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Kinetic study of the reaction of pyridoxal 5′-phosphate with hydrazine

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Abstract

The kinetics of the reaction between pyridoxal 5′-phosphate (PLP) and hydrazine in aqueous solution at a variable pH and a constant strength of 0.1 M was studied spectrophotometrically. The rate constants of formation and hydrolysis of the resulting Schiff base and its stability were also determined in a wide range of pH. A comparison of the formation rate constants with those for the models of PLP with *n*-hexylamine and with poly-L-lysine revealed that hydrazine formed Schiff base more quickly than lysine below pH 7 and than *n*-hexylamine below pH 8. The reactivity shows the sequence poly-L-lysine > *n*-hexylamine > hydrazine in whole studied range of pH. Schiff bases formed by hydrazine with PLP are more stable than the ones formed by *n*-hexylamine in the pH range studied and more stable than the formed by poly-L-lysine at pH < 7.0. © 1998 Elsevier Science B.V.

Keywords: Schiff base; PLP; Hydrazine

1. Introduction

Pyridoxal 5′-phosphate (PLP) is the coenzyme of a wide range of different reactions [1–3] and plays an important role in the metabolic reactions of aminoacids by forming a carbinolamine intermediate by bonding its carbonyl group to the ε-amino group of an L-lysine residue of the polypeptide chain [1,4]. The carbinolamine loses a molecule of water to yield the Schiff bases in an acid catalyzed process [4,5].

In attempts to obtain information about the PLP dependent enzymatic process, several reactions models have been studied, using different amine groups as primary amines [6–11], aminoacids [11–13] and polypeptides [14–17]. Pyridoxal (PL) and 5′-deoxypyridoxal (DPL) have been used as model of PLP [10,12,18,19]. In order to emulate the more or less hydrophobic environment of the enzymatic process, the reactions models have been studied in aqueous (water–ethanol, water–dioxane) [20–23] and non-aqueous media (pure and mixed solvents) [24–26]. These studies have provided many information about the formation and hydrolysis of Schiff bases and also about the stability.

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It is interesting to take into account that many hydrazine ($H_2N–NH_2$) derivatives are used as therapeutic chemicals, i.e., hydralazine (1-hydrazinophthalazine), isoniazid (4-pyridinecarboxylic acid hydrazide) or as protectors of medicines as carbidopa (α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)propionic acid). It is known that in most cases, hydrazine compounds behave as inhibitory compounds towards PLP-dependent enzymes because they react with PLP forming Schiff bases competing with the enzymatic process [27,28]. The first step of all PLP-dependent enzymes is a transimination reaction, namely, the conversion of the PLP-lysine imine in the substrate-PLP imine [29].

In this paper, we report as a model of hydrazinic compounds the kinetics results of the reaction of pyridoxal 5′-phosphate with hydrazine (PLP-HY system) and the results are compared with those of pyridoxal 5′-phosphate with *n*-hexylamine (PLP-HEX system) and with poly-L-lysine (PLP-LYS system).

2. Materials and methods

Hydrazine sulfate, pyridoxal 5′-phosphate and all the other chemicals were reagent grade and purchased from Merck.

Acetate, phosphate and carbonate buffers were used in appropriate pH ranges. The ionic strength was kept constant and equal to 0.1 mol/l.

PLP and hydrazine solutions were prepared daily in appropriate buffers and were stored in the dark. The exact concentrations of PLP were determined by dilution [30] with 0.1 mol/l HCl.

The kinetics of formation of the Schiff bases was monitored by measuring the variation of the absorption at 340 nm as a function of pH using a Uvikon 941-Plus spectrophotometer furnished with thermostated cells of 1 cm lightpath. The reaction was started by adding a known volume of PLP buffered solution to prethermostated hydrazine solutions at 25 ± 0.05°C. The observed pseudo-first-order rate constants (k_{obs}) were determined by infinite method. The difference between the initial and final pH in the reaction cell never exceeded 0.03 pH units. The pH measurements were made by using a Crison pH-meter furnished with a Metrohm EA120 electrode that was previously calibrated with aqueous buffers at 25 ± 0.05°C.

The overall reaction between an aldehyde and an amine can be schematized as follows:

$$R_1–CHO + NH_2–R_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} R_1–CH=N–R_2 + H_2O,$$

where k_1 and k_2 are the overall rate constants of formation and hydrolysis of the Schiff base, respectively. The procedure used to calculate these two constants from the k_{obs} values is described in detail elsewhere [9]. The ratio between them represent the equilibrium constant ($K_{pH} = k_1/k_2$). Nucleophilic rate constants (k_N) were obtained from slopes of linear plots k_{obs} vs. free amine concentration or by dividing the k_1 values by their corresponding free amine molar fraction.

