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Journal of Molecular Catalysis A: Chemical 101 (1995) 137–142

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Determination of the rates of formation and hydrolysis of the Schiff bases of pyridoxal with polyallylamine [1]

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Received 13 January 1995; accepted 28 March 1995

Abstract

The rate constants of formation (k_1) and hydrolysis (k_2 of the Schiff bases formed between pyridoxal (PL) and polyallylamine were determined as a function of pH at a temperature of 25°C and an ionic strength $I = 0.1$ M. The k_1 values obtained at every pH studied exceeded those for the Schiff bases of PL with poly-L-lysine and *n*-hexylamine, which is consistent with a favoured intramolecular acid catalysis of the dehydration of the intermediate carbinolamine formed in the process. The Schiff bases examined in this work lie in a hydrophobic environment and the pK for their imine group is smaller than 9.2.

Keywords: Hydrolysis; Polyallylamine; Pyridoxal; Schiff base

1. Introduction

The Schiff bases formed by pyridoxal 5′-phosphate (PLP, denoted by I in Scheme 1) and its analogues with various amino group bearers have been used as models for the binding of PLP to the enzymes for which it is a cofactor [1].

Recently, we reported a more realistic model for the binding of PLP to enzymes based on the use of homopolypeptides and copolypeptides containing L-lysine as amino group bearers, and studied the effect of the extent of polymerization and copolymer composition [2–4]. We found Schiff bases to be highly stable as a result of specific interactions with the polypeptide skeleton via strengthening of a hydrogen bond by interaction with the CO or NH group in the skeleton [3]. In order to explain this behaviour, we studied the reactions of PLP with polyallylamine (PAA), a polymer that contains no peptide groups in its skeleton, so it could hinder stabilization of the Schiff bases concerned by the above-mentioned mechanism [5].

The results show that the Schiff bases of PLP with polyallylamine (the PLP–PAA system) are more stable and formed in a shorter time than the previous ones; however, their kinetic behaviour is markedly divergent from that of previously stud-

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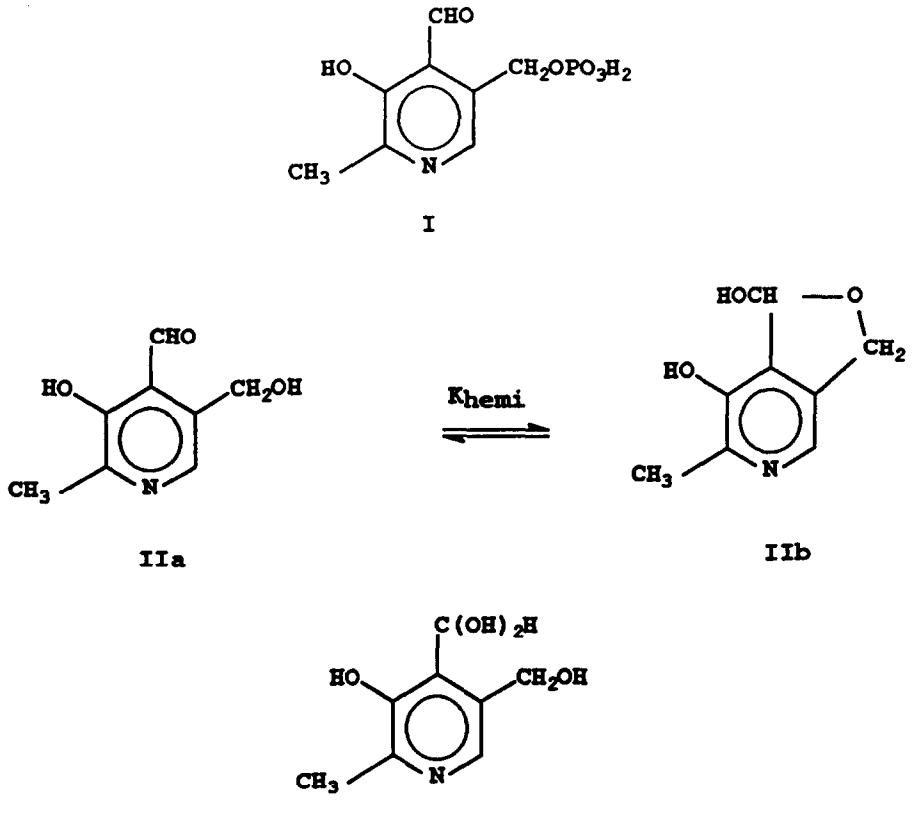
[1] Financially supported by the Spanish DGICyT (Project PB91-0161-C02-01) and the Programa para la Cooperación con Iberoamérica.

