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Determination of the rates of formation and hydrolysis of the Schiff bases formed by pyridoxal 5′-phosphate and hydrazinic compounds

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Abstract

The macroscopic rate constants of formation (k_1) and hydrolysis (k_2) for the reactions of pyridoxal 5′-phosphate (PLP) with hydrazine (PLP-HY system), carbidopa (α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)propionic acid, PLP-CD system), hydralazine (1-hydrazinophthalazine, PLP-HL system) and isoniazid (4-pyridinecarboxylic acid hydrazide, PLP-ISO system) were fitted to a kinetic scheme that considers the different ionic species present in the medium, their protonation constants, and their individual rates of formation (k_1^i) and hydrolysis (k_2^i). The results obtained for the molecules bearing the hydrazine group are compared with those for the reactions of PLP with *n*-hexylamine (PLP-NHA system) and poly-L-lysine (PLP-LYS system). Some structural effects on the individual rate constants are also examined.

Keywords: Schiff base; PLP; Hydrazine; Hydralazine; Isoniazid; Carbidopa

1. Introduction

Pyridoxal 5′-phosphate (PLP) is an indispensable cofactor for a number of enzymes involved in amino acid metabolism including transaminases and decarboxylases [1–3]. PLP binds to the enzyme via a carbinolamine formed between the carbonyl group in PLP and the ε-amino group in an L-lysine residue of the polypeptide chain [1,4]. The carbinolamine releases one water molecule and becomes a Schiff base (an imine) in an acid-catalysed process [4,5]. The first process undergone by PLP-dependent enzymes is the transimination reaction, viz. the conversion of the PLP-lysine imine into a PLP-substrate imine [6].

Some hydrazinic derivatives (Scheme 1) are widely used as therapeutical chemicals. Very often, hydrazinic compounds behave as inhibitors for PLP-dependent enzymes in reacting

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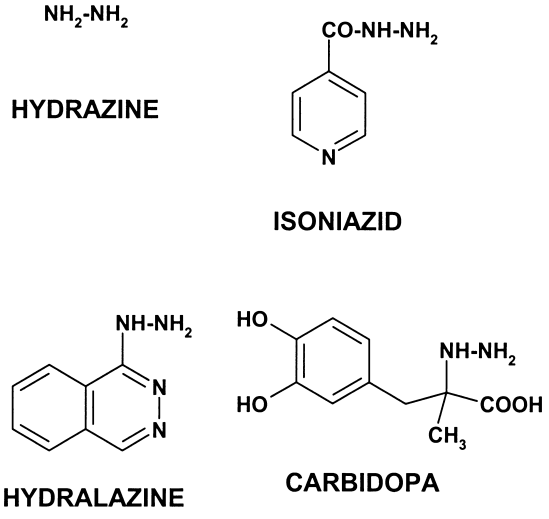


Scheme 1.

with PLP to form Schiff bases that inhibit the enzymatic process [7,8].

Recently, our group reported the kinetic results [9,10] for the reactions of PLP with the compounds in Scheme 1. The reactivity sequence found over a broad pH range was isoniazid > hydrazine > carbidopa > hydralazine.

This paper discusses the kinetic results obtained in the reaction of PLP with hydrazine (PLP-HY system), carbidopa (α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)propionic acid, PLP-CD system), hydralazine (1-hydrazinophthalazine, PLP-HL system) and isoniazid (4-pyridinecarboxylic acid hydrazide, PLP-ISO system) as a function of the different ionic species present in the medium, their protonation constants, and the individual rate constants of formation and hydrolysis (see Scheme 2). The results thus obtained are compared with those of the reactions of PLP with *n*-hexylamine (PLP-NHA system) [11] and poly-L-lysine (PLP-LYS system) [12].

2. Materials and methods

Hydrazine sulphate, 5′-pyridoxal phosphate, and all other chemicals used in the buffer solutions (acetate, phosphate, and carbonate) were of reagent grade and purchased from Merck. Carbidopa, hydralazine, and isoniazid were from Laboratorio Chile and were used as purchased.

Acetate, phosphate, and carbonate buffers were used over appropriate pH ranges. The ionic strength was kept constant and equal to 0.1 mol/l. PLP and hydrazinic solutions were prepared daily in appropriate buffers and were stored in the dark. The PLP concentration in the solutions was determined spectrophotometrically by dilution with 0.1 mol/l HCl [13].

pH measurements were made with a CRISON pH-meter furnished with a Metrohm EA120 electrode or a Radiometer PHM-62 with GK-2401C electrode that were previously calibrated by using aq. buffer solutions at 25 ± 0.05°C.

