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TRANSIMINATION REACTION BETWEEN PYRIDOXAL-5′-PHOSPHATE SCHIFF BASES WITH DODECYLAMINE AND AMINO ACIDS

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Some transimination processes were studied using an intermolecular model formed by pyridoxal-5′-phosphate (PLP) an amino acid and dodecylamine (DOD) in an aqueous medium. All the kinetic constants for the reversible reaction were determined. The results show that in these cases transimination proceeds through an addition–elimination by forming a diamine geminal intermediate. Equilibria are always shifted to dodecylamine–PLP Schiff base formation. Differences between the stability of this Schiff base and the ε-aminocaproic Schiff base cannot be explained only on the basis of the different nucleophicities of amine groups and therefore differences in the imine double bond environment must be taken into account to explain this behaviour.

INTRODUCTION

It is generally accepted that the first reaction catalysed by a pyridoxal-5′-phosphate (PLP)-dependent enzyme is one of transimination, viz. one involving the displacement of the amino group of lysine in the enzyme by an amino group in the substrate. This reaction converts amines into imines and *vice versa*.

From a chemical point of view, transimination is a symmetrical, reversible process that can take place via two reaction pathways (Scheme 1).[1–3] One involves free PLP and two carbinolamines (CA-E and CA-L) as intermediates, whereas the other involves only one intermediate (a geminal diamine, GD, which is structurally analogous to a carbinolamine). Some workers claim that transimination takes place via a geminal diamine[4–7] whereas others believe it involves a twofold addition–elimination.[8,9] This fast, reversible process is of great biochemical interest insofar as it plays a crucial role in cellular reactions.[10]

Studies on this type of reaction in enzyme systems are made extremely difficult by its high rate and complexity.[10]

Non-enzymatic transimination has so far been studied by using intramolecular and intermolecular chemical model systems. The intramolecular displacement reaction can be used as a reliable model in conformational studies on enzyme active sities. In fact, geminal diamines have been used as model compounds in a number of UV and NMR spectroscopic studies[11–18] which have allowed the determination of the rate and equilibrium constants of various cyclization and tautomerization processes involving geminal diamines as intermediates. Acid–base catalysis and the effect of the solvent on the proton transfer involving the geminal diamine have also been studied in this way.[10,19,20]

The bimolecular displacement reaction has the advantage of being slow enough for UV spectroscopic monitoring. In addition, the concentration of the intermediate geminal diamine is usually so low that its actual occurrence cannot be confirmed by UV spectroscopy. However, it poses problems arising from the great similarity of the UV spectra of the Schiff bases of a number of amines and/or amino acids. This problem can be circumvented by using hydroxylamine or a semicarbazide as attacking amine in order to obtain a reaction product absorbing at a different wavelength.[15,21–23] However, the low basicity of these amines makes them poor enzyme models.[10,23] Weng

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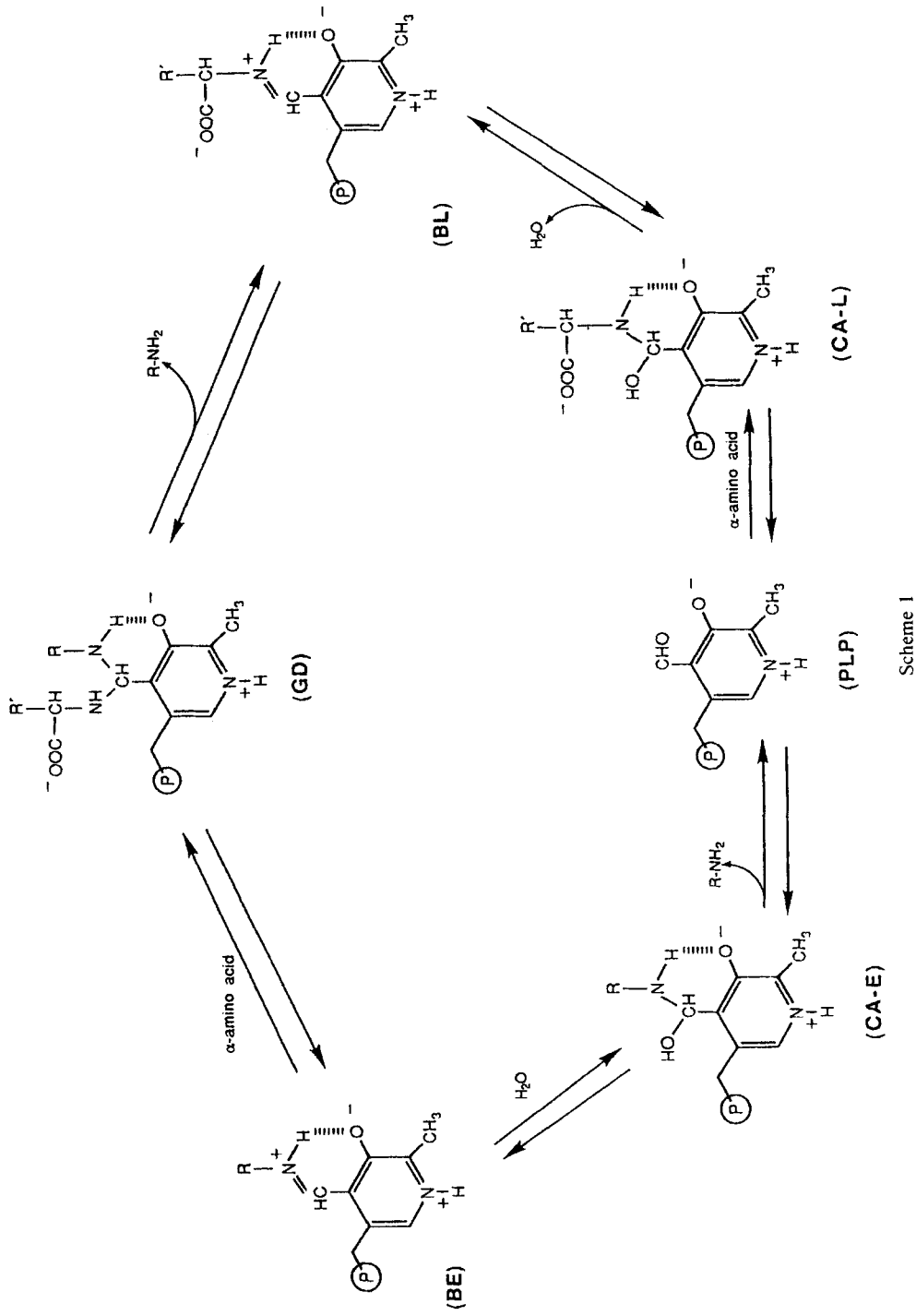


Scheme 1

and Leussing[24] used Zn(II) to resolve the spectrum of the Schiff base of ethylamine from that of an α-amino acid, the latter of which was complexed by the metal ion to form a chelate that absorbs at a different wavelength. This type of kinetic study is complex owing to the large number of solution equilibria involved.

