ELSEVIER

Journal of Molecular Catalysis A: Chemical 123 (1997) 9–13

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Schiff bases of poly-L-lysine and some compounds of the vitamin B-6 group. Influence of polypeptidic structure

M. Angeles García del Vado [a], Gerardo R. Echevarría Gorostidi [a,*], José G. Santos Blanco [b], Francisco García Blanco [c]

[a] *Department of Physical Chemistry, University of Alcalá, E-28871 Alcalá de Henares, Spain*
[b] *Faculty of Chemistry, Pontificia Universidad Católica de Chile, Casilla 306, Santiago 22, Chile*
[c] *Faculty of Pharmacy, Complutense University, E-28040 Madrid, Spain*

Received 21 November 1996; accepted 3 February 1997

Abstract

The apparent rate constants of formation (k_1) and hydrolysis (k_2) of the Schiff bases formed between pyridoxal and poly-L-lysine has been fitted to a kinetic scheme that involves the different protonated species in the reaction medium and the individual rate constants of formation (k_1^i) and hydrolysis (k_2^i). A Brönsted plot with $\alpha = 0.67$ is in accord with an acid catalytic intramolecular process. The effects of hydrophobic medium due to the presence of the macromolecule on the formation and hydrolysis of Schiff bases from PL, DPL and PLP with poly-L-lysine is discussed.

Keywords: Schiff base; Pyridoxal 5'-phosphate; 5'-Deoxypyridoxal; Pyridoxal; Poly-L-lysine

1. Introduction

Pyridoxal 5'-phosphate (PLP) and pyridoxal (PL) are two of the different forms of vitamin B-6 and are involved in different enzymatic process, as transaminations, deaminations, decarboxylations and others. 5'-deoxypyridoxal (DPL) is a very good analog of PL and PLP as it possesses the three chemical groups which are considered to be fundamental to catalytic activity (–CH=O, –OH, =N–) [1,2].

All PLP dependent enzymes bind the carbonyl group of PLP through an internal Schiff base with a lysine residue of polypeptide chain usually in more or less hydrophobic medium [3–5].

In order to evaluate the influence of the polarity of the environment on the formation and hydrolysis of Schiff bases, the reaction of PLP and analogues with *n*-hexylamine has been carried out in several media of different polarity [6–16].

Recently we reported a more realistic model for the binding of PLP to enzymes, based in the use of homopolypeptides and copolypeptides containing L-lysine as bearers of the NH_2 groups [17–20]. Thus, we studied the formation and hydrolysis of the Schiff bases of PLP with poly-L-lysine in various degrees of polymerization [17,18], as well as those formed by PLP with L-lysine copolymers [18–20] and also that

* Corresponding author. E-mail: qfechevar@quifis.alcala.es; fax: (+34-1) 8854763.

PII S1381-1169(97)00039-3

corresponding to the formation and hydrolysis on poly-L-lysine with PL and DPL [21,22]. The dehydratation of the corresponding carbinolamine has always been the rate determining step of the formation of these Schiff bases.

In this work we study the effects on the formation and hydrolysis of Schiff bases from PL, DPL and PLP with poly-L-lysine, of the hydrophobic medium derived on the presence of the macromolecule, in comparison with the behavior of the systems derived of the reaction between *n*-hexylamine and the same aldehydes [9].

2. Experimental

The polypeptides were purchased from Sigma Chemical. Pyridoxal 5′-phosphate, pyridoxal and all the other chemicals were reagent grade and purchased from Merck. 5′-Deoxypyridoxal was synthesized from pyridoxine hydrochloride [23].

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, PL and DPL solutions were prepared daily in appropriate buffers and were stored in the dark. Their exact concentrations were determined by dilution [24,25] with 0.1 mol/l HCl (PLP, DPL) or 0.1 mol/l NaOH (PL). Polypeptide solutions were also daily prepared by diluting the appropriate amount of polymer in the corresponding buffer.

