot. This correlation, however, is based on the analysis of only three Vitamin B_6 substance–amine–solvent systems. A comparative study of many related systems (including several Vitamin B_6 substances, different amines and different types of mixed solvents) is required to ascertain the general validity of the correlation (or to propose modified correlations). We believe that the experimental approaches employed in this and the preceding paper[1] make this comparative study feasible.

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