Bull. Chem. Soc. Jpn., ***76***, *523–528 (2003)*

Schiff's Bases Formed between Pyridoxal 5′-Phosphate and 4-Aminobutanoic Acid. Kinetic and Thermodynamic Study

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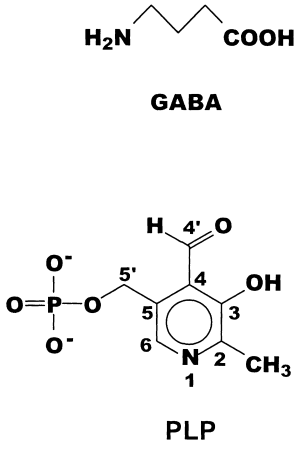
(Received July 15, 2002)

The overall and individual kinetic constants of formation (k_1 and $k_1{}^i$) and hydrolysis (k_2, k_{OH} and $k_2{}^i$) of the Schiff's bases formed between pyridoxal 5′-phosphate (PLP) and 4-aminobutanoic acid (GABA) at 10, 20, 25, 30, and 37 °C, a variable pH and a constant ionic strength of 0.1 M (1 M = 1 mol dm^{-3}) were determined. The formation of a Schiff's base is an intramolecular acid-catalyzed process. The activation and thermodynamic parameters for the formation and hydrolysis of the Schiff's bases were also determined. ΔH and ΔS for the individual processes were all found to be negative.

Pyridoxal 5′-phosphate (PLP, Scheme 1) is a form of vitamin B_6 and a cofactor (coenzyme) for a wide variety of enzymes involved in amino acid metabolism, including aminotransferases, decarboxylases and racemases.[1–3] While the role of this coenzyme varies among processes, it invariably binds to the protein at the active site to form an internal Schiff's base between its aldehyde group at position 4′ (Scheme 1) and the terminal ε-amino group in a lysine residue of the polypetide chain.[4] The first step in the formation of a Schiff's base (an imine) from PLP is the formation of an intermediate aminomethanol, which subsequently releases a molecule of water to yield the corresponding imine in an acid-catalyzed process.[5,6]



Scheme 1.

All PLP-dependent enzymes, except for glycogen phosphorylases, form an external Schiff's base[4] that subsequently undergoes cleavage of the carbon-imine nitrogen bond normal to the pyridine ring (this is so-called Dunathan's hypothesis[7]). The conversion of a Schiff's base into another is called "transimination". One of the more important transimination processes occurring in living organisms is that involving the aminotransferase of 4-aminobutanoic acid (or γ-aminobutyric acid, GABA, Scheme 1) [EC 2.6.1.19, GABA-T].[8,9] A number of studies suggest that epilepsy, and various degenerative diseases of motor control as a result, are associated to GABA-T hyperfunction (i.e. to low GABA levels in the central nervous system).[8] Knowledge of the thermodynamic parameters for the formation of the Schiff's base between PLP and GABA is therefore essential.

In this work, we studied the formation and hydrolysis of the Schiff's bases formed between pyridoxal 5′-phosphate and 4-aminobutanoic acid at different temperatures and pH values at a constant ionic strength. The activation parameters for both processes and the thermodynamic parameters for the reaction were examined in the light of the individual reaction rate constants (Scheme 2) corresponding to the different ionic species involved in the process. The results were compared with the scant thermodynamic data available for the Schiff's bases of PLP and its analogues with aminoacids and amines.[10–15]

Material and Methods

4-Aminobutanoic acid was purchased from Sigma Chemical Co, and pyridoxal 5′-phosphate and all other chemicals were supplied by Merck.

Acetate, phosphate and carbonate buffer solutions were used in the appropriate pH ranges. The buffer concentration

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was normally 0.02 M and the ionic strength was kept constant at 0.1 M by adding appropriate amounts of KCl.

Buffered PLP solutions were stored in the dark prior to use. The exact concentration of PLP in them was obtained by dilution[16] with 0.1 M HCl and was always in the region of 2×10^{-5} M. GABA solutions were prepared immediately prior to their use by diluting appropriate volumes of the stock solution in the corresponding buffer; their concentrations ranged from 1×10^{-3} M to 5×10^{-2} M.