SSDI 1381-1169(95)00074-7



Scheme 1.

ied models involving the Schiff bases of PLP and its analogues with various amino group bearers, so no quantitative comparison between the models is possible. The differences in the behaviour of these systems have been ascribed to a shift in the rate-determining step (rds) to the formation of the tetrahedral intermediate, followed by rapid dehydration –this last is believed to be the rds in the reactions of PLP and its analogues with amino group bearers other than PAA. While the dehydration step for the PLP–PAA system must obviously be much faster, the lack of rate constant values for the other systems precludes quantitative comparison.

Based on the low reactivity of pyridoxal (PL) towards n-hexylamine [6] and poly-L-lysine [7] (the PL–HEX and PL–LYS systems) relative to the PLP–HEX [8,9] and PLP–LYS bases [2], we studied the reaction between pyridoxal and polyallylamine in order to determine the influence of a skeleton precluding stabilization by interaction with the groups in the peptide bond (as in polypeptides).

This paper reports on the kinetics of formation and hydrolysis, and stability, of PL–PAA Schiff bases, and compares the results obtained with those previously reported for the PL–HEX [6] and PL–LYS systems [7].

2. Experimental

Polyallylamine hydrochloride (MW = 60 000 dalton as determined viscometrically) was purchased from Polysciences, Inc. Pyridoxal (PL) and all other chemicals were reagent-grade and supplied by Merck. Acetate, phosphate and carbonate buffers of ionic strength 0.01 M were used throughout. The final ionic strength was adjusted to 0.1 M with KCl.

PL solutions were made daily in the appropriate buffer and stored in the dark. Their exact concentrations were determined by dilution with 0.1 M HCl and measurement of the absorbance at 288 nm ($\epsilon = 8600\ l\ mol^{-1}\ cm^{-1}$) [10]. Polypeptide solutions were also prepared daily at concentrations from 3×10^{-4} to 10^{-1} M by dissolving the required amount of polymer in the appropriate buffer.

The kinetics of formation of the Schiff base was monitored by measuring changes in the absorption at 420 nm as a function of pH using a Perkin Elmer Lambda 3 or a Hewlett-Packard 8452A spectrophotometer furnished with thermostated cells of 1 cm path length. The imines were obtained by adding 10 μl of PL solution to 3 ml of a buffered solution of the polymer previously thermostated at 25 ± 0.05°C.

The observed rate constant (k_{obs}) of the Schiff base was determined at various PAA concentrations and pH values using the infinite dilution method.

The experimental rate constants of formation (k_1) and hydrolysis (k_2), and the equilibrium constant (K_{pH}), for the PL–PAA system were obtained by using the same procedure as for simple amines described elsewhere [8,9].

$$R_1\text{–CHO} + R_2\text{–NH}_2 \underset{k_1}{\overset{k_2}{\rightarrow}} R_1\text{–CH=N–}R_2 + H_2O \qquad (1)$$

The pK for polyallylamine was determined by viscometric titration using a Desreux–Bischoff viscometer at 25 ± 0.1°C. The value thus obtained, p$K = 9.0$, was consistent with that found by potentiometric titration.

3. Results and discussion

Figs. 1–3 show the variation of k_1, k_2 and K_{pH} for the formation of the Schiff bases of PL with PAA, as well as those for the PL–HEX [6] and PL–LYS systems [7] in aqueous solutions at the same temperature and ionic strength ($I = 0.1$ M). As can be seen, the kinetic behaviour of the PL–PAA system at a variable pH resembles that of the PL–LYS and PL–HEX systems, but departs from that of the PLP–PAA system [5].

