

On the L-DOPA and Carbidopa Reactivity against Pyridoxal 5′-Phosphate. A Kinetic Study

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The apparent rate constants of the formation (k_1) and hydrolysis (k_2) of Schiff bases formed by pyridoxal 5′-phosphate (PLP) with L-3,4-dihydroxyphenylalanine (L-DOPA) at a variable pH, 25 °C and an ionic strength of 0.1 M was determined. The individual rate constants for the formation and hydrolysis of Schiff bases corresponding to the different chemical species present in the medium as a function of its acidity were also determined, as were the pK_a values for the Schiff bases. The formation and hydrolysis rate constants of the Schiff bases were compared with those of the reaction of PLP with carbidopa (CD), showing that the reactivity of L-DOPA and carbidopa on PLP are the same over the whole pH range studied, and that the hydrolysis rate is somewhat greater for the Schiff bases between PLP and CD than those between PLP and L-DOPA.

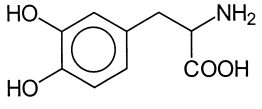
Pyridoxal 5′-phosphate (PLP) is one form of vitamin B_6 that plays a central role as a coenzyme in a wide range of reactions (e.g. transaminations, transiminations, dealdolations, eliminations, and decarboxylations) involved in amino acid metabolism.[1–3] Its action relies on the formation of a α-hydroxy amine intermediate by bonding of its carbonyl group to the ε-amino group of the L-lysine residue in the peptide chain.[1,4] The α-hydroxy amine then releases one molecule of water to give the Schiff base in an acid-catalyzed process.[4,5]

In virtually all PLP-dependent enzymes, the first step of the process is a transimination reaction, viz. conversion of the PLP-lysine imine into a PLP-substrate imine;[6] in other words, the covalent linkage in the Schiff base must be broken during the course of the catalytic cycle and a new base be formed between the coenzyme and the substrate (usually an amino acid).[1]

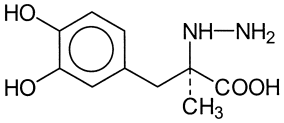
Parkinson disease is caused by a decrease of 3,4-dihydroxyphenethylamine (dopamine) in the central nervous system;[7] in order to increase it, the cerebral level, L-3,4-dihydroxyphenylalanine (L-DOPA, Scheme 1), a dopamine precursor, is used. L-DOPA reacts with a PLP-dependent enzyme (dopa-decarboxylase), forming a corresponding Schiff base which by decarboxylation yields dopamine. Dopamine generated in the intestine can not cross the blood-brain barrier; nevertheless, L-DOPA can without difficulty get to the brain by means of an active transport system; consequently, L-DOPA is transported into the brain where it is decarboxylated by dopa decarboxylase to dopamine.[7] A strategy frequently used is to administrate the patient L-DOPA in combination with carbidopa (3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methyl propionic acid, Scheme 1) in order to permit that L-DOPA gets as such to the brain.[7]

In the literature there are references to Schiff bases formed by PLP and compounds bearing amino groups such, as amines, amino acids, and polypeptides.[8–10]

In this work, the kinetics of formation and hydrolysis of the Schiff bases of pyridoxal 5′-phosphate with L-DOPA (viz. the PLP–DOPA system) was determined and the results compared with those previously obtained for the reaction of PLP with carbidopa (PLP–CD system),[10] in order to shed additional light on the use of carbidopa in the L-DOPA formulations in Parkinson's disease. The results for both systems were examined in terms of the individual rate constants for those species involved in the process (see Scheme 2).