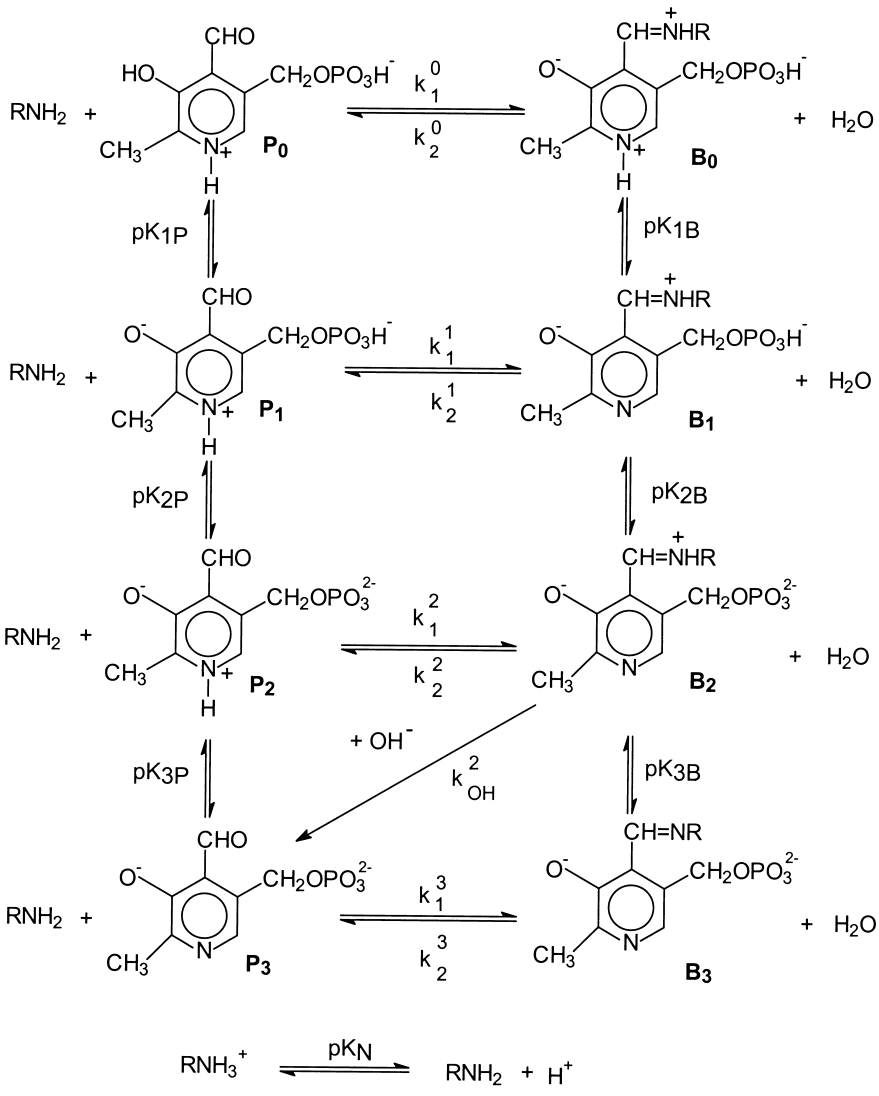
^{13}C NMR spectra were recorded on a Bruker AC-200 spectrophotometer, using 0.1 mol/l solutions of PLP containing excess hydrazine in a 80:20 v/v H_2O/D_2O mixture and dioxane as internal standard.

The kinetics of formation of the Schiff bases in the 3.50–11.50 pH range, were monitored by measuring the variation of the absorption at 340 nm for hydrazine, at 395 nm for hydralazine, in the range 295–370 nm for isoniazid, and 400–425 nm for carbidopa, using a Perkin-Elmer Lambda 3 or a Uvikon 941-Plus spectrophotometer furnished with thermostated cells of 1-cm light path. The observed pseudo-first-order (k_{obs}) was determined by the infinite method.

The overall reaction between the carbonyl group and an amine can be schematized as follows:

$$R^1\text{–CHO} + NH_2\text{–}R^2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} R^1\text{–CH=N–}R^2 + H_2O$$

where k_1 and k_2 are the macroscopic or overall rate constants of formation and hydrolysis, respectively, of the Schiff base. The procedure used to calculate these two rate constants from the experimental k_{obs} values is described in detail elsewhere [6]. The ratio between both rate



Scheme 2.

constants is the equilibrium constant ($K_{pH} = k_1/k_2$).