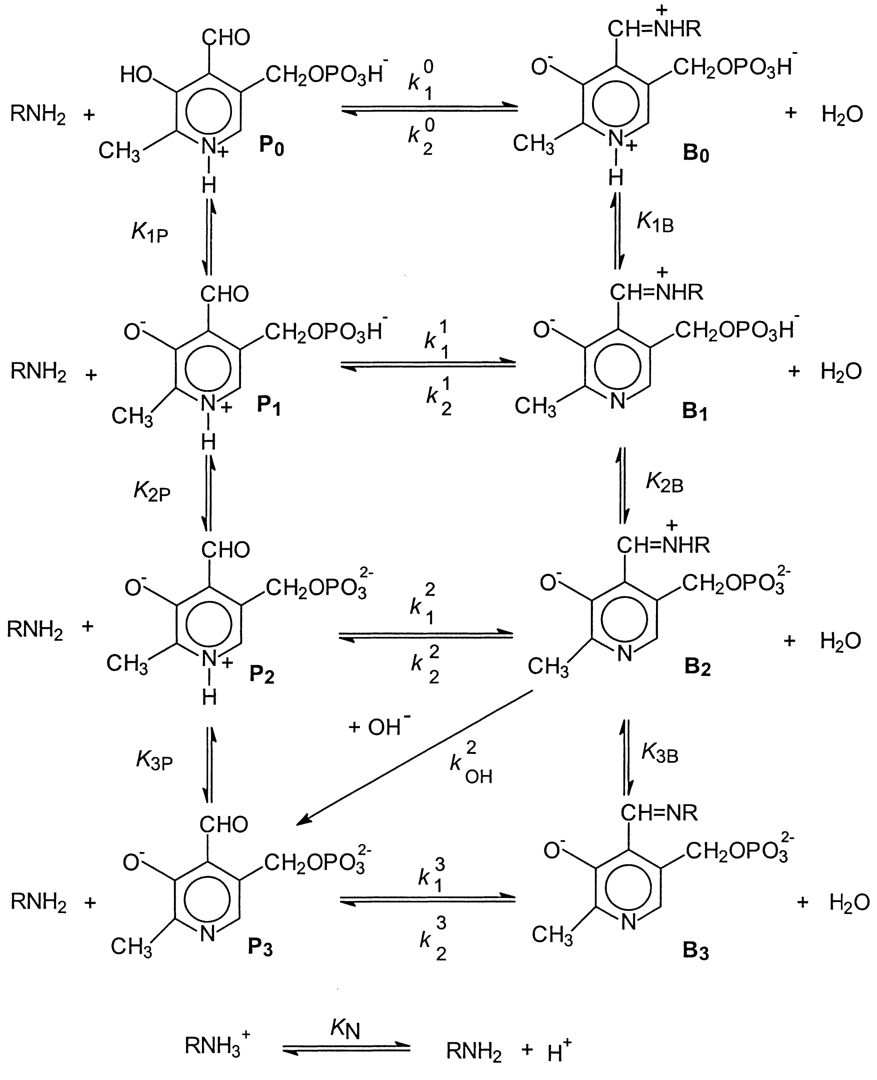
The kinetics of formation of the Schiff's bases were monitored by using a Uvikon 941-Plus spectrophotometer furnished with a thermostated support for cells of 1 cm light path. In all experiments, the reaction was started by adding a known volume of PLP solution at the desired pH over a pre-thermostated GABA solution at the same pH and the desired temperature. *Pseudo* first-order kinetics were obtained (GABA was used at a concentration of more than 10-times higher than the stoichiometric value) by monitoring either the decrease in the absorbance of PLP or the increase in that of the Schiff's base at appropriate wavelengths. The rate constant, k_{obs}, was determined by using the infinite method. The difference between the initial and final pH values never exceeded 0.03 units. The pH values were measured with a Crison 2001 pH-meter equipped with a Crison 59-02 combined electrode that was calibrated prior to use.