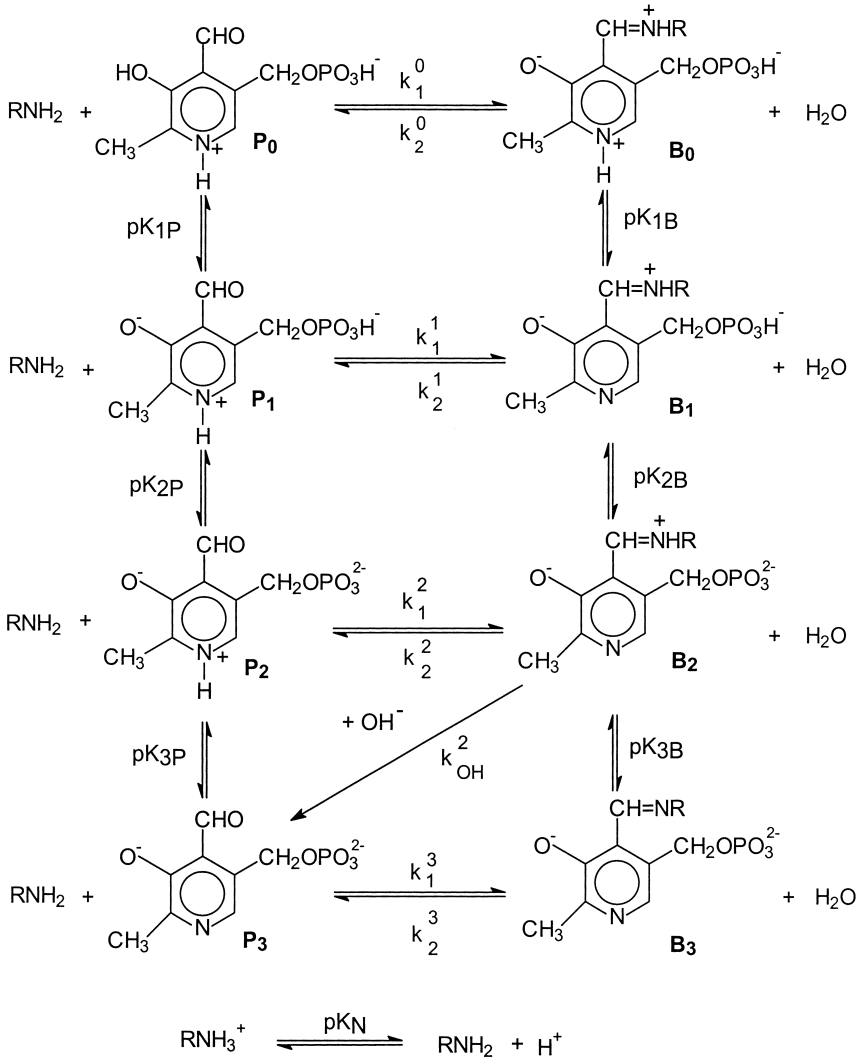


L-Dopa



Carbidopa

Scheme 1.



Scheme 2.

Material and Methods

L-DOPA was purchased from Sigma Chemical Co. Pyridoxal 5′-phosphate and all other chemicals used were of reagent-grade and purchased from Merck.

Acetate, phosphate, and carbonate buffers were used over appropriate pH ranges. The buffer concentration used was typically 0.02 M and the ionic strength was maintained at 0.1 M by adding appropriate amounts of KCl to the medium.

PLP solutions were made in appropriate buffers and stored in the dark. Their exact concentrations were determined by dilution with 0.1 M HCl,[11] and were found to be in the region of 5×10^{-5} M. L-DOPA solutions spanning the concentration range from 5×10^{-4} to 2×10^{-2} M were also prepared on a daily basis by diluting appropriate amounts of stock solutions in the corresponding buffer.

Kinetic measurements were made at various pH by using a Hewlett–Packard 8453 diode array spectrophotometer furnished with thermostated cells of 1-cm light path. In each case, the reaction was started by adding a known volume of PLP buffered solution to prethermostated L-DOPA solutions at (25 ± 0.1) °C. The difference between the initial and final pH in the reaction cell never exceeded 0.03 units. pH measurements were made by using a Crison pH-meter equipped with a Metrohm EA120 electrode that was previously calibrated with aqueous buffers at 25.0 °C.