We recently used an enzyme–PLP model in an aqueous medium formed by the Schiff base of PLP with dodecylamine, the UV spectrum of which is different from those of the Schiff bases of PLP and α-amino acids and amines.[25] Our kinetic studies revealed a significant increase in the rate of formation of the Schiff base and a decrease in the rate of hydrolysis, both as a result of the dual hydrophilic–hydrophobic character of the system in question.[26] On the other hand, the analysis of the UV spectral bands showed enol tautomers to prevail over their keto counterparts over wide pH ranges.[25]

In this work we carried out a kinetic study on transimination by using an intermolecular model formed by PLP, an amino acid and dodecylamine in an aqueous medium in order to establish the mechanism of the process and to determine the influence of pH on its rate constant and whether the different protonatable groups in the Schiff base exert any catalytic effects. This is the first reported model system made up of amines and amino acids of similar basic strength that allows the transimination process to be studied without the need to use a metal ion.

EXPERIMENTAL

Materials. L-Leucine, glycine, ε-aminocaproic acid and dodecylamine were purchased from Sigma and were used without further purification. All other chemicals were supplied by Merck. The ionic strength was kept at 0·1 M by using either an acetate or a HEPES buffer.

PLP stock solutions (*ca* 3×10^{-5} M) were prepared daily in an appropriate buffer and stored in the dark. Their exact concentrations were determined from absorbance measurements after dilution with 0·1 M HCl.[27]

Amino acid solutions were also prepared daily by diluting the appropriate volumes of the concentrated solutions with buffer and adjusting the pH with HCl or NaOH as required. Their concentrations ranged between 1×10^{-2} and 8×10^{-2} M. Dodecylamine solutions were prepared from the hydrochloride as described elsewhere.[25]

pH measurements were made with a Crison pH-meter. As checked in every experiment, the initial and final pH values in the reaction cell never differed by more than ±0·03.

A Uvikon 940 spectrophotometer furnished with thermostated cells of 1 cm light path was used to obtain UV spectra. The temperature was kept constant at 25·00 ± 0·05 °C throughout the experiments.

Methods. The Schiff base of PLP and the amino acid was pre-formed in solution by adding to the already buffered PLP solution the amino acid at the desired pH. In order to shift the equilibrium as much as possible towards imine formation, the initial amino acid concentration was 500–1000 times higher than that of PLP.

The transimination reaction was started by adding a few millilitres of the dodecylamine solution to a cell which already contained the pre-thermostated Schiff base solution in the same buffer and at the same pH. The dodecylamine concentration in the measuring cell was 200–800 times higher than that of the Schiff base. The reaction was monitored by measuring the decrease in the absorbance at 420 nm.

Transimination between a Schiff base and an amine takes place according to the following reaction:

$$R_1CH=NR_2 + R_3NH_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R_1CH=NCHR_3 + R_2NH_2$$

which is second order in both directions. However, since R_2NH_2 and R_3NH_2 were in excess with respect to the Schiff bases under the experimental conditions used, both can also be considered to be of pseudo-first order. Thus, on the basis of the Beer–Lambert law, the rate of the process can be expressed by

$$\ln(A_0 - A_\infty)/(A - A_\infty) = k_{obs}t \quad (1)$$

$$k_{obs} = k_1 a + k_2 b \quad (2)$$

where a and b are the initial concentrations of dodecylamine and the amino acid, respectively, and A_0, A_∞ and A are the absorbances at time zero, infinity and t, respectively.

Constant k_{obs} was obtained from the slopes of the plots of $\ln(A - A_\infty)$ vs time. The correlation coefficient obtained in the fitting was always $\geqslant 0{\cdot}999$. The k_{obs} values thus obtained were in turn used to calculate the rate constants k_1 and k_2.

The overall rate constants of hydrolysis and formation of the aldimine can be described in terms of the rate constants for the individual ionic species involved in each case.

Scheme 2 shows all the possible chemical species of the Schiff bases of PLP with an amino acid (BL) and dodecylamine (BD). Subscripts denote the number of net negative charges of the different molecules. As the working pH ranged between 3·3 and 8·5, the fully deprotonated species, which occurs above pH 11 only, was excluded from the study. Species BL_1 was only considered in relation the Schiff base PLP–CA, in which the pK of the carboxyl group is 4 (that of the carboxyl group of the Schiff bases with α-amino acids ranges between 2.1 and 2.2). According to Scheme 2, species

BD_{-1} can also be attacked by the monoprotonated amino acid (CA). Constants k_1^i ($i = -1, 0, 1, 2$ or 3) are the specific rate constants for the transimination between the Schiff base of BL with dodecylamine, while k_2^i ($i = -1, 0, 1$ or 2) and $k_2'^{-1}$ are their counterparts for the Schiff base of BD with an amino acid.

The rate of transimination of the Schiff base of an amino acid (BL) with dodecylamine is given by

$$v_1 = [D_0] \sum_{i=-1}^{3} k_1^i [BL_i] = k_1 [BL]_T [DOD]_T \quad (3)$$

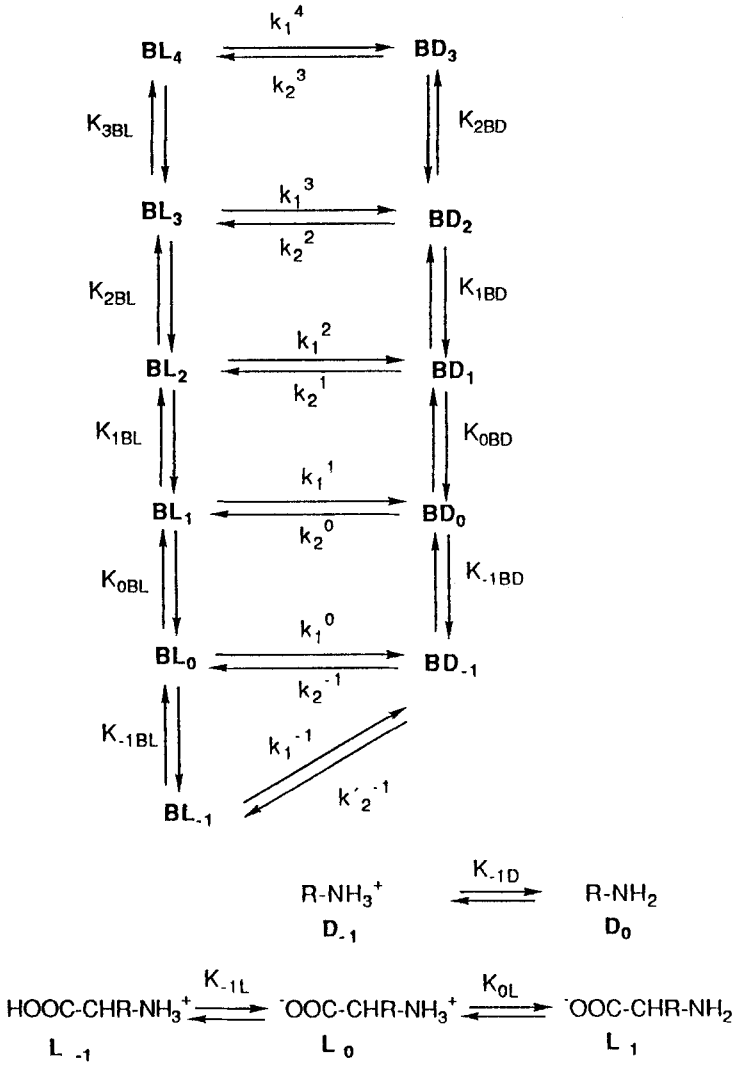
where T denotes the total concentration of all species. On the other hand, the rate of transimination of the Schiff base of dodecylamine (BD) and an amino acid (L) is given by