The overall reaction between the carbonyl group in an aldehyde and an amino group can be schematized as follows:

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

where k_1 and k_2 are the overall rate constants of formation and hydrolysis, respectively, of the Schiff's base. The procedure used to determine these rate constants from k_{obs} is described elsewhere.[10] The equilibrium constant at each pH studied was calculated as the k_1/k_2 ratio.

The ionic species present in solution over the pH range studied are shown in Scheme 2, where P and B denote PLP and the Schiff's base, respectively; subscripts 0–3 the net negative charges of each ionic species; p$K_{i\mathrm{P}}$ and p$K_{i\mathrm{B}}$ (with i = 1, 2, 3) the pK values for PLP and the Schiff's base, respectively; and



Scheme 2.

$R\mathrm{NH_2}$ and $R\mathrm{NH_3^+}$ the deprotonated and protonated form, respectively, of GABA (K_N being its ionization constant).

The overall rates of formation and hydrolysis of Schiff's bases can be expressed in terms of the individual rate constants for the different ionic species involved in the processes. Thus, $k_1{}^i$ ($i = 0, 1, 2, 3$) and $k_2{}^i$ ($i = 0, 1, 2, 3$) are the individual rate constants of formation of the Schiff's base and that of hydrolysis by $\mathrm{H_2O}$, respectively; k^2_OH is the rate constant of hydrolysis of species $\mathrm{B_2}$ by $\mathrm{OH^-}$ ions. The hydrolysis of species $\mathrm{B_1}$ and $\mathrm{B_0}$ by $\mathrm{OH^-}$ was not considered because the hydroxide ion concentrations in the pH region where both species exist were virtually negligible.

The rate of formation of the Schiff's base can be expressed as

$$v_1 = [\mathrm{RNH_2}]_T \sum_{i=0}^{3} k_1^i[\mathrm{P}_i] = k_1[\mathrm{RNH_2}]_T[\mathrm{PLP}]_T, \qquad (1)$$

where subscript T denotes the concentration of all species.

Similarly, the rate of disappearance of the Schiff's base can be expressed as

$$v_2 = k^2_\mathrm{OH}[\mathrm{B_2}][\mathrm{OH^-}] + \sum_{i=0}^{3} k_2^i[\mathrm{B}_i] = k_2[\text{Schiff's Base}]_T, \qquad (2)$$

and the equilibrium constant as

$$K_\mathrm{pH} = [\text{Schiff's Base}]_T/([\mathrm{RNH_2}]_T[\mathrm{PLP}]_T), \qquad (3)$$

Taking into account the equilibria in Scheme 2 and the fact that the equilibrium constant of formation of the Schiff's base at very high pH values is $K_\mathrm{M} = k_1{}^3/k_2{}^3$, Eqs. 1–3 can be rewritten as

$$k_1 = \frac{k_1^3 + k_1^2 \cdot a/K_\mathrm{3P} + k_1^1 \cdot a^2/(K_\mathrm{3P} \cdot K_\mathrm{2P}) + k_1^0 \cdot a^3/(K_\mathrm{3P} \cdot K_\mathrm{2P} \cdot K_\mathrm{1P})}{(1 + a/K_\mathrm{N})(1 + a/K_\mathrm{3P} + a^2/(K_\mathrm{3P} \cdot K_\mathrm{2P}) + a^3/(K_\mathrm{3P} \cdot K_\mathrm{2P} \cdot K_\mathrm{1P}))}, \qquad (4)$$

$$k_2 = \frac{k_\mathrm{OH} + k_2^2 \cdot a/K_\mathrm{3B} + k_2^1 \cdot a^2/(K_\mathrm{3B} \cdot K_\mathrm{2B}) + k_2^0 \cdot a^3/(K_\mathrm{3B} \cdot K_\mathrm{2B} \cdot K_\mathrm{1B})}{1 + a/K_\mathrm{3B} + a^2/(K_\mathrm{3B} \cdot K_\mathrm{2B}) + a^3/(K_\mathrm{3B} \cdot K_\mathrm{2B} \cdot K_\mathrm{1B}))}, \qquad (5)$$