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

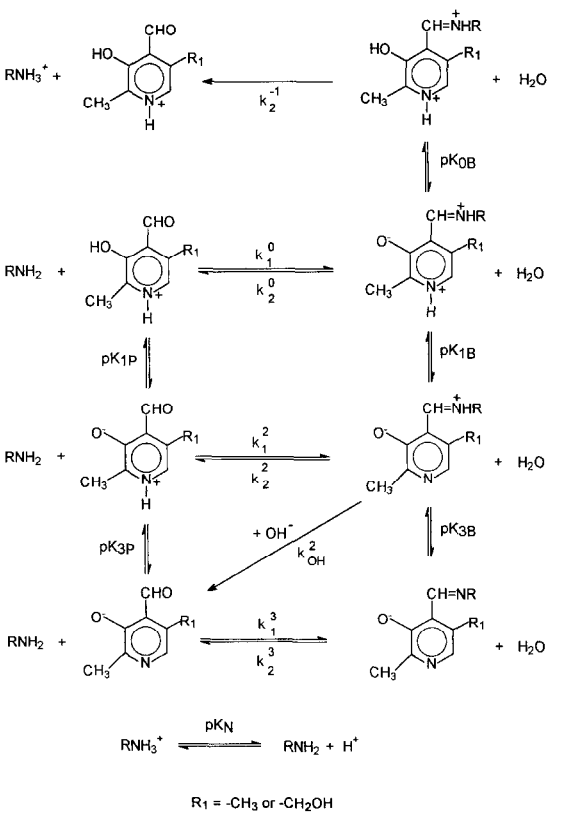
$$R^1\text{-CHO} + NH_2\text{-}R^2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R^1\text{-CH=N-}R^2 + H_2O$$

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

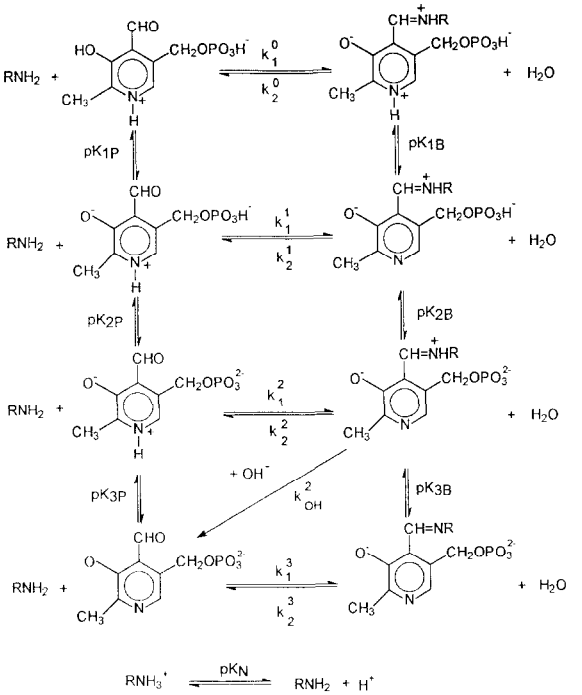
Table 1
Best kinetics constants and pK obtained in the fitting experimental k_1 and k_2 values

	PL–NHA [a]	PL–Lys	DPL–Lys [b]	PLP–Lys [c]
$\log k_1^0$	6.15	5.80	7.20	8.74
$\log k_1^1$	—	—	—	6.13
$\log k_1^2$	3.80	2.98	4.60	5.45
$\log k_1^3$	3.22	3.99	4.50	3.53
$\log k_2^{-1}$	−1.00	−1.06	1.05	—
$\log k_2^0$	−0.50	−0.39	−0.75	−0.17
$\log k_2^1$	—	—	—	−2.29
$\log k_2^2$	−0.95	−1.15	−0.57	−0.42
$\log k_{OH}$	1.03	0.65	0.15	1.04
pK_{1P}	4.26	4.34	4.14	3.46
pK_{2P}	—	—	—	6.02
pK_{3P}	8.61	8.52	7.98	8.22
pK_N	10.75	10.03	10.03	10.03
pK_{0B}	3.15	3.15	3.24	—
pK_{1B}	6.12	6.22	6.68	6.62
pK_{2B}	—	—	—	7.74
pK_{3B}	11.16	9.25	9.21	10.92

[a] Taken from Ref. [9].
[b] Taken from Ref. [22].
[c] Taken from Ref. [19].



Scheme 1.



Scheme 2.

these two constants is described in detail elsewhere [7]. Table 1 gives the pK values and those of the microscopic rate constants, k_1^i and k_2^i, obtained by fitting the experimental k_1 and k_2 values to the equations derived from Scheme 1 [19,22]. The table also shows the pK values and those of the microscopic rate constants for the PL–*n*-hexylamine (PL–NHA) and DPL–poly-L-lysine (DPL–Lys) systems, which are conform to Scheme 1, and for the PLP–poly-L-lysine (PLP–Lys) system, which conforms to Scheme 2. In the table $k_{OH} = k_2^3 + k_{OH}^2(K_W/K_{3B})$ and K_W is the ionic product of water.