$$v_2 = k_2'^{-1} [BD_{-1}] [L_0] + [L_1] \sum_{i=-1}^{2} k_2^i [BD_i] = k_2 [BD]_T [L]_T \quad (4)$$

The equilibrium constant, K_{pH}, can be expressed as

$$K_{pH} = k_1/k_2 = \frac{[BD]_T [L]_T}{[BL]_T [D]_T} \quad (5)$$

Taking into account the equilibrium in Scheme 2,



Scheme 2

equations (3)–(5) can be readily transformed into

$$k_1 = \frac{k_1^3 + k_1^2\frac{h}{K_{2BL}} + k_1^1\frac{h^2}{k_{2BL}K_{1BL}} + k_1^0 \frac{h^3}{K_{2BL}K_{1BL}K_{0BL}} + k_1^{-1}\frac{h^4}{K_{2BL}K_{1BL}K_{0BL}K_{-1BL}}}{\left(1+\frac{h}{K_{-1D}}\right)\left(1+\frac{h}{K_{2BL}}+\frac{h^2}{K_{2BL}K_{1BL}} + \frac{h^3}{K_{2BL}K_{1BL}K_{0BL}} + \frac{h^4}{K_{2BL}K_{1BL}K_{0BL}K_{-1BL}}\right)} \quad (6)$$

$$k_2 = \frac{k_2^2 + k_2^1\frac{h}{K_{2BD}} + k_2^0\frac{h^2}{K_{2BD}K_{1BD}} + (k_2^{-1} + k_2'^{-1}h/K_{0L})\frac{h^3}{K_{2BD}K_{1BD}K_{0BD}}}{\left(1+\frac{h}{K_{0L}}+\frac{h^2}{K_{0L}K_{-1L}}\right)\left(1+\frac{h}{K_{2BD}} + \frac{h^2}{K_{2BD}K_{1BD}} + \frac{h^3}{K_{2BD}K_{1BD}K_{0BD}}\right)} \quad (7)$$

$$K_{pH} = \frac{\left(1+\frac{h}{K_{0L}}+\frac{h^2}{K_{0L}K_{-1L}}\right)\left(1+\frac{h}{K_{2BD}} + \frac{h^2}{K_{2BD}K_{1BD}} + \frac{h^3}{K_{2BD}K_{1BD}K_{0DB}}\right)K_M}{\left(1+\frac{h}{K_{-1D}}\right)\left(1+\frac{h}{K_{2BL}}+\frac{h^2}{K_{2BL}K_{1BL}} + \frac{h^3}{K_{2BL}K_{1BL}K_{0BL}} + \frac{h^4}{K_{2BL}K_{1BL}K_{0BL}K_{-1BL}}\right)} \quad (8)$$

$$K_M = \frac{[BD_2][L_1]}{[BL_3][D_0]} \quad (9)$$

where h is the proton concentration.

Experimental data for k_1, k_2 and K_{pH} were fitted to the corresponding theoretical equations by means of a non-linear regression method and minimizing the function U_i:

$$U_i = \Sigma(\log k_{i,e} - \log k_{i,t})^2 \quad (10)$$

where subscripts e and t refer to experimental and theoretical data, respectively.

RESULTS AND DISCUSSION

Transimination between a Schiff base of PLP (BE) and an amine or amino acid can take place through two different pathways (see Scheme 1), namely, addition–elimination via an intermediate geminal diamine (GD) and twofold addition–elimination yielding two carbinolamines (CA-E, CA-L) and free PLP.

As noted in the Introduction, the main difficulty encountered in studying transimination reactions on intermolecular model systems lies in the similarity between the UV spectra of the Schiff bases of PLP with amines and amino acids.[10] Figures 1(A) and (B) show the analysis of the bands yielded by the monoprotonated species of the Schiff bases of PLP with L-leucine and dodecylamine.[25,28] As can be seen, there are major differences in molar absorptivity between the two in the regions of 335 and 425 nm throughout the pH range, which allows the process involved to be studied kinetically.

As stated in the Experimental section, the k_{obs} values of the transimination process can be obtained from fitting experimental absorption results to equation (1). Different values of this parameter can be obtained when the initial concentration of dodecylamine and/or amino acid is changed [equation (2)]. Table 1 gives some values of k_{obs} at different pH values for the transimination reaction between the Schiff base of glycine and PLP and the dodecylamine, and also k_1 and k_2 values obtained from fitting the experimental k_{obs} to equation (2). Values of k_1 and k_2 obtained in every case from this kind of fitting present errors similar to those shown in Table 1, but they were never more than 4% of the total value.

Figures 2 and 3 show the second-order rate constants of transimination for the Schiff bases of PLP with amino acids (BL) and dodecylamine (BD). The symbols denote experimental values and the theoretical curves were obtained from equations (6) and (7), respectively, and the data listed in Tables 2 and 3. Such rate constants are always larger than the corresponding hydrolysis rate constants, which range between 0·5 and 0·005 min^{-1}. This behaviour indicates that transimination in our model system must take place via a geminal diamine.

The rate constant k_1 increases with increasing pH as a result of the increase in the unprotonated DOD concentration ($pK_{1D} = 10{\cdot}63$) up to pH 7–8, above which log k_1 remains constant despite the increase in the free amine concentration. This behaviour can be ascribed to the variation in the deactivating power of the pyridine ring with the degree of protonation. Since the pyridine nitrogen is deprotonated at pH 6·5, the electrophilic character of the imine double bond is diminished as a result. This varying reactivity has also been observed in imines from PLP analogues, the pyridine nitrogen of which was found to decrease the reactivity of the imine bond by a factor of 40–600 on deprotonation.[1,4,29]

On comparing the values of k_2 with those of the reverse process (k_1), it is seen that the former are considerably smaller. Such a great difference in the rate constants cannot be explained by taking into account only the differences in the nucleophilicities of the different amines, as discussed below some aspects must be pointed out.