$$K_\mathrm{pH} = \frac{(1 + a/K_\mathrm{3B} + a^2/(K_\mathrm{3B} \cdot K_\mathrm{2B}) + a^3/(K_\mathrm{3B} \cdot K_\mathrm{2B} \cdot K_\mathrm{1B}))K_\mathrm{M}}{(1 + a/K_\mathrm{N})(1 + a/K_\mathrm{3P} + a^2/(K_\mathrm{3P} \cdot K_\mathrm{2P}) + a^3/(K_\mathrm{3P} \cdot K_\mathrm{2P} \cdot K_\mathrm{1P}))}, \qquad (6)$$

where $k_\mathrm{OH} = k_2{}^3 + k^2{}_\mathrm{OH}(K_\mathrm{W}/K_\mathrm{3B})$ and $a = 10^{-\mathrm{pH}}$, K_W being the ionic product of water.

The experimental k_1, k_2 and K_pH values were simultaneously fitted to Eqs. 4–6 using a nonlinear regression method that minimized the following functions:

$$\mathrm{U_1} = \Sigma(\log k_{1,e} - \log k_{1,t})^2, \qquad (7)$$

$$\mathrm{U_2} = \Sigma(\log k_{2,e} - \log k_{2,t})^2, \qquad (8)$$

$$\mathrm{U_{pH}} = \Sigma(\log K_{\mathrm{pH},e} - \log K_{\mathrm{pH},t})^2, \qquad (9)$$

where subscripts e and t denote the experimental and theoretical data, respectively. The p$K_{i\mathrm{P}}$ values for PLP at the different temperatures studied were obtained from the literature,[10,17] and are given in Table 1. The pK_N values for GABA under the same experimental conditions as those used in the kinetic measurements were determined potentiometrically, and are also given in Table 1. The initial p$K_{i\mathrm{B}}$ values for the Schiff's bases of PLP with GABA, which were required for the fitting, were estimated from spectrophotometric measurements and reported data for similar systems.[18,19]

Table 1. pK Values for PLP and 4-Aminobutanoic Acid as Calculated Using Various Techniques

T/°C	pK_1P	pK_2P	pK_3P	pK_N
10	3.61[b]	6.15[b]	8.68[b]	10.66[c]
20	3.57[a]	6.12[a]	8.37[a]	10.45[c]
25	3.46[a]	6.02[a]	8.22[a]	10.36[c]
30	3.46[a]	6.02[a]	8.16[a]	10.20[c]
37	3.39[a]	6.05[a]	8.13[a]	10.15[c]

a) Determined calorimetrically.[17] b) Determined spectrophotometrically.[10] c) Determined potentiometrically.

Results and Discussion

Table 2 gives the experimental results for the overall rate constants of formation (k_1) and hydrolysis (k_2) of the Schiff's bases of PLP with GABA, as obtained at different pH values and temperatures (10, 20, 25, 30, and 37 °C). As can be seen, k_1 increased with increasing pH, whereas k_2 exhibited a minimum at pH 7–8. These results are consistent with those found in the formation and hydrolysis of the Schiff's bases of PLP and DPL (5′-deoxypyridoxal) with hexylamine.[10,11,20] The k_1 values for the Schiff's bases of PLP with GABA are smaller than those for the bases of PLP and DPL with hexylamine at any pH and temperature; on the other hand, the k_2 values for the former are all greater than those for the latter. As a result, the K_pH values (Table 2) are all smaller than those for the Schiff's bases between PLP and hexylamine. However, the k_1, k_2 and K_pH values obtained for the Schiff's bases of PLP with GABA are similar to those for the bases of PLP with α-amino acids.[19]

By fitting the data of Table 2 to Eq. 4, as derived from Scheme 2, the individual rates of formation for the Schiff's bases of PLP with GABA, $k_1{}^i$ (Table 3), were obtained.