3. Results and discussion

In previous work [9,10], we reported global kinetic data for the reactions of PLP with the compounds in Scheme 1.

The overall rate constants for formation and hydrolysis of the Schiff base can be described in terms of the individual rate constants for the different chemical species present in the medium as a function of pH [11–14]. For the PLP system, the ionic species existing in solution over the pH range studied are given in Scheme 2 (P_3, P_2, P_1, P_0) with subscripts (3, 2, 1, 0) indicating the number of net negative charges on the molecule. P_3 must be identified with the completely deprotonated aldehyde molecule, which bears three net negative charges: two on the phosphate group and one on the 3′-hydroxy group. Successive addition of proton gives the P_2, P_1 and P_0 forms and pK_{3P}, pK_{2P} and pK_{1P}

are the different pK values that relate them [15,16]. For the Schiff base, molecule must be considered four different imine ionic species: B_3, B_2, B_1 and B_0, and pK_{3B}, pK_{2B}, and pK_{1B}, the pK values that relate them. Their structural formulae have been assigned according to the literature, which exists for this sort of compound [17–19]. K_N is the deprotonation equilibrium constant for the $-NH_3^+$ group in hydrazine or its derivative.

Thus, k_1^i and k_2^i (with $i = 0$, 1, 2 or 3) are the individual rates of formation of the Schiff base and those of its hydrolysis by water, respectively, and k_{OH}^2 is the rate constant of hydrolysis of species B_2 by OH^- ions. The equilibrium formation constant of the Schiff base in highly alkaline media is $K_M = k_1^3/k_2^3$.

The rate of formation of the Schiff base can be expressed as

$$V_1 = [RNH_2] \sum_{i=0,1,2,3} k_1^i [P_i] = k_1 [RNH_2]_T [PLP]_T \quad (1)$$

where subscript T denotes all species as a whole.

On the other hand, the rate of hydrolysis of the Schiff base can be expressed as

$$V_2 = k_{OH}^2 [B_2][OH^-] + \sum_{i=0,1,2,3} k_2^i [B_i] = k_2 [\text{Schiff Base}]_T. \quad (2)$$

The equilibrium constant will be given by

$$K_{pH} = [\text{Schiff Base}]_T / ([RNH_2]_T [PLP]_T). \quad (3)$$

Taking into account the equilibria of Scheme 2, Eqs. (1) and (2) can be readily transformed into the following

$$k_1 = \frac{k_1^3 + \frac{k_1^2 a}{K_{3P}} + \frac{k_1^1 a^2}{K_{3P}K_{2P}} + \frac{k_1^0 a^3}{K_{3P}K_{2P}K_{1P}}}{\left(1 + \frac{a}{K_N}\right)\left(1 + \frac{a}{K_{3P}} + \frac{a^2}{K_{3P}K_{2P}} + \frac{a^3}{K_{3P}K_{2P}K_{1P}}\right)} \quad (4)$$

$$k_2 = \frac{k_{OH} + \frac{k_2^2 a}{K_{3B}} + \frac{k_2^1 a^2}{K_{3B}K_{2B}} + \frac{k_2^0 a^3}{K_{3B}K_{2B}K_{1B}}}{1 + \frac{a}{K_{3B}} + \frac{a^2}{K_{3B}K_{2B}} + \frac{a^3}{K_{3B}K_{2B}K_{1B}}} \quad (5)$$

where $a = 10^{-pH}$, $k_{OH} = k_2^3 + (k_{OH}^2 K_w / K_{3B})$ and K_W is the ionic product of water.

The experimental results for k_1 and k_2 were simultaneously fitted to Eqs. (4) and (5) using a non-linear regression method that minimizes the following functions:

$$U_i = \sum (\log k_{i,e} - \log k_{i,t})^2 \qquad i = 1 \text{ or } 2$$

where subscripts e and t denote experimental and theoretical data, respectively.

The values of the protonation constants of PLP, hydrazine, and its derivatives were obtained from the literature [9,10,15]. The deprotonation constants for the Schiff bases required for the initial fitting were estimated from reported data for related systems [20].