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

$$R_1 - CHO + NH_2 - R_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R_1 - CH{=}N - R_2 + H_2O, \quad (1)$$

where k_1 and k_2 are the overall rate constants for the formation and hydrolysis, respectively, of the Schiff base. The procedure used to calculate these two constants is described in detail elsewhere.[12] Nucleophilic rate constants (k_N) were obtained from the slopes of linear plots of k_{obs} vs free amine concentration, or by dividing the k_1 values by the corresponding free amine molar fractions.

Results and Discussion

The kinetic law obtained in the present reactions is given by Eq. 2, where k_{obs} is the pseudo-first-order rate coefficient, k_1 and k_2 are the rate constants for the formation and hydrolysis of the Schiff base, respectively, and [L-DOPA] represents the total L-DOPA concentration. The values of k_1 and k_2 were obtained as the slope and intercept, respectively, of linear plots of

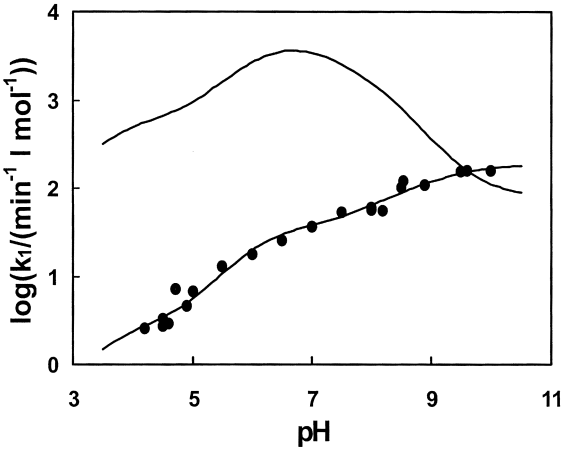


Fig. 1. Plot of log k_1 vs pH for the PLP–DOPA (●) system. Curves (—) for PLP–DOPA and (----) for PLP–CD systems were calculated using Eq. 3 and data from Table 1.

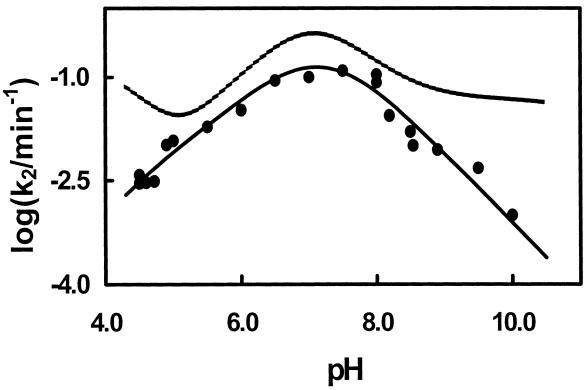


Fig. 2. Plot of log k_2 vs pH for the PLP–DOPA (●) system. Curves (—) for PLP–DOPA and (----) for PLP–CD systems were calculated using Eq. 4 and data from Table 2.

k_{obs} vs [L-DOPA] at constant pH,

$$k_{obs} = k_1[\text{L-DOPA}] + k_2. \quad (2)$$

Figures 1 and 2 show the experimental results in the form of the variation of the logarithmic overall rate constants of formation (k_1) and hydrolysis (k_2) for the Schiff bases of PLP with L-DOPA as a function of pH. The figures also include the calculated curve for the Schiff bases of the PLP–CD system.[8,10]

Reaction measurements could only be made up to pH 10.5 because more alkaline media resulted in the oxidation of L-DOPA,[13] and thus hindering the reaction and any precluding examination beyond this pH.

Figure 1 shows that log k_1 increases with increasing pH for the PLP–DOPA system; nevertheless, for the PLP–CD system, log k_1 increases to a maximum at pH = 7 and then decreases again as the pH increases. Below pH = 9, the k_1 values are greater for the latter system.