The decrease in $k_1{}^i$ with the increase in the pH observed at all of the temperatures studied was the result of all protonable groups in PLP taking part in the formation of the Schiff's base via an intramolecular acid-catalysed process that facilitates the dehydration of the intermediate aminomethanol. The corresponding Brønsted plot ($\log k_1{}^{i-1}$ versus p$K_{i\mathrm{P}}$, not shown) confirmed the presence of intramolecular acid catalysis, with an α value (where α is the slope of the Brønsted slope with its sign changed) of ca 0.76 (Table 3). This α value is greater than those reported for the Schiff's bases of PLP with hexylamine[10,20] (0.57–0.68); and the difference cannot be ascribed to a differential nucleophilic character in the amine, because the pK_N values for hexylamine and GABA at 25 °C (viz. 10.7 and 10.36) are very similar. On the other hand, our α values are nearly identical to those reported for the Schiff's bases of PLP with α-amino acids[19] (viz. 0.74–0.79). Auld and Bruice[21] suggested that the acid catalysis process might be promoted by the 3-hydroxy group in PLP (Scheme 1) and must therefore be favoured by the presence of the deprotonated carboxyl group in the molecule that bears the –$\mathrm{NH_2}$ group[19] (as is the case with GABA, L-leucine, L-isoleucine, L-glycine, and ε-aminocaproic

Table 2. Kinetic Parameters for the Formation of the Schiff's Base[a]

10 °C				20 °C				25 °C				30 °C				37 °C			
pH	k_1	k_2	K_{pH}	pH	k_1	k_2	K_{pH}	pH	k_1	k_2	K_{pH}	pH	k_1	k_2	K_{pH}	pH	k_1	k_2	K_{pH}
4.27	1.675	0.114	14.69	4.14	2.690	0.512	5.760	4.46	3.607	0.640	5.636	4.09	4.240	0.988	4.291	4.68	11.92	1.743	6.838
5.33	2.720	0.128	21.25	5.23	5.320	0.407	13.07	5.23	6.800	0.690	9.855	4.67	6.000	1.001	6.000	5.07	13.56	1.721	7.880
5.60	2.681	0.134	20.07	5.94	7.570	0.486	15.58	6.02	7.803	0.777	10.01	5.24	7.400	0.990	7.475	5.44	18.41	1.388	13.26
5.90	3.475	0.127	27.36	6.64	10.74	0.355	30.25	6.87	11.40	0.480	23.75	5.63	9.900	1.176	8.418	5.88	21.30	1.582	13.47
6.42	4.440	0.132	33.66	6.97	14.40	0.234	61.54	7.04	18.28	0.338	54.08	5.88	9.400	1.030	9.100	6.26	22.61	1.478	15.27
6.97	5.344	0.099	53.98	7.51	17.10	0.122	140.2	7.46	20.14	0.254	79.29	6.32	18.83	0.895	21.04	6.60	31.18	1.215	25.66
7.43	5.248	0.069	75.86	7.89	23.55	0.101	235.5	8.07	34.39	0.406	84.70	7.06	23.63	0.665	35.55	6.99	44.80	0.725	61.80
7.72	7.482	0.027	276.6	8.30	33.56	0.116	289.3	8.45	48.23	0.492	98.03	7.53	32.80	0.360	91.00	7.57	66.71	0.519	128.5
8.01	8.251	0.022	375.9	8.96	53.33	0.178	314.1	8.88	60.00	0.750	80.00	8.00	55.50	0.270	205.6	7.99	94.49	0.526	179.6
8.17	12.64	0.036	347.1	9.11	78.22	0.158	495.1	9.28	119.0	0.952	125.3	8.46	71.22	0.384	185.5	8.05	87.94	0.547	160.8
9.24	57.64	0.135	427.0	9.95	173.9	0.666	400.0	9.44	121.8	1.002	121.6	9.19	126.2	0.801	157.7	8.18	97.51	0.791	123.3
9.49	71.53	0.160	447.1	10.21	218.7	0.825	261.2	9.65	188.3	1.372	137.2	9.84	352.0	1.500	152.3	8.35	120.9	0.962	125.7
10.09	124.3	0.321	443.2					9.99	295.3	1.603	184.2					8.99	212.0	1.295	156.0
								10.03	300.3	1.807	166.2					9.29	212.5	3.757	56.28
								10.39	388.0	4.925	78.78					9.58	200.8	5.761	34.75

a) k_1 in ($\mathrm{l\,mol^{-1}\,min^{-1}}$); k_2 in ($\mathrm{min^{-1}}$); K_{pH} in ($\mathrm{l\,mol^{-1}}$). The estimated errors of k_1 and k_2 are not greater than 5%.