Table 1 gives the pK_a values and those for the individual rate constants k_1^i and k_2^i obtained in the fitting. It also gives the pK_a values and the individual rate constants for the Schiff bases of PLP with *n*-hexylamine (PLP-NHA system) [11] and poly-L-lysine (PLP-LYS system) [12].

As can be seen, the k_1^i values for the PLP-HY, PLP-CD, and PLP-HL systems were smaller than those for the PLP-NHA system, consistent with the reportedly decreased reactivity of the PLP-HY system [9]. However, the individual rate constants k_1^i (with $i = 1$, 2 or 3) for the PLP-ISO system were very similar to those for the PLP-NHA system; also, k_1^0 was about 100 times greater than for the PLP-NHA system. Consequently, the decreased reactivity of the hydrazine group in isoniazid was offset by the effect of some groups in its molecule that increased its reactivity. Thus, the k_1^i values for the hydrazine compounds followed the same

Table 1
Best kinetic constants and pK_{iB} values obtained in the fitting of experimental values of k_1 and k_2 to Scheme 2, and those corresponding to PLP-NHA [11] and PLP-LYS [12] systems (k_1 in l mol^{-1} min^{-1}, k_2 in min^{-1})

	PLP-ISO	PLP-HY	PLP-CD	PLP-HL	PLP-NHA	PLP-LYS
Log k_1^0	10.08	6.87	6.51	5.79	7–7.7[a]	8.74[b]
Log k_1^1	6.21	5.17	4.89	3.90	5.96[a]	6.13[b]
Log k_1^2	4.52	3.41	3.32	2.66	4.60[a]	5.45[b]
Log k_1^3	3.51	2.42	1.90	1.65	3.62[a]	3.53[b]
pK_{1P}	3.46[c]	3.46[c]	3.46[c]	3.46[c]	3.60[a]	3.46[b]
pK_{2P}	6.02[c]	6.02[c]	6.02[c]	6.02[c]	5.95[a]	6.02[b]
pK_{3P}	8.22[c]	8.22[c]	8.22[c]	8.22[c]	8.21[a]	8.22[b]
Log k_2^0	−3.65	−2.97	−4.63	−0.97	−0.57[a]	−0.17[b]
Log k_2^1	−2.80	−0.16	−0.04	−1.22	−0.33[a]	−2.29[b]
Log k_2^2	−2.10	−1.02	−1.32	< −3.0	−1.18[a]	−0.42[b]
Log k_{OH}	0.43	−0.60	−3.85	< −3.0	0.84[a]	1.04[b]
pK_{1B}	6.25	6.50	6.97	6.43	5.00[a]	6.62[b]
pK_{2B}	8.00	8.00	7.15	8.23	6.60[a]	7.74[b]
pK_{3B}	10.80	11.50	11.35	–	11.21[a]	10.92[b]
pK_N	11.10[d]	8.23[d]	7.20[d]	7.25[d]	10.70[a]	10.03[b]

[a] Ref. [11].
[b] Ref. [12].
[c] Calorimetric data [15].
[d] Potentiometric data [9,10].

sequence as for their macroscopic counterparts, viz.

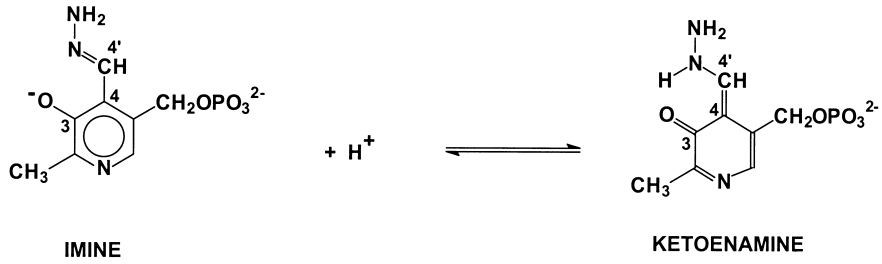
isoniazid > hydrazine > carbidopa > hydralazine

which also coincided with the sequence of the pK_a values for the $-NH_2$ group in the hydrazinic compounds. Despite the similarity of the pK_a values for hydralazine and carbidopa, the k_1^i values for the PLP-HL system were smaller than those for the PLP-CD system. These reactivity differences were observed throughout the pH range studied, so it held for all P_i species, whether protonated or otherwise; this behaviour is thus probably related to a sterically hindered attack of the amine group in hydralazine on the carbonyl group of PLP resulting from the presence of the polycyclic aromatic ring bonded to the hydrazine group in hydralazine.