On the other hand, k_2 was greater for the PLP–CD system than the PLP–DOPA system throughout the pH range studied (Fig. 2). Both systems show a maximum at pH = 7 in the log k_2 vs pH plot.

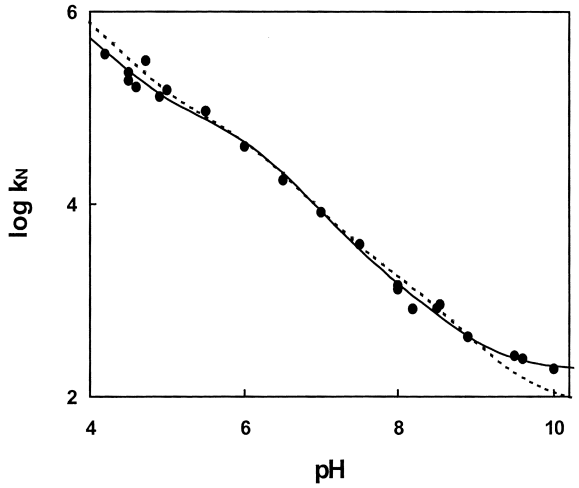


Fig. 3. Plot of log k_N vs pH for the PLP–DOPA (●) system. Curves (—) for PLP–DOPA and (----) for PLP–CD systems were calculated using Eq. 5 and data of Table 1.

The apparently increased reactivity of carbidopa with L-DOPA relative to PLP below pH 9 (see Fig. 1) arises from the differential values of pK_a for the amino group in the two compounds. One way to compare their reactivity is by making the formation rate constants for the Schiff bases independent of pK_a of the amino group. Figure 3 shows the variation of the nucleophilic rate constant (k_N) with the pH; as can be seen, both compounds show practically the same reactivity throughout the pH range studied.

As shown in Scheme 2, the overall rate constants of formation and hydrolysis of the Schiff bases can be described in terms of the individual constants for the different chemical species present in the medium at each pH.

Thus, k_1^i and k_2^i (with i = 0, 1, 2, or 3) are the individual rate constants of formation of the Schiff bases and of their hydrolysis by H_2O, respectively, and k_{OH}^2 is the rate constant of hydrolysis of species B_2 (a Schiff base with a net charge of -2) by OH^- ions. P_i (with i = 0, 1, 2 or 3) denotes the different chemical species of PLP, and pK_{3P}, pK_{2P}, and pK_{1P} the different pK_a values that relate them. B_i (with i = 0, 1, 2, or 3) are the different chemical species of the Schiff bases, and pK_{3B}, pK_{2B}, and pK_{1B} the pK_a values that relate them. Finally, K_N is the deprotonation equilibrium rate constant of the NH_3^+ group in L-DOPA. The experimental k_1 and k_2 values for the Schiff bases were fitted to Eqs. 3 and 4, derived from Scheme 2,[12]

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

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

$$k_2 = \frac{k_{OH} + \dfrac{k_2^2 a}{K_{3B}} + \dfrac{k_2^1 a^2}{K_{3B}K_{2B}} + \dfrac{k_2^0 a^3}{K_{3B}K_{2B}K_{1B}}}{1 + \dfrac{a}{K_{3B}} + \dfrac{a^2}{K_{3B}K_{2B}} + \dfrac{a^3}{K_{3B}K_{2B}K_{1B}}} \quad (4)$$

The nucleophilic rate constant k_N is

$$k_N = k_1\left(1 + \frac{a}{K_N}\right)$$

$$= \frac{k_1^3 + \frac{k_1^2 a}{K_{3P}} + \frac{k_1^1 a^2}{K_{3P}K_{2P}} + \frac{k_1^0 a^3}{K_{3P}K_{2P}K_{1P}}}{1 + \frac{a}{K_{3P}} + \frac{a^2}{K_{3P}K_{2P}} + \frac{a^3}{K_{3P}K_{2P}K_{1P}}} \quad (5)$$