Table 3. Best Kinetics Constant (Scheme 2) Values Obtained by Fitting Experimental Values of k_1 to Eq. 4 and α Values (Slope of Brønsted Plot)

Temperature	10 °C	20 °C	25 °C	30 °C	37 °C
$\log k_1^0$	7.40 ± 0.05	7.45 ± 0.03	7.53 ± 0.05	7.46 ± 0.04	7.84 ± 0.03
$\log k_1^1$	5.18 ± 0.05	5.45 ± 0.03	5.46 ± 0.05	5.50 ± 0.04	5.64 ± 0.03
$\log k_1^2$	3.53 ± 0.07	3.82 ± 0.03	3.91 ± 0.05	3.98 ± 0.05	4.21 ± 0.04
$\log k_1^3$	2.79 ± 0.08	2.74 ± 0.04	2.90 ± 0.04	2.95 ± 0.06	3.13 ± 0.14
α	0.76	0.76	0.76	0.74	0.77

Table 4. Best Kinetics Constants and pK Values (Scheme 2) Obtained by Fitting Experimental Values of k_2 to Eq. 5 and K_{pH} to Eq. 6

Temperature	10 °C	20 °C	25 °C	30 °C	37 °C
$\log k_2^0$	−0.86 ± 0.11	−0.27 ± 0.09	−0.10 ± 0.11	0.03 ± 0.07	0.32 ± 0.10
$\log k_2^1$	−0.99 ± 0.12	−0.48 ± 0.07	−0.36 ± 0.12	−0.03 ± 0.06	0.09 ± 0.06
$\log k_2^2$	−1.44 ± 0.10	−1.11 ± 0.11	−0.58 ± 0.08	−0.52 ± 0.13	−0.38 ± 0.14
$\log k_{OH}$	1.96 ± 0.14	2.12 ± 0.08	2.46 ± 0.08	2.49 ± 0.08	3.07 ± 0.16
pK_{1B}	5.99 ± 0.27	5.38 ± 0.15	5.40 ± 0.23	5.20 ± 0.15	5.08 ± 0.36
pK_{2B}	7.29 ± 0.26	7.23 ± 0.12	7.26 ± 0.13	7.28 ± 0.13	7.23 ± 0.22
pK_{3B}	12.46 ± 0.26	12.35 ± 0.14	12.22 ± .18	12.10 ± 0.14	12.01 ± 0.30
$\log K_M$	1.14 ± 0.11	0.84 ± 0.05	0.47 ± 0.05	0.54 ± 0.06	0.28 ± 0.13

acid).

By fitting the data of Table 2 to Eqs. 5 and 6, as derived from Scheme 2, the individual rate constants of hydrolysis, k_2^i and k_{OH}, and the different pK_{iB} values for the Schiff's base of PLP with GABA at different temperatures, were determined (see Table 4). No intramolecular acid catalysis was apparent in the hydrolysis reaction.

The rate constant of hydrolysis, k_2^2, was found to be the smallest among those found at each temperature. This indicates that species B_2 is the most resistant to water hydrolysis, i.e. the entry of a proton in B_3 stabilizes the Schiff's base because the imine nitrogen is protonated and the proton in question forms a hydrogen bond with the phenoxide group at the pyridine ring.[22] However, the entry of the second proton to form B_1 facilitates its hydrolysis by the water.