We recently showed that the different possible chemical species of the Schiff base of PLP with dodecylamine (BD) occur preferentially in their enol forms (Scheme 3), in which the imine double bond lies in a hydrophobic environment made up of the non-polar residues of the dodecylamine chain.[25,26] On the other hand, as can be seen in Scheme 4, which shows the predominant tautomers of the different chemical species of the Schiff bases of PLP with amino acids,[16,28] and bearing in mind the data in Table 2, the imine nitrogen was always protonated and hence highly electrophilic and polar throughout the pH range studied.

On the other hand, and according to other workers, the rate-determining step of the transimination process is the addition–elimination of the less basic amine.[10] Therefore, k_2 must correspond to the addition and k_1 to the elimination of the amino acid to the Schiff base of dodecylamine in the glycine and leucine cases.

ε-Aminocaproic acid and dodecylamine present very similar ionization constants (see Table 2), and therefore similar values must be expected for k_1 and k_2 in this case. Nevertheless, k_1 is very much higher than k_2, which can be explained by assuming that the dodecylamine Schiff base is embedded in a very hydrophobic environment where the imine double bond is protected from the attack of amino acid and the small values obtained for k_2 in all the cases studied must therefore be due to the low accessibility of the imine double bond to the polar amino acid molecule since the imine lies in a non-polar environment.

The variation of log k_2 with pH is in principle anomalous since, as with log k_1, log k_2 should increase with increasing pH as a result of the increase in the free amine concentration. However, log k_2 initially decreases sharply between pH 3 and 5·5–6, then

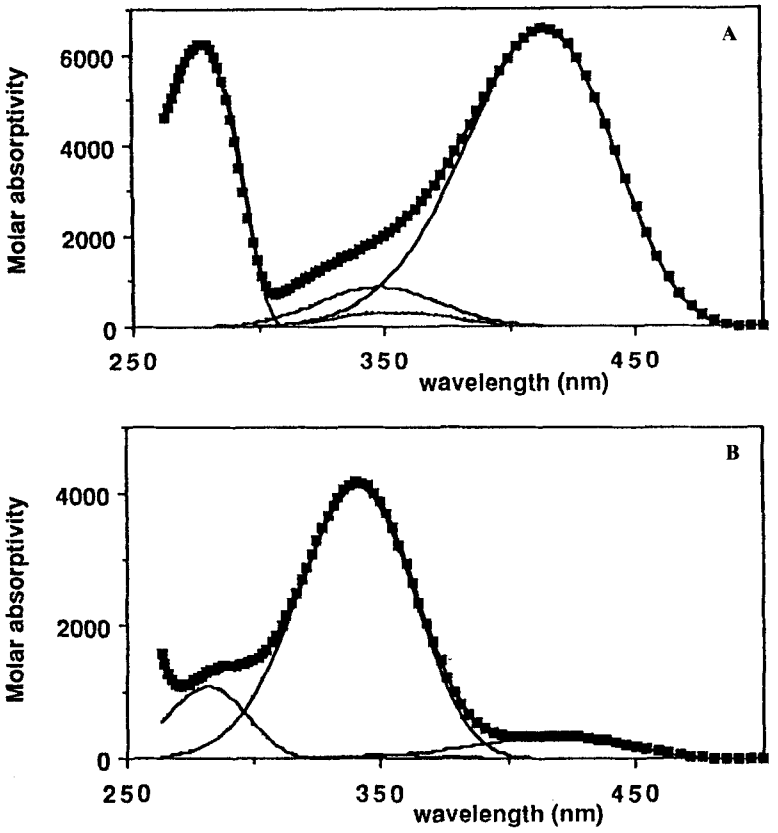


Figure 1. Absorption spectra of monoprotonated ionic species of the Schiff bases, (A) PLP and leucine and (B) PLP and dodecylamine, in aqueous solution fitted with log-normal distribution curves

Table 1. k_{obs} values obtained by fitting experimental absorption data to equation (1) for the transimination reaction between the PLP and glycine Schiff base and dodecylamine, and best kinetic constants k_1 and k_2 obtained by fitting experimental values to equation (2)

pH	Glycine concentration (10^{-2} M)	k_{obs} (min^{-1})	DOD concentration (10^{-3} M)	Parameters[a]
4·76	4·99	4·48	6·10	$k_1 = 721 \cdot 45$
		5·93	7·93	$k_2 = 1 \cdot 77$
		7·38	10·3	$\rho = 0 \cdot 999$
		8·83	12·1	$\delta = 0 \cdot 1226$
		10·28	14·3	
		11·73	15·9	
		13·19	18·2	
6·26	3·47	10·75	5·93	$k_1 = 1765 \cdot 8$
		14·23	8·01	$k_2 = 1 \cdot 51$
		17·86	10·2	$\rho = 1 \cdot 000$
		21·42	12·3	$\delta = 0 \cdot 231$
		24·97	14·1	
		28·52	16·0	
		32·07	18·1	
8·15	2·82	14·21	6·08	$k_1 = 2205 \cdot 9$
		18·80	8·03	$k_2 = 23 \cdot 39$
		23·37	10·6	$\rho = 0 \cdot 999$
		27·96	12·5	$\delta = 0 \cdot 485$
		32·54	14·3	
		37·11	16·7	
		41·68	18·4	

[a] ρ is the correlation coefficient and δ is the standard deviation of fitting to equation (2).

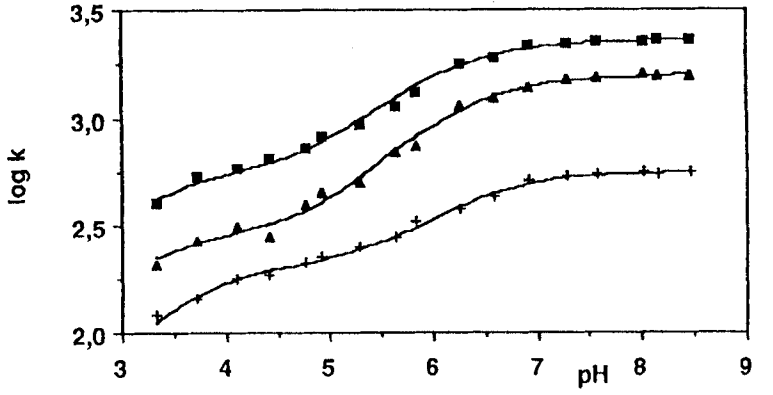


Figure 2. Variation of log k_1 as a function of pH for the transimination of Schiff bases of PLP–amino acids and dodecylamine. Amino acids: (■) glycine; (▲) leucine; (+) ε-aminocaproic acid. Symbols are experimental values and continuous lines are the theoretical fitting to equation (6)

increases above pH 7. This behaviour can be explained on the basis of the pK_{iBD} value of each chemical species involved. Thus, pK_{0BD} for the pyridine nitrogen in the Schiff base of PLP with DOD (Scheme 4) is 3·64, i.e. much lower than that of the Schiff base of PLP with an amino acid (BL, $pK_{1BL} = 6 \cdot 5$).[16,29] This deprotonation at such a low pH value results in a decrease in the electrophilic character of the carbon atom in the imine double bond and hence in a decrease in the rate constant, despite the increase in the free amine concentration. The subsequent deprotonation of the Schiff base (phosphate group, $pK = 7 \cdot 91$) and the increase in the free amine concentration result in an increase in the rate constant. On comparing the log k_2 values for the transimination of leucine and glycine, both of which feature the same basic strength ($pK = 9 \cdot 76$), it is seen