It should be noted that PL can exist as both a free aldehyde (IIa in Scheme 1), an internal hydrate or hemiacetal (IIb in Scheme 2) and an aldehyde hydrate (IIc in Scheme 1) in aqueous solutions, depending on the pH. This posed no special problem in our study inasmuch as all the systems were compared at the same pH values. The experimental k_1, k_2 and K_{pH} values obtained for the PL systems were comparable.

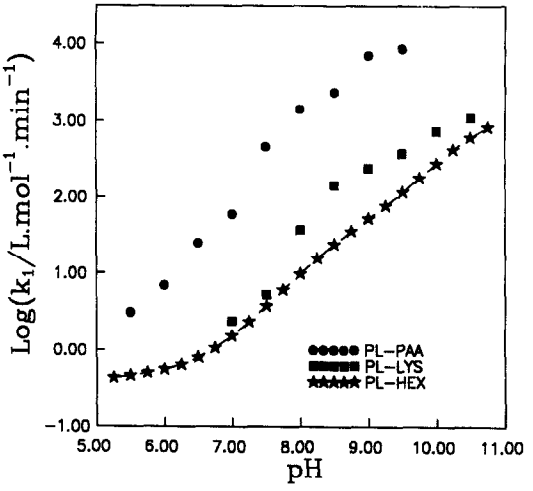


Fig. 1. Variation of log k_1 as a function of pH for different systems. PL–PAA (●) PL–HEX (from [6]) (★). PL–LYS (from [7]) (■).

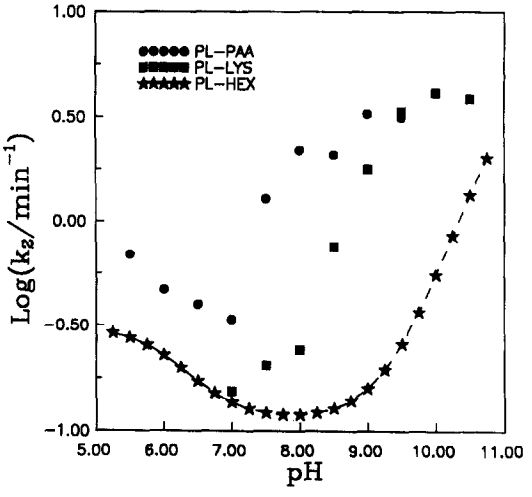


Fig. 2. Variation of log k_2 as a function of pH for different systems. PL–PAA (●). PL–HEX (from [6]) (★). PL–LYS (from [7]) (■).