The protonation constants for PLP[14] are pK_{1P} = 3.46, pK_{2P} = 6.02, and pK_{3P} = 8.22, and that for the Schiff bases required in the initial fitting were estimated from reported data for related systems.[9,10]

Tables 1 and 2 give individual rate constants of formation (k_1^i), the individual rate constants of hydrolysis (k_2^i) and the pK values obtained in the fitting. For a comparison, the values for the Schiff bases of PLP with other amino acids[9,15] and of PLP–CD system[10] are also given. The pK_N value for L-DOPA obtained in the fitting is 9.33, which agrees with the pK value determined spectrophotometrically at λ = 245 nm, and the same experimental condition of pK = 9.35

Due to the pH range studied and the expected value for pK_{3B} (about 11), there is no precision in fitting the k_2^2 and k_{OH} rate constants nor the pK_{3B} values.

As can be seen from Table 1, the k_1^i values for the reactions of the PLP–DOPA system and those for the reaction of PLP with different amino acids are quite similar; consequently, the side chains in the amino acids play no significant role in the formation of Schiff bases with PLP, as has been described.[9] A comparison of the k_1^i values obtained for the PLP–CD and PLP–DOPA systems (Table 1) reveals the absence of significant differences in the corresponding k_1^i. Accordingly, the greater reactivity expected in L-DOPA than CD, due to the presence of a hydrazine group in the latter,[8,10] is compensated for the presence of a methyl group in the CD. This effect is also apparent from a comparison of the k_N curves of Fig. 3, which do not exhibit any appreciable differences.

The rate-determining step for the formation of a Schiff base is known to be the dehydration of an intermediate α-hydroxy amine formed by an attack of the amine on the carbonyl group.[4,5] It is also known that, with PLP, the dehydration is subject to intramolecular acid catalysis, and that the phenol group at C-3 on the pyridine ring plays an especially prominent role in acid media.[16] Each k_1^i value in the PLP–DOPA system is very similar to the corresponding one in the PLP–CD system, resulting in Brönsted plots (log $k_1^{(i-1)}$ vs. pK_{iP}) for the PLP–DOPA and PLP–CD systems being very similar; because both are linear with α = 0.66, in both reactions the dehydration step is rate-determining.

Table 1. Best Kinetic Constant Values Obtained in the Fitting of Experimental k_1 Values to Scheme 2, and Those Corresponding to the PLP–GLY,[9] PLP–LEU,[9] PLP–ILE,[9] PLP–CD,[10] PLP–PHE,[15] and PLP–ALA[15] Systems

	Log k_1^3	Log k_1^2	Log k_1^1	Log k_1^0
PLP–DOPA	2.27	3.16	4.90	6.32
PLP–CD	1.90	3.32	4.89	6.51
PLP–GLY	2.50	3.79	5.52	7.30
PLP–LEU	2.25	3.36	5.05	7.16
PLP–ILE	2.34	3.54	5.20	7.31
PLP–PHE	2.23	2.96	4.75	—
PLP–ALA	2.39	3.23	5.01	—

(k_1 in L·mol^{-1}·min^{-1}). PHE and ALA denote L-phenylalanine and L-alanine, respectively.

At this point it is interesting to notice that the reaction mechanism is through an α-hydroxy amine intermediate, as in Eq. 6 (R_1–CHO is P_i (with i = 0, 1, 2 or 3)) and R_1–CH=N–R_2 is B_i (with i = 0, 1, 2, or 3); therefore the k_1^i rate constants in Scheme 2 involve the formation of α-hydroxy amine rate constants (k_{ci}) and those from the intermediate α-hydroxy amine to form the Schiff base by water elimination (k_{di}) and to return to reactants by amine elimination (k_{-ci}), besides $k_2^i = k_{-di}$.

$$R_1-CHO + NH_2-R_2 \underset{k_{-ci}}{\overset{k_{ci}}{\rightleftharpoons}} R_1-CH(OH)-NH-R_2 \underset{k_{-di}}{\overset{k_{di}}{\rightleftharpoons}} R_1-CH{=}N-R_2 + H_2O \quad (6)$$

If $K_{ci} = k_{ci}/k_{-ci}$ is defined as the equilibrium formation constant for α-hydroxy amine, provided this is formed and split fairly rapidly, and this is completely shifted to the reactants, then $k_1^i = K_{ci}k_{di}$ and $k_2^i = k_{-di}$. This is in accord with linear plots of k_{obs} vs [L-DOPA] obtained at different pH values.