3. Results and discussion

Figs. 1 and 2 show the experimental results for the formation (k_1) and hydrolysis (k_2) obtained for the Schiff bases from poly-L-lysine and PL [21], DPL [22]. and PLP [19] and PL with *n*-hexilamine [9], PL–Lys, DPL–Lys,

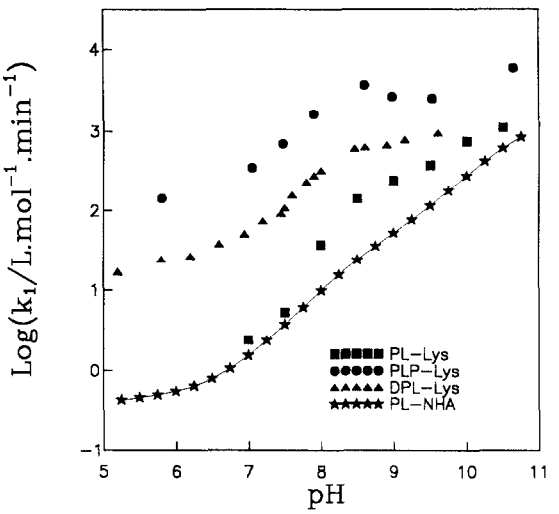


Fig. 1. Plot of log k_1 vs. pH for the Schiff bases of 5′-deoxypyridoxal with poly-L-lysine (▲), pyridoxal with poly-L-lysine (■), pyridoxal 5′-phosphate with poly-L-lysine (●) and pyridoxal with *n*-hexylamine (★).

PLP–Lys and PL–NHA systems respectively, as a function of pH, at the same experimental conditions.

For the three lysine systems (see Fig. 1), about pH = 8, it can be observed a discontinuity of log k_1 vs. pH curve, as the result of the formation of α-helix in the poly-L-lysine. This conformation is more stable at basic pH [26].

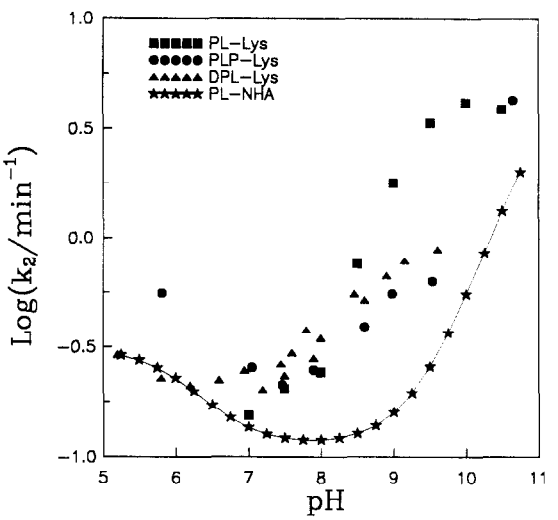


Fig. 2. Plot of log k_2 vs. pH for the Schiff bases of 5′-deoxypyridoxal with poly-L-lysine (▲), pyridoxal with poly-L-lysine (■), pyridoxal 5′-phosphate with poly-L-lysine (●) and pyridoxal with *n*-hexylamine (★).

From Fig. 1 it can be observed that the k_1 values for PL–Lys system always exceeded those for PL–NHA system. These differences were attributed to specific effects of the polymer such as those arising from charges on the side chains, the conformation of the peptide chain and the formation of hydrogen bonds with the solvent, which favor intramolecular acid catalysis in the intermedia carbinolamine, that is the rate determining step [21]. Nevertheless from Table 1 it can be observed that the k_1^0 and k_1^2 values for the PL–NHA system are greater than that for PL–Lys system but for the last, the k_1^3 value is greater. Wherever the experimental conditions are those that permits an important population of nonprotonated PL, the contribution of k_1^3 on k_1 is diminished due to apparition of α-helix conformation, that is less reactive than the statistical conformation [19].

The greater reactivity of PL–Lys system since pH = 7.5, must be attributed to the differences in pK_N and to the differences in hydrophobicity in the reaction medium.