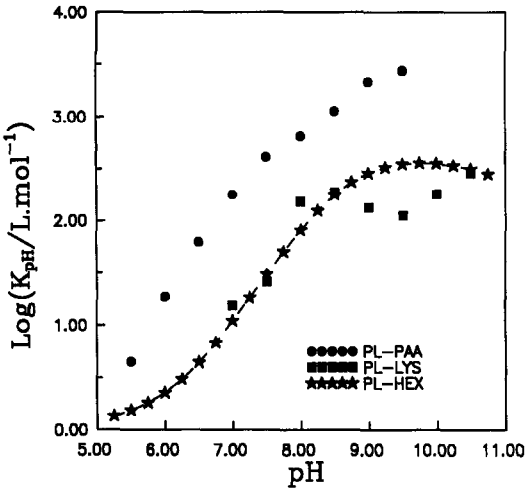


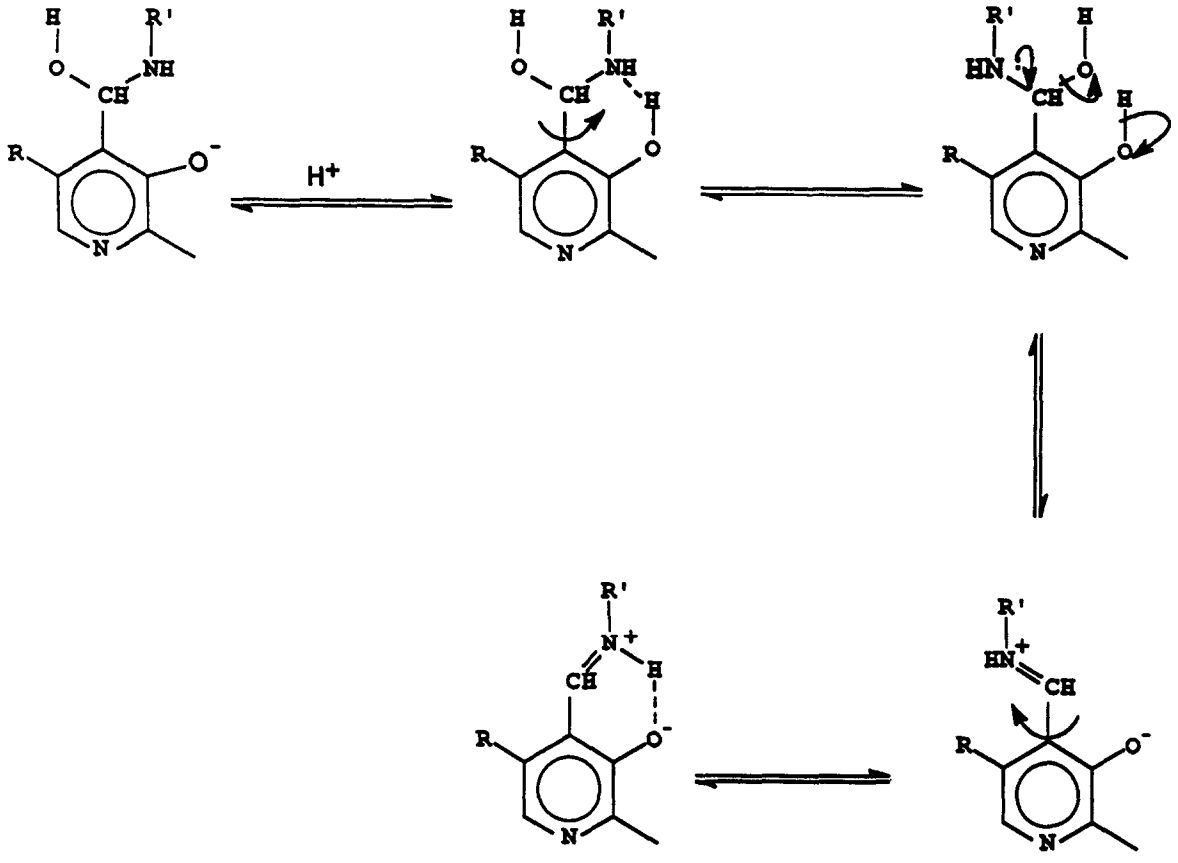
Fig. 3. Variation of log K_{pH} as a function of pH for different systems. PL–PAA (●). PL–HEX (from [6]) (★). PL–LYS (from [7]) (■).

Fig. 1 illustrates the significant role played by the molecular size of the amino bearer in the formation of this type of Schiff base. Thus, poly-L-lysine and polyallylamine macromolecules are more reactive than are *n*-hexylamine molecules. As with PLP, the higher reactivity of the PL–LYS system relative to the PLP–HEX system can be ascribed to specific effects of the polymer such as those arising from charges on side chains, the conformation of the peptide chain and hydrogen bonding to the solvent, all of which favour intramolecular acid catalysts [7]. In addition, the rate constants for the PL–PAA system were greater by about two orders of magnitude than those for the PL–HEX system.

Several studies have shown the significance of the phenoxy group at position 3 in both PL, PLP and their analogues (see Scheme 2) as an intramolecular catalyst for the dehydration of the intermediate carbinolamine [9,11,12]. Intramolecular catalysis in the reactions of PLP and its analogues with amines has also been found to be promoted by other, proton-releasing molecular groups [6,8,9]. In addition, the polypeptide chain was recently reported to presumably play some role in stabilizing the resulting Schiff base in the reactions of PLP with homo-and copolypeptides containing L-lysine [3]. The higher reactivity of polyallylamine towards PL relative to L-lysine suggests that the carbinolamine dehydration is greatly favoured; however, the effect cannot be ascribed to an interaction with the macromolecular skeleton as it contains no peptide bonds. The presence of a high concentration of $-NH_3^+$ groups in the vicinity of the carbinolamine may be responsible for this behaviour since such groups can act as intramolecular catalysts for the carbinolamine dehydration and also interact (by release of a proton) with the phenoxy group in the pyridine ring, thereby favouring the process. This is also probably the origin of the above-mentioned shift in the rate-determining step of the reaction between PLP and PAA.