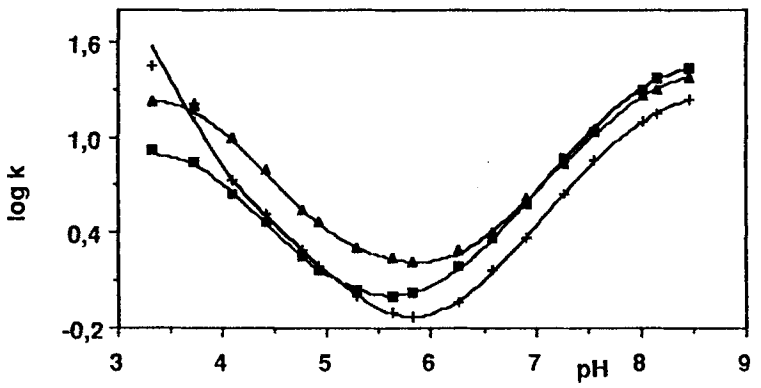


Figure 3. Variation of log k_2 as a function of pH for the transimination of Schiff bases of PLP–dodecylamine and (■) glycine, (▲) leucine and (+) ε-aminocaproic acid. Symbols are experimental values and continuous lines are the theoretical fitting to equation (7)

Table 2. Ionization constants of glycine (Gly), L-leucine (Leu), ε-aminocaproic (CA) and dodecylamine (DOD) and their Schiff bases with PLP (BL and BD, see text), taken from Refs 25, 26 and 29

Constant	Gly	Leu	CA	DOD
pK_{3BL}	11·35	11·61	11·70	
pK_{2BL}	6·36	6·65	6·42	
pK_{1BL}	5·46	5·68	5·69	
pK_{0BL}	2·84	2·83	3·93	
pK_{-1BL}	2·16	2·25	2·99	
pK_{0L}	9·76	9·76	10·45	
pK_{-1L}	2·37	2·31	4·07	
pK_{2BD}				11·31
pK_{1BD}				7·91
pK_{0BD}				3·64
pK_{-1BD}				2·96
pK_{0D}				10·63

that leucine yields greater values as a result of the presence of a non-polar carbon residue (isopropyl), which makes this amino acid more accessible than glycine to the hydrophobic environment of the imine double bond.

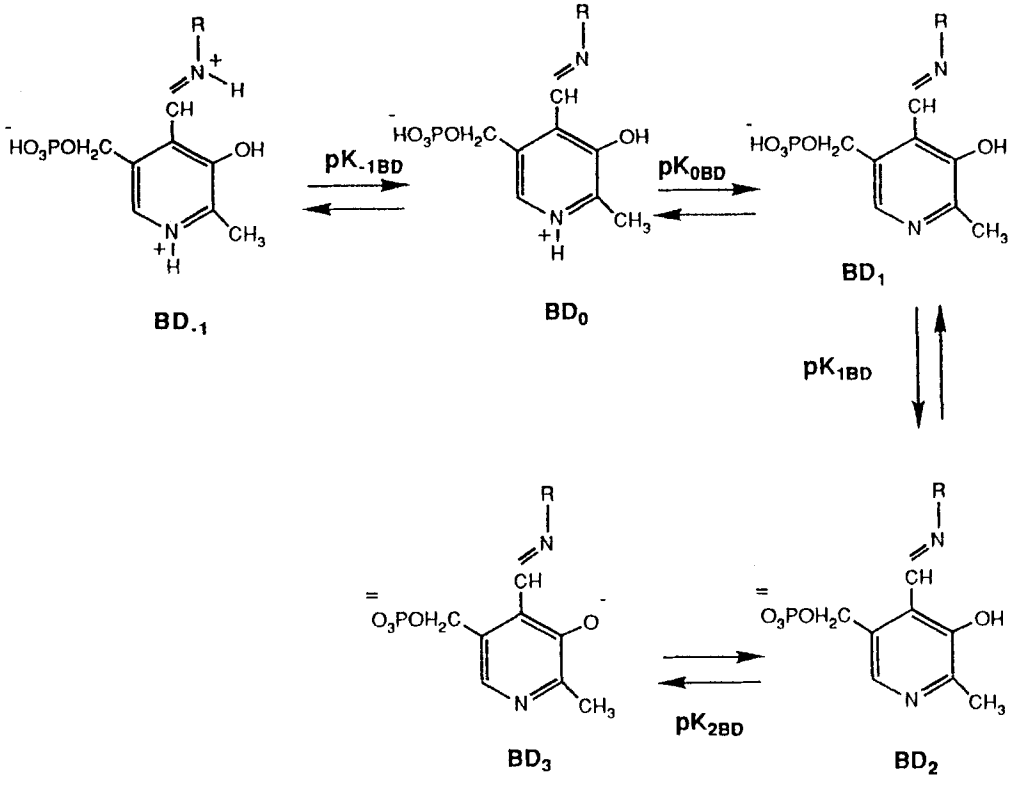
The k_2 values for the transimination of ε-aminocaproic acid above pH 4 are greater than those for other amino acids despite the greater basic strength of its amino group ($pK = 10·4$). This behaviour can be ascribed to the fact that the carboxyl group in ε-aminocaproic acid (CA) is more distant from the amine group and its pK (4.07) is higher than those of α-amino acids. Thus, below pH 4, the carboxyl group is protonated and must favour the addition of the amino acid by providing a non-polar environment from the carbon residue. With α-amino acids the carboxyl group is always deprotonated throughout the pH range studied, thereby inducing some polarity that disfavours the interaction with the dodecyl group. Above pH 7, the k_2 value for CA is smaller than it is for glycine and leucine as a result of the greater basic strength of the amino group and hence the lower free amine concentration available.