In order to appreciate the magnitude of polarity of environment originated by the macromolecule in the site where of Schiff base is formed, we compare the log k_1^2 and log k_1^3 differences in DPL–NHA system, in different reaction media. These differences are 0.88 in water [9], 0.62 in 50%/50% dioxane/water (v/v) [11]. and 0.28 in 70%/30% dioxane/water (v/v) [11]. Taking in the account that the difference in the DPL–Lys system it is only 0.1, it is possible to conclude that the polypeptide chain provide a less polar medium (more hydrophobic) than 70%/30% dioxane/water (v/v).

The results for the PL–Lys system (Table 1) show that $k_1^3 > k_1^2$. This is the opposite that the obtained for all the systems studied for us, and it can be explained considering the above discussion relative to the hydrophobicity that leads to similar values for both constants and also to the presence of the hemiacetal form in PL, because the experimental k_1 included the equilibrium constant of hemiacetal formation [21], and this equilibrium constant could be different in front of the different polarity of reaction media.

From the pK_{iP} and k_1^i values for PL–Lys system (Table 1) it is possible to observed an intramolecular acid catalysis process, in fact, drawing a Brönsted plot, a slope of $\alpha = 0.67$ is obtained, close similar to those for the other systems $\alpha = 0.69$ for DPL–Lys [22], 0.77 for PLP–Lys [19] and 0.54 for PL–NHA [9].

Fig. 2 show that in the range of pH = 8.0–10.5, the PL–Lys system exhibits greater k_2 values than the PL–NHA system. On the other hand, the Schiff base of poly-L-lysine is more readily hydrolyzable. A comparison of microscopic rate constants (Table 1) show very similar values for both systems, therefore it is not possible to attribute the behavior previously expound to a greater reactivity of Schiff bases with poly-L-lysine; moreover the k_{OH} value for PL–NHA system is about twice than that of PL–Lys system. The behavior consigned in Fig. 2 can be attributed to the more hydrophobic environment provided by the macromolecule that decrease the pK of the imino group of PL–Lys system giving a greater population of nonprotonated Schiff bases at more acidic pH values, increasing k_2.

The minor pK_{3B} values obtained for the poly-L-lysine systems with DPL and PLP, referred to the corresponding *n*-hexylamine systems, it is also attributed to the hydrophobic environment provides by poly-L-lysine chain [22]. In order to give an idea of the magnitude of hydrophobic environment provided by poly-L-lysine we can compare the effects on pK of imine group (pK_{3B}) of Schiff base in DPL–NHA system in different media; in water the value is 11.69 and decreased to 11.38 and 11.31 in 50%/50% and 70%/30% dioxane/water (v/v) respectively [11]; for the Schiff base of DPL–Lys the pK_{3B} value decrease to 9.21 [22].

It is also remarkable that the pK_{3B} differences, obtained by comparison of the values of NHA systems [9] with those of poly-L-lysine systems are 0.3, 1.9 and 2.4 for PLP, PL and

DPL respectively, suggesting environments of different hydrophobicity for each Schiff base, probably due to the different C-5 substitution in each substrate. The difference in PLP systems [19] has been compared with that of PLP–NHA system in going from water to a 30%/70% ethanol/water (w/w) media [10], nevertheless the difference in DPL systems suggest a more hydrophobic environment than that of 70%/30% dioxane/water (v/v). For PL–Lys system the hydrophobicity could be minor than DPL–Lys system due to the interaction with the hydroxylic group in PL.

The differences found in k_{OH} for PL systems (Table 1) are related to the hydrophobicity of media. The values of $\log k_{OH} = 1.03$ for PL–NHA and $\log k_{OH} = 0.65$ for PL–Lys are in the line of our discussion. From Schiff bases derived of n-hexylamine with PLP, PL and DPL, the less hydrolizables (the smallest k_{OH} value [9]) are from PLP. Nevertheless the Schiff bases derived from poly-L-lys and PLP show the greatest k_{OH}, probably due to the greater polarity of PLP–Lys system.

The hydrophobicity derived from the presence of the polypeptide chain is localized in the environments of the C-4 carbon atom because no significant differences were founded in pK_{0B} or pK_{1B}.

Acknowledgements

Financially supported by the Spanish DGICyT (Proyect PB91-0161-C02-01 and Proyect PB93-0073) and Programa para la Cooperación con Iberoamérica.