3. Results and discussion

Figs. 1–3 show the experimental results, logarithm of the rate of formation (k_1), of the rate of hydrolysis (k_2) and of the equilibrium con-

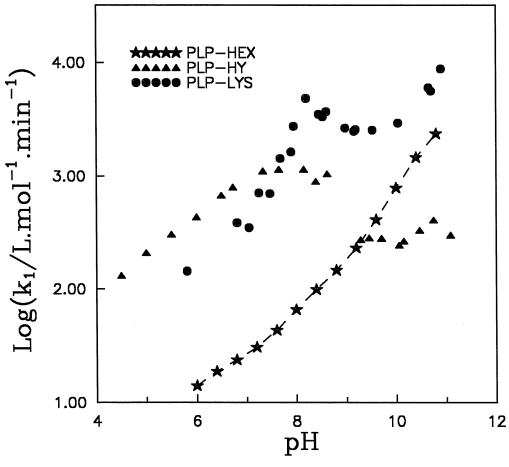


Fig. 1. Plot of $\log k_1$ vs pH for different adducts of PLP with *n*-hexylamine (★) [10], poly-L-lysine (●) [14] and hydrazine (▲).

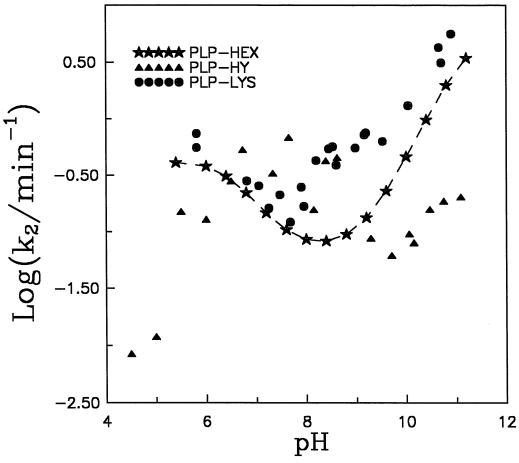


Fig. 2. Plot of $\log k_2$ vs pH for different adducts of PLP with *n*-hexylamine (★) [10], poly-L-lysine (●) [14] and hydrazine (▲).

stant (K_{pH}) as a function of pH, obtained for the Schiff bases of PLP with hydrazine. The figures also include the results obtained for the Schiff bases of PLP-HEX and PLP-LYS systems at the same temperature and ionic strength.

As can be observed from Fig. 1, the formation rate constants of PLP-HY system increases with pH at acid and neutral media, as expected. Nevertheless, a maximum value in k_1 is obtained around pH 8. The k_1 values for PLP-HY system are greater than the other systems in acid and moderate basic media (below pH 8 and pH 9 for PLP-LYS and PLP-HEX, respectively), i.e., at pH = 6 the k_1 value for PLP-HY is ten times greater than PLP-HEX system and twice for the PLP-LYS system.

This results suggest a greater reactivity of hydrazine than *n*-hexylamine and poly-L-lysine toward PLP. Nevertheless, this result can also be due to the different pK of nucleophiles (pK = 10.7 for *n*-hexylamine, pK = 10.03 for poly-L-lysine and pK = 8.23 for hydrazine).

In order to obtain an independence of pK of amine and to give a more quantitative comparison, the k_N values for the three systems were calculated. The behavior of $\log k_N$ in function of pH for the three systems (Fig. 4) shows the sequence

poly-L-lysine > *n*-hexylamine > hydrazine,

in the whole studied pH range, reveling a lesser reactivity for hydrazine. Since the mechanistic point of view, it is known that Schiff bases (SB) are formed via a two-step mechanism involving the initial formation of a carbinolamine (CA) by attack of an amine (AM) to the carbonylic group of an aldehyde (ALD), followed by dehydratation [4] (Scheme 1). If $K_b = k_b/k_{-b}$ is defined as the equilibrium formation constant of carbinolamine, the following expression will hold for the observed rate constant:

$$k_{obs} = \frac{k_c \cdot K_b \cdot [AM]}{(1 + K_b \cdot [AM])} + k_{-c}.$$

The linearity of plots k_{obs} against [AM] permit to assume that $K_b \cdot [AM] < 1$, therefore the overall rate constant of formation is $k_1 = k_c \cdot K_b \cdot [AM]$, and involves the carbinolamine formation equilibrium constant. Fig. 4 shows that the k_N increases as pH decreases, for the three showed systems, in accord with an intramolecular catalytic process [31], therefore it is possible to conclude that in the PLP-HY system the carbinolamine dehydratation is the rate determining step, as in most of the PLP and analogues studied reactions [6,9,10,12,16,18,19].