Scheme 5 shows the intermediate geminal diamines involved in the transimination process. The attack of dodecylamine (DOD) on the Schiff base of an amino acid begins with a rapid addition of the dodecyl residue to the Schiff base (BL) to yield a highly polar geminal diamine (GD1) that is converted into another geminal diamine (GD2) by proton transfer. This latter has a

Table 3. Best kinetic constants and K_M values obtained from fitting of experimental values of k_1 (l mol^{-1} min^{-1}) and k_2 (l mol^{-1} min^{-1}) to equations (6)–(8) for the transimination of PLP Schiff bases

Parameter[1]	PLP–Gly	PLP–Leu	PLP–CA
Log k_1^4	—	—	—
Log k_1^3	3·52	3·23	3·01
Log k_1^2	7·63	7·18	6·95
Log k_1^1	8·35	7·99	7·45
Log k_1^0	10·53	10·26	9·02
Log k_1^{-1}	—	—	9·78
$\delta(k_1^i)$	0·013	0·010	0·017
Log k_2^3	—	—	—
Log k_2^2	0·85	0·66	1·43
Log k_1^2	3·41	3·35	3·88
Log k_2^0	5·95	6·20	6·43
Log k_2^{-1}	8·01	8·34	8·93
Log k_2^{-1}	—	—	12·72
$\delta(k_2^i)$	0·008	0·007	0·010
Log K_M	2·67	2·57	1·58
$\delta(K_M)$	0·022	0·018	0·029

[a] $\delta(i)$ is the standard deviation of fitting of the respective equation.



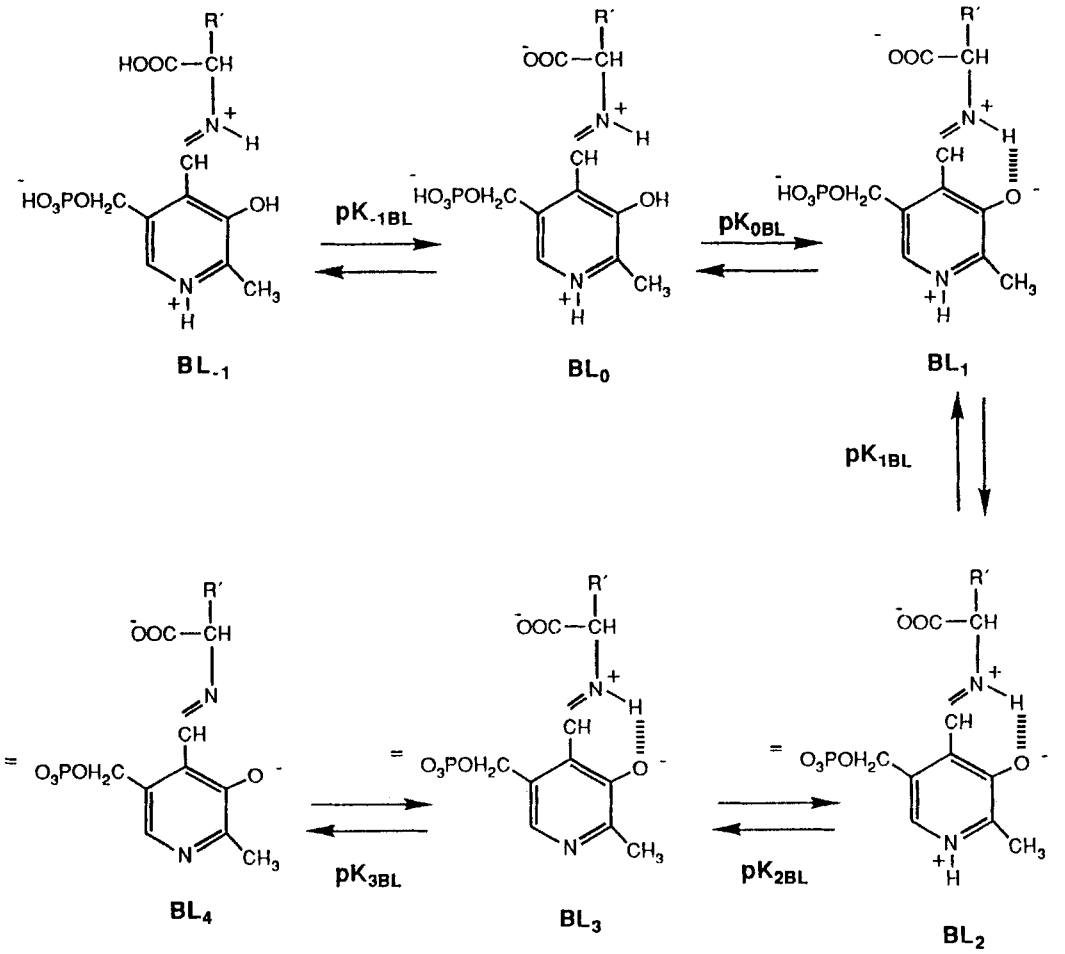
Scheme 3

non-polar structure as a result of the amphiphilicity of DOD. Rotation about C-4′ yields a new geminal diamine (GD3) which possesses an appropriate conformation to undergo a new proton transfer to yield a fourth geminal diamine (GD4) that loses one amino acid molecule to yield the Schiff base of dodecylamine. As the rate-determining step of this process is the elimination of the amino acid from GD4, k_1 must correspond to this step. As can be seen, the portion of the diamine next to the would-be imine bond is highly polar, so it will interact more readily with polar amino acids (Scheme 5). As can be seen in Figure 2, the largest k_1 values were obtained for the elimination of glycine, viz. the most polar of the three amino acids assayed, its pK being identical with that of leucine. The log k_1 values obtained for the elimination of ε-aminocaproic acid are substantially smaller than those of leucine. As the only difference between CA and LEU is the relative position of carboxyl group (in ε- and α-positions, respectively), such a great difference must be the result of the geminal diamine being unstabilized by polar or charged groups close to C-4′. Hence, the ε-carboxyl group of CA will interact with greater difficulty with the non-polar dodecyl residue, particularly above pH 5, where the carboxyl group is fully deprotonated (see Figure 2).

Table 4 lists the K_{pH} values of the transimination process calculated as k_1/k_2 and as the ratios between the formation equilibrium constants of the Schiff bases BD and BL from PLP and the respective amine or amino acid. As can be seen, the two sets of values are fairly consistent, which testifies to the reliability of the kinetic results obtained for the transimination process.

Figure 4 shows the log K_{pH} values for the different transimination equilibria studied. The symbols denote experimental values and the theoretical curves were obtained from equation (8) and the data listed in Tables 2 and 3. The variation of these equilibrium constants with pH is the result of the different stabilities of the Schiff bases of BD and BL with the acidity of basicity of the medium. Thus, K_{pH} is maximum at pH 6 (the pH at which the Schiff base of BD is most stable) and decreases above this value as a result of the increase in the equilibrium constant of the Schiff base of BL.