The PLP-HY, PLP-CD, and PLP-HL systems gave linear Bronsted plots with slopes of $\alpha = 0.74$, consistent with the presence of intramolecular acid catalysis involving all protonable groups in the PLP molecule. These α values are consistent with those for other PLP systems and various molecules bearing poly-L-lysine ($\alpha = 0.77$) [12], *n*-hexylamine ($\alpha = 0.64$–0.68) [11] and L-alanine amino groups ($\alpha = 0.80$) [14].

The PLP-ISO system gives a non-linear Bronsted plot because its log k_1^0 value, 10.08, is much greater than expected in relation to other hydrazinic compounds (a straight line drawn from the k_1^1 and k_1^2 values only exhibits a slope of $\alpha = 0.74$ extrapolation of which yields a value about 8 for log k_1^0). This positive deviation can be ascribed to the presence of intramolecular acid catalysis, which adds to that exerted by the phenol group in PLP ($pK_1 = 3.46$) and is promoted by the protonated nitrogen atom in isoniazid ($pK_a = 3.61$) [10].

In order to verify pK_{3B} value obtained in the fitting for the Schiff base of PLP and hydrazine, the ^{13}C NMR spectra for solutions containing a large excess of hydrazine relative to PLP were recorded over the pH range 8.5–12.4 where the formation equilibrium of a Schiff base is strongly displaced to the base as no signals for PLP are observed in the spectrum [21,22]. However, the spectrum thus recorded contained more signals than expected (10 in all) but not that for carbinolamine (at 66–69 ppm) [21], the presence of which can thus be excluded. The reason



Scheme 3.

probably lies in the presence of the acid–base equilibrium between the imine form of B_3 and the ketoenamine of B_2 (Scheme 3), which is supported by the presence of two signals of the methyl group, both in H NMR and ^{13}C NMR spectra, and the presence of the iminic C–H of B_3 and the vinilic C–H of B_2 in the H NMR spectra. As can be seen from Fig. 1, an important shift (132–138 ppm) of one of the signals, probably due to C-4′, permit us to determine the $pK = 10.9$, corresponding to the protonation in the imine nitrogen and is consistent with the pK_{3B} value obtained by fitting the experimental data (Table 1). The H NMR spectra obtained a pH = 10.9, shows practically the same integral value for both methyl signals, confirming the obtained pK.

In recent work, we ascribed the differences in the pH-dependence of log k_2 for the PLP-HY, PLP-CD, and PLP-HL systems, which exhibit a maximum near pH 7, from that for the PLP-ISO system, which exhibits a minimum in that pH region, to the differences in the pK_a values for their respective Schiff bases resulting from those in pK_a for the amino group in the hydrazinic compounds [10]. The pK_{iB} values obtained in this work for the Schiff bases are very similar (see Table 1), so they cannot be used to justify the behaviour observed. Although the rate constants of hydrolysis, k_2^i (with $i = 0$, 1 or 2) are small, their differences are consistent with such a behaviour; also, the minimum in the log k_2 vs. pH plot for the PLP-ISO system can be ascribed to the large value of k_{OH}.

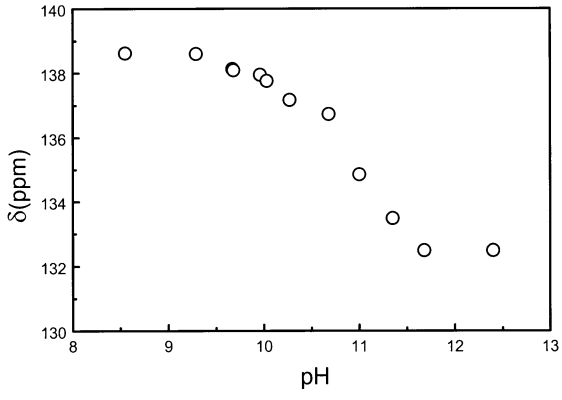


Fig. 1. pH dependence of the C-4′ signal of the ^{13}C NMR of Schiff base from PLP and hydrazine.

A comparison of the k_1^i values obtained for the hydrazinic systems and those for PLP-LYS with the exception of k_1^3 for isoniazid for the above described reasons reveals that the Schiff bases of PLP-LYS are formed the fastest but also that these are more readily hydrolysed than those of the hydrazinic systems (see k_2^i values in Table 1); as a result, these systems exhibit similar stability over the physiological pH range. On the other hand, in acid and alkaline media, the hydrazinic systems are more stable.

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