The maximum of the k_1 curve for the PLP–CD system (Fig. 1) is a result of the pK_a of the amino group of carbidopa (pK_a = 7.2) and of the sequence $k_1^0 > k_1^1 > k_1^2$ (the reactivity de-

Table 2. Best Kinetic Constant Values Obtained in the Fitting of Experimental k_2 Values to Scheme 2, and pK Values for the Schiff Bases, and Those Corresponding to the PLP–GLY,[9] PLP–LEU,[9] PLP–ILE,[9] and PLP–CD[10] Systems

	PLP–DOPA	PLP–CD	PLP–GLY	PLP–LEU	PLP–ILE
Log k_2^0	−1.75	−4.63	0.584	0.170	−0.010
Log k_2^1	−0.596	−0.04	0.676	0.080	−0.170
Log k_2^2	—	−1.32	−0.550	−0.900	−1.000
Log k_{OH}	—	−3.85	1.160	1.080	1.020
pK_{1B}	5.85	6.97	5.46	5.68	5.67
pK_{2B}	6.37	7.15	6.36	6.65	6.66
pK_{3B}	—	11.35	11.35	11.61	11.57

(k_2 in min^{-1}).

creases with increasing pH). The net balance between these two opposing effects (the free amine increases while the reactivity decreases with increasing pH) leads to the maximum in Fig. 1. Due to the higher pK_a value of the NH_3^+ value of L-DOPA (pK_a = 9.33) and the pH range studied, no maximum is observed for the k_1 values of the PLP–DOPA system.

Figure 2 reveals an increased stability of the Schiff base of L-DOPA relative to that of carbidopa, consistent with the values of the individual k_2^i constants given in Table 2. The presence of non-polar groups in the vicinity of the hydrolysis site reportedly protects the imine bond from hydrolysis,[9] as found, for example, by comparing the Schiff bases of PLP with L-leucine (LEU) and L-isoleucine (ILE), and those of the same substrate with glycine (GLY) and LEU (Table 2). This effect has also been observed in the Schiff bases of PLP with poly L-lysine,[17] where the polypeptide chain provides a less-polar environment, and leads to bases of increased stability. Accordingly, the methyl group in carbidopa should result in less readily hydrolyzed bases. However, the behavior of the Schiff bases studied here leads to the opposite conclusion, probably due to other differences in the structure of the corresponding Schiff base (viz. the amine or hydrazine group).

In Scheme 2 the hydrolysis reactions of the forms B_i (i = 0, 1) for OH^- has been omitted because of the very small concentration of OH^- at the pH at which the concentration of B_i (i = 0, 1) is important, as can be obtained from the pK values for the various forms of the Schiff bases derived from both systems (Table 2).

The conclusions of this study are: i) The k_1^i and k_N values for PLP–DOPA and PLP–CD systems show the same reactivity to PLP by both amine group bearer in the whole of the pH range studied. ii) The greater values for the overall rate constant of formation k_1 for the reaction of PLP with carbidopa than L-DOPA are a consequence of the different pK_a of both amine groups. iii) At physiological pH, the k_1 value for the PLP–CD system is about 100-times greater than that for the PLP–DOPA system. iv) Probably the same effect can occur at the enzymatic level (i.e. transimination reaction), thus inhibiting dopa-decarboxylase and, consequently, the carbidopa presence permits that a greater L-DOPA quantity can get to the brain.

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