The kinetic constants of transimination are the result of the different microscopic kinetic constants (k_1^i and k_2^i, Table 3) and ionization constants of the Schiff bases, dodecylamine and amino acid, according to

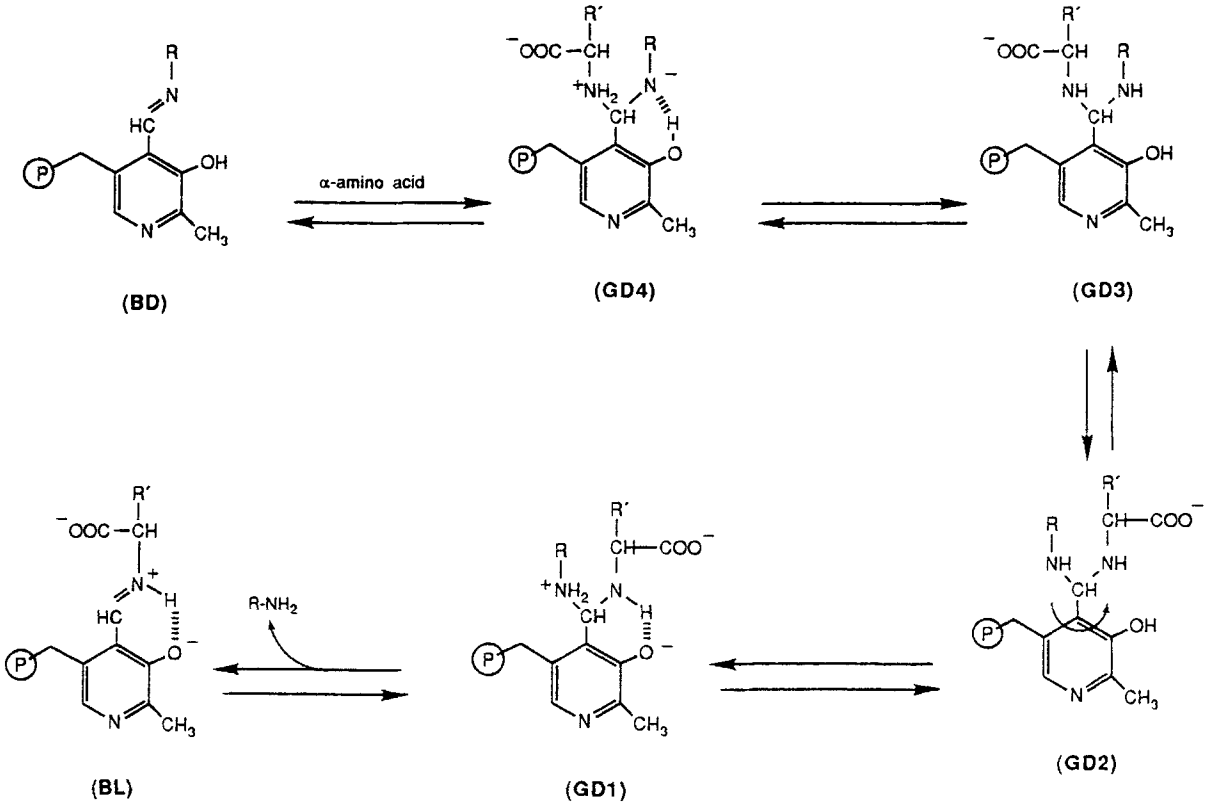


Scheme 4

Scheme 2. The k_1^i values increased with decreasing pH for all the Schiff bases studied. The Brønsted plots of log k_1^i against the pK values of the Schiff bases of BL were fitted to a straight line of slope 0·8 with a high correlation coefficient. This indicates that the more protonated species, with lower pK values, react more rapidly and/or the presence of acid catalytic effects, which in this case seems to be improbable.

On the other hand, the largest specific constants were obtained for the transimination of the Schiff base of glycine, which can be attributed to the higher polarity of this amino acid compared with the others studied. On comparing the specific rate constant for each chemical species of the three Schiff bases studied, it is seen that the trend is identical with that of the pH-dependent overall rate constants as a result of their similar pK values.

The specific rate constants (k_2^i) for the attack of the different amino acids on the Schiff base of BD confirm the assumption that the attack of an α-amino acid on the imine double bond is disfavoured by the non-polar environment of the imine. As can be seen in Table 2, the addition of an amino acid to any of the chemical species of the Schiff base of dodecylamine is always faster for CA than it is for an α-amino acid. Accordingly, at any pH, the greater the distance between the carboxyl and amino groups the readier the addition of the latter to the imine double bond will be. It should be noted that the rate constant for the attack of the monoprotonated species of CA (with a protonated carboxyl group), k_2^{i-1}, on species BL_{-1} is larger than that of the unprotonated species of CA by about four orders of magnitude. This supports our previous assumption that the nucleophilic attack on the double bond of BD is



Scheme 5

Table 4. Logarithms of equilibrium constants (log K_{pH}) of the overall transimination reaction between some PLP and amino acid Schiff bases and dodecylamine at several pH values[a]

pH	PLP–Gly	PLP–Leu	PLP–CA
3·33	1·69 ± 0·09(1·73)	1·10 ± 0·10(1·12)	0·71 ± 0·10(0·58)
3·72	1·89 ± 0·08(1·86)	1·23 ± 0·12(1·26)	1·04 ± 0·10(1·05)
4·10	2·11 ± 0·09(1·91)	1·50 ± 0·09(1·49)	1·52 ± 0·08(1·52)
4·41	2·34 ± 0·07(2·33)	1·76 ± 0·09(1·73)	1·75 ± 0·07(1·78)
4·76	2·61 ± 0·06(2·60)	2·05 ± 0·09(2·02)	1·81 ± 0·07(2·02)
4·92	2·75 ± 0·09(2·72)	2·19 ± 0·06(2·15)	2·05 ± 0·09(2·14)
5·28	2·93 ± 0·06(2·95)	2·40 ± 0·08(2·42)	2·20 ± 0·09(2·39)
5·63	3·04 ± 0·08(3·08)	2·60 ± 0·07(2·62)	2·44 ± 0·06(2·56)
5·82	3·10 ± 0·05(3·12)	2·65 ± 0·09(2·69)	2·63 ± 0·09(2·61)
6·26	3·06 ± 0·10(3·07)	2·76 ± 0·07(2·75)	2·61 ± 0·05(2·62)
6·59	2·91 ± 0·07(2·92)	2·68 ± 0·07(2·68)	2·48 ± 0·08(2·50)
6·91	2·74 ± 0·08(2·73)	2·53 ± 0·10(2·54)	2·33 ± 0·08(2·31)
7·27	2·47 ± 0·10(2·48)	2·33 ± 0·09(2·33)	2·08 ± 0·10(2·08)
7·57	2·32 ± 0·15(2·28)	2·16 ± 0·13(2·16)	1·89 ± 0·11(1·88)
8·02	2·05 ± 0·11(2·05)	1·90 ± 0·13(1·94)	1·65 ± 0·15(1·64)
8·15	1·99 ± 0·16(2·00)	1·82 ± 0·14(1·89)	1·60 ± 0·13(1·59)
8·46	1·92 ± 0·15(1·92)	1·75 ± 0·14(1·82)	1·51 ± 0·13(1·51)

[a] The intervals (±) are the standard errors for the 95% confidence level. Values in parentheses calculated as the ratio between the formation equilibrium constants of the Schiff bases BD and BL (see text) taken from Refs 26 and 29.