Above pH 10 the $\log k_N$ value for PLP-HEX system remains constant at $\log k_N = 3.6$, the same value obtained for the formation rate con-

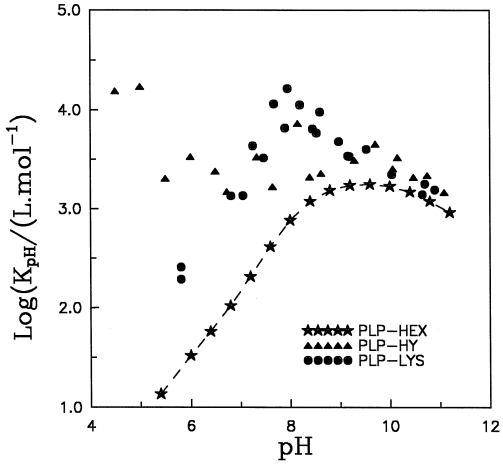


Fig. 3. Plot of $\log k_{pH}$ vs pH for different adducts of PLP with *n*-hexylamine (★) [10], poly-L-lysine (●) [14] and hydrazine (▲).

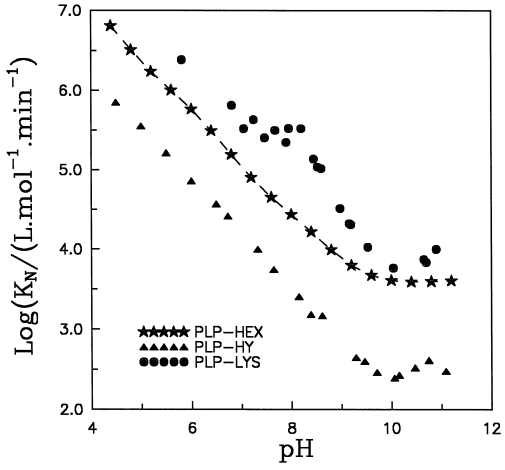
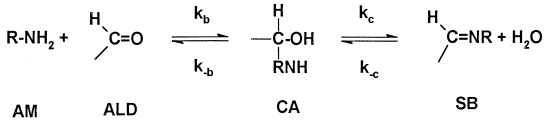


Fig. 4. Plot of log k_N vs pH for different adducts of PLP with *n*-hexylamine (★) [10], poly-L-lysine (●) [14] and hydrazine (▲).

stant for the less protonated specie of PLP with *n*-hexylamine [10]. The log k_N value above pH = 10 for the reaction with hydrazine remains constant approximately at log $k_N = 2.5$ showing that *n*-hexylamine is about ten times more reactive than hydrazine toward PLP in basic media. For this reason the k_1 values behaves as is shown in Fig. 1 at pH greater than 9, when the most of hydrazine it is as free amine.

The shape of the curve log k_2 vs. pH for PLP-HY system is different than the PLP-HEX system [9,10], PLP-LYS system [14], and other described systems involving PLP or analogues [10,12], as can be observed in Fig. 2. In fact, while for PLP-HEX, PLP-LYS and the other systems the log k_2 value decreases with pH to a minimum and then increase, the values for hydrazine increase with pH to a maximum at about pH 7.5 and then decrease until pH 10 and then slowly increases again for more basic pH. Also it can be observed that in the 6.5–9.0 pH range, the Schiff bases formed in the reaction of PLP with hydrazine are more hydrolyzed than



Scheme 1.

of PLP-HEX but k_2 values are similar than those of PLP-LYS system. For pH below 6 and above 9, the Schiff bases of PLP-HY system show minor values of k_2. This behavior is related with the reactivity and dissociation equilibrium constants of the different protonated forms of Schiff base in media.

As a result of the differences observed in k_1 and k_2 already discussed, Fig. 3 summarizes the results on the stability of the Schiff bases derived from the three systems. The Schiff bases of PLP-HY system are more stable than those of the PLP-HEX system in the whole studied pH range, and more stable than those of the PLP-LYS system at pH below pH 7. Very significant differences are obtained in acid media between the PLP-HY and PLP-HEX systems (K_{pH} for PLP-HY Schiff base is more than three orders of magnitude greater than the K_{pH} of Schiff base of PLP-HEX at pH 5), but minor differences at pH > 9 are found. In acid media the Schiff bases of the PLP-HY system are also more stable than those of the PLP-LYS system (about ten times at pH 6).

It should be noted that near physiological pH (pH 7–8) where many PLP-dependent enzymes exhibit their greatest activity, the formation of the Schiff bases of the PLP-HY system is greatly favored (about one order of magnitude greater than the PLP-HEX system and similar to the PLP-LYS system), and the Schiff bases of hydrazine show high stability. Therefore, depending on the concentrations, some therapeutic chemicals derived from hydrazine, can interfere with the enzymatic system, because at physiological pH they can form Schiff bases very quickly (capturing PLP) and these can be rather stable.

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