The increase in k_1 obtained on increasing the size of the amino bearer (from *n*-hexylamine to poly-L-lysine) was less than one order of magnitude for PL and only appreciable over the pH range 7.5–10.0 (Figs. 2 and 1). The increase in this rate constant was greater (1–2 orders of magnitude) for PLP (Fig. 4) and is appreciable throughout the pH range studied, before the polypeptide α-helix was formed [2]. These results suggest that the phosphate group also plays some role in the carbinolamine dehydration, in contradiction with the results for small molecules [6], i.e. the phosphate group of PLP and the macromolecule interact in some way that also favours the carbinolamine dehydration.

We obtained a reactivity increase of 1–2 orders of magnitude in switching from *n*-hexylamine to polyallylamine as the amino bearer. While the carbinolamine dehydration was dramatically favoured via the above-described potential mechanisms, the effect does not warrant a shift in the rate-determining step. In fact, the dehydration step is so favourable in the reaction between PLP and PAA that the shift in question actually takes place [5]. For example, since $k_{obs} = 840\ min^{-1}$ for the PLP–PAA system at $[PAA] = 6.9 \times 10^{-3}$, k_1 must be greater than $1.2 \times 10^5\ l\ min^{-1}\ mol^{-1}$ for the rds to change. This value is three and four orders of magnitude greater than that for the PLP–LYS and PLP–HEX system (Fig. 4), respectively, which is quite feasible since both the amino



Scheme 2.

groups of polyallylamine and the phosphate group of PLP contribute to the dehydration step.

The k_2 values obtained for the PL–PAA system (Fig. 2) are greater than those for the PL–HEX and PL–LYS systems, so the latter two are more readily hydrolysed over the pH range shown in the figure. On the other hand, the k_2 values estimated for PL–LYS and PL–PAA at very low pH values are similar and close to 0.65, whereas log $k_2 = 1.03$ for PL–HEX [6]. These results demonstrate that the Schiff bases produced by macromolecules lie in a similarly hydrophobic, though less polar, environment than does the PL–HEX system. The shift to higher pH values at the minimum of the log k_2 vs. pH plot for the PL–PAA system is consistent with the presence of a hydrophobic medium; hence, the pK for the imine group of the Schiff base must be lower than 9.2–9.4, which is the estimated value for the PL–LYS system.

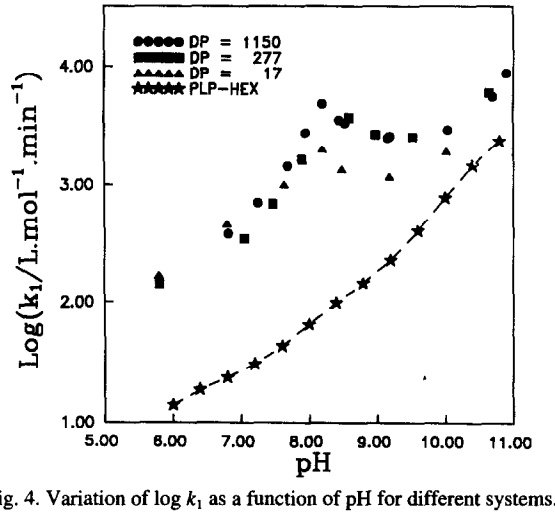


Fig. 4. Variation of log k_1 as a function of pH for different systems. PLP–LYS (from [2]) (●, ■, ▲). PLP–HEX (from [8,9]) (★).

The greater k_1 values for the PL–PAA system also result in greater K_{pH} values (Fig. 3). While, unlike the PL–LYS system, no maximum is obtained as no α-helix conformation exists, the

Schiff bases formed are roughly one order of magnitude more stable. This additional stability may be provided by the side chains or polymeric skeleton interacting with some group (phenol, pyridine ring) in the Schiff base. Because the polymer chain only contains CH groups, the interactions presumably involve terminal groups in the side chains that do not react with the aldehyde. On the other hand, the Schiff base probably interacts via a phenol group.

Acknowledgements

This work was financially supported by the Spanish DGICyT (Project PB91-0161-C02-01) and the 'Programa para la Cooperación con Iberoamérica'.

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