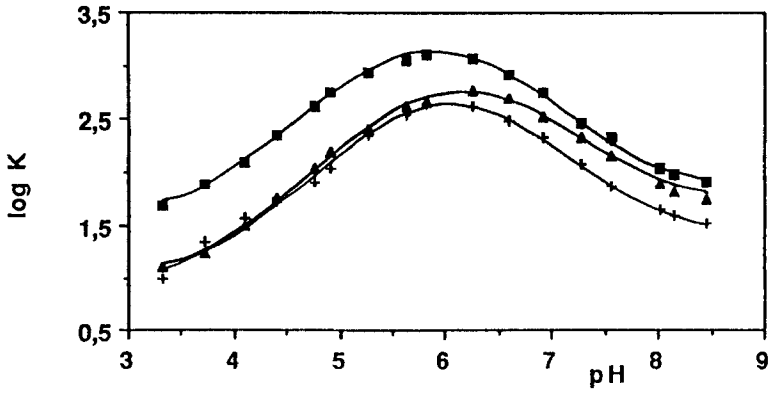


Figure 4. Variation of log K_{pH} as a function of pH for the transimination of Schiff bases of PLP–amino acids and dodecylamine. Amino acids: (■) glycine, (▲) leucine and (+) ε-aminocaproic acid. Symbols are experimental values and continuous lines are the theoretical fitting to equation (8)

favoured by the presence of uncharged and/or non-polar molecules.

The specific kinetic constants of transimination (k_2^i) to the Schiff base of dodecylamine (BD) also increase with decreasing pH. The Brønsted plots are highly correlated; however, there are some deviations in the specific rate constants of the most protonated chemical species, which may arise from the fact that the enol form accounts for 67% of species BD_0 and for 95% of all other chemical species.[25] The greater contribution of the zwitterionic form of BD_0 results in a much larger increase in k_1^i than one would expect if the initial proportion were to be maintained. Overall, the specific rate constants of transimination of the Schiff bases of PLP and DOD (BD) are considerably smaller than those of the Schiff bases of PLP with amino acids (BL). This must be ascribed to the prevalence of enol forms in the former, where the imine double bond lies in a non-polar environment that reduces the electrophilic character and accessibility of the imine carbon.

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REFERENCES

1. E. E. Snell and S. J. Di Mari, in *The Enzymes*, edited by P. D. Boyer, Vol. II, Chapt. 7. Academic Press, New York (1970).
2. W. P. Jencks, in *Catalysis in Chemistry and Enzymology*, Chapt. 10. McGraw-Hill, New York (1969).
3. E. E. Snell and W. T. Jenkins, *J. Cell. Comp. Physiol., Part 1* **54**, 161 (1959).
4. P. S. Tobias and R. G. Kallen, *J. Am. Chem. Soc.* **97**, 6530 (1975).
5. H. Fisher, F. X. De Candis, S. D. Ogden and W. P. Jencks, *J. Am. Chem. Soc.* **102**, 1340 (1980).
6. L. Schirch, *J. Biol. Chem.* **250**, 1939 (1975).
7. R. J. Ulévitch and R. G. Kallen, *Biochemistry* **16**, 5355 (1977b).
8. S. Hershey and D. L. Leussing, *J. Am. Chem. Soc.* **99**, 1992 (1977).
9. Byeong, H. Jo, Vansur Nair and L. Davis, *J. Am. Chem. Soc.* **99**, 4467 (1977).
10. M. Mäkelä and T. Korpela, *Chem. Soc. Rev.* **12**, 309 (1983).
11. T. Korpela, M. Mäkelä and H. Lönnberg, *Arch. Biochem. Biophys.* **212**, 581 (1981).
12. E. M. Abbott and A. E. Martell, *J. Am. Chem. Soc.* **92**, 1754 (1970); **93**, 5852 (1971).
13. M. H. O'Learly and J. R. Payne, *J. Biol. Chem.* **251**, 2248 (1976).
14. R. D. Lapper, H. H. Mansch and I. C. P. Smith, *Can. J. Chem.* **53**, 2406 (1975).
15. J. L. Hogg, D. A. Jencks and W. P. Jencks, *J. Am. Chem. Soc.* **99**, 4772 (1977).
16. C. M. Metzler, A. Cahill and D. E. Metzler, *J. Am. Chem. Soc.* **102**, 6075 (1980).
17. P. M. Robitaille, R. D. Scott, J. Wang and D. E. Metzler, *J. Am. Chem. Soc.* **111**, 3034 (1989).
18. A. J. Kresge, *Pure Appl. Chem.* **53**, 189 (1981).
19. C. D. Ritchie, R. J. Minasz, A. A. Kamego and M. J. Sawada, *J. Am. Chem. Soc.* **93**, 3747 (1977).
20. M. Lehtokari, J. Puisto, R. Raunio and T. Korpela, *Arch. Biochem. Biophys.* **202**, 533 (1980).
21. E. H. Cordes and W. P. Jencks, *Biochemistry* **1**, 773 (1962).
22. E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.* **84**, 826 (1962).
23. B. Chance, in *Techniques of Chemistry*, edited by G. G. Hammes, p. 171. Wiley, New York (1974).

24. S. H. Weng and D. L. Leussing, *J. Am. Chem. Soc.* **105**, 4082 (1983).
25. M. A. Vázquez, F. Muñoz, J. Donoso and F. García, Blanco, *Biochem. J.* **279**, 759 (1991).
26. M. A. Vázquez, F. Muñoz, J. Donoso and F. García Blanco, *Int. J. Chem. Kinet.* in press (1992).
27. E. H. Peterson and H. A. Sober, *J. Am. Chem. Soc.* **76**, 169 (1954).
28. M. A. Vázquez, F. Muñoz, J. Donoso and F. García Blanco, *J. Chem. Soc., Perkin Trans. 2* 275 (1991).
29. M. A. Vázquez, F. Muñoz, J. Donoso and F. García Blanco, *Int. J. Chem. Kinet.* **22**, 905 (1990); M. A. Vázquez, G. Echevarría F. Muñoz, J. Donoso and F. García Blanco, *J. Chem. Soc., Perkin Trans. 2* 1617 (1989).