References

[1] E.E. Snell, in: D. Dolphin, R. Poulson, O. Avramivic (Eds.), Vitamin B-6 Pyridoxal Phosphate, Chemical, Biochemical and Medical Aspects, Part A, Wiley, New York, 1986, p. 1–12.
[2] A.E. Braunstein, in: P. Christen, D.E. Metzler (Eds.), Transaminases, Wiley, New York, 1985, ch. 1.
[3] S. Shaltiel, M. Cortijo, Biochem. Biophys. Res. Commun. 41 (1970) 594.
[4] M. Cortijo, J. Llor, J.S. Jiménez, F. García Blanco, Eur. J. Biochem. 65 (1976) 521.
[5] D.E. Metzler, Adv. Enzymol. 50 (1979) 1.
[6] C.M. Metzler, A. Cahill, D.E. Metzler, J. Am. Chem. Soc. 102 (1980) 6075.
[7] M.A. García del Vado, J. Donoso, F. Muñoz, G. Echevarría, F. García Blanco, J. Chem. Soc. Perkin Trans. 2 (1987) 445.
[8] J. Donoso, F. Muñoz, M.A. García del Vado, G. Echevarría, F. García Blanco, Biochem. J. 238 (1987) 137.
[9] M.A. Vázquez, J. Donoso, F. Muñoz, F. García Blanco, M.A. García del Vado, G. Echevarría, Bull. Soc. Chim. France (1988) 361.
[10] M.A. García del Vado, G. Echevarría, A. García-Espantaleon, J. Donoso, F. Muñoz, F. García Blanco, J. Mol. Catal. 44 (1988) 313.
[11] M.A. Vázquez, J. Donoso, F. Muñoz, F. García Blanco, M.A. García del Vado, G. Echevarría, J. Mol. Catal. 59 (1990) 137.
[12] M.A. Vázquez, J. Donoso, F. Muñoz, F. García Blanco, M.A. García del Vado, G. Echevarría, Helv. Chim. Acta 73 (1990) 1991.
[13] M.A. Vázquez, J. Donoso, F. Muñoz, F. García Blanco, M.A. García del Vado, G. Echevarría, J. Chem. Soc. Perkin Trans. 2 (1991) 1143.
[14] M.A. García del Vado, G. Echevarría, M.A. Vázquez, F. García Blanco, J. Chem. Soc. Perkin Trans. 2 (1992) 915.
[15] I.M. Plaza del Pino, J.M. Sánchez-Ruiz, J. Chem. Soc. Perkin Trans. 2 (1993) 573.
[16] I.M. Plaza del Pino, J. Llor, J.M. Sánchez-Ruiz, J. Chem. Soc. Perkin Trans. 2 (1993) 581.
[17] M.A. García del Vado, G.R. Echevarría, F. García Blanco, J.G. Santos, M. Blázquez, J.M. Sevilla, M. Dominguez, J. Mol. Catal. 68 (1991) 379.
[18] M.A. García del Vado, G.R. Echevarría, F. García Blanco, J.G. Santos, J. Laynez, J.L. García de Paz, Helv. Chim. Acta 74 (1991) 1749.
[19] M.A. García del Vado, G.R. Echevarría, J.G. Santos, F. García Blanco, J. Mol. Catal. 78 (1993) 379.
[20] M.A. García del Vado, M.P. Martín Pérez, A.F. Rodríguez Cardona, G. Echevarría, J.G. Santos, F. García Blanco, J. Mol. Catal. A 111 (1996) 193.
[21] M.A. García del Vado, F. Rodríguez Cardona, G. Echevarría, M.C. Martínez González, J.G. Santos, F. García Blanco, J. Mol. Catal. 87 (1994) 361.
[22] M.A. García del Vado, G. Echevarría, J.G. Santos, F. García Blanco, J. Mol. Catal. A 111 (1996) 193.
[23] C. Iwata, Biochem. Prep. 12 (1968) 117.
[24] E.A. Peterson, H.A. Sober, J. Am. Chem. Soc. 76 (1954) 169.
[25] K. Nakamoto, A.E. Martell, J. Am. Chem. Soc. 81 (1959) 5863.
[26] G.D. Fasman, in: G.D. Fasman (Ed.), Poly-a-aminoacids, M. Dekker, New York, 1967, pp. 499–604.