Table 5. Energies of Activation (kJ/mol) for the Elemental Processes

	Hexylamine (Ref. 10)		GABA	
i	E_a (k_1^i)	E_a (k_2^i)	E_a (k_1^i)	E_a (k_2^i)
0	35.1	67.3	23 ± 11	72 ± 6
1	31.8	56.7	27 ± 4	69 ± 6
2	56.4	68.6	40 ± 3	71 ± 12
3	29.3	8.0	23 ± 7	74 ± 12

The Arrhenius plots (not shown) of the logarithmic rate constants, k_1^i and k_2^i, against the reciprocal temperature provided the energies of activation for the individual processes of formation and hydrolysis of the Schiff's bases of PLP with GABA (Table 5). The k_2^3 values required were obtained from the equation $K_M = k_1^3/k_2^3$ (see Scheme 1). Table 5 also gives the energies of activation for the Schiff's bases of PLP with hexylamine.[10] As can be seen, the energies obtained for the formation process are slightly lower than those for the Schiff's bases of PLP with *n*-hexylamine,[10] and roughly similar to those reported by Wiesinger and Hinz[12] for the bases of PLP with ε-aminocaproic acid (28 and 54 kJ/mol) and for the Schiff's bases of PLP with L-serine (in the region of 34 kJ/mol). The energies of activation for species P_0, P_1, and P_3 (Scheme 1) are similar; by contrast, that for species P_2 (where only the pyridine nitrogen is protonated) is higher. This effect is also observed in the Schiff's bases of PLP with hexylamine.[10]

The energies of activation for the hydrolysis of the Schiff's bases of PLP with GABA are higher than those for the bases of PLP with hexylamine;[10] the values for species B_0, B_1, B_2, and B_3 (Scheme 2) are very similar, mutually.

Table 6 gives the values of the activation parameters obtained from Eyring plots ($\log(k_1{}^i/T)$ vs $1/T$ and $\log(k_2{}^i/T)$ vs $1/T$, not shown) for the individual processes of formation and hydrolysis of the Schiff's bases of PLP with GABA. The enthalpies of activation for the hydrolysis processes, $\Delta H^{\#}(k_2{}^i)$, were always higher than those for the corresponding formation processes, which is consistent with the energies of activation of Table 5. All $\Delta S^{\#}$ values (except for $k_2{}^i$) were negative, consistent with a decreased number of degrees of freedom in the transition state. The formation of a Schiff's base requires the elimination of a molecule of water from the intermediate aminomethanol with the aid of a catalyst; also, the hydrolysis of the Schiff's base is a bimolecular process. These results coincide with those previously reported for the Schiff's bases of PLP with hexylamine,[10] confirming the proposed mechanism.

Table 7 gives the thermodynamic parameters (ΔH^0 and ΔS^0) for the elemental processes as obtained from the values of the activation parameters and the calculated values of ΔG^0 for the process at 25 °C. The table also includes the logarithmic values of the equilibrium constant, as calculated from ΔG^0 ($\log K_{\mathrm{eq}}$), and those obtained from the $k_1{}^i$ and $k_2{}^i$ values in Tables 3 and 4 ($\log K^i{}_{\mathrm{pH}} = k_1{}^i/k_2{}^i$). As can be seen, the reaction is exothermal at any pH. The ΔH^0 values obtained for the Schiff's bases of PLP with GABA are similar to those for the bases of PLP with hexylamine,[10] and range from −32.2 to 21.3 kJ/mol; the differences are larger in alkaline media, where the formation of the Schiff's bases of PLP with hexylamine is endothermal. ΔS^0 increases when the first proton is bonded to Schiff's base totally deprotonated (specie B_3 Scheme 2), and is then maintained practically constant. The increment of ΔS^0 is the result of the smaller charge of the products that than reactants, and the fact that a hydrogen bond is formed between the hydrogen bonded to the iminic nitrogen and the group O^- in position 3. For the Schiff's bases of PLP with hexylamine, ΔS^0 ranges[17] from −92 to 80 J/mol^{-1} K^{-1}; for those of PLP with ε-aminocaproic acid, Wiesinger and Hinz[12] obtained a value of −40 J/mol^{-1} K^{-1}. The equilibrium constants calculated from the thermodynamic and kinetic data exhibit no appreciable differences.

Table 6. Activation Parameters for the Elemental Processes

i	$\Delta H^{\neq}(k_1{}^i)$	$\Delta H^{\neq}(k_2{}^i)$	$\Delta S^{\neq}(k_1{}^i)$	$\Delta S^{\neq}(k_2{}^i)$
0	20 ± 10	69 ± 6	−67 ± 35	−50 ± 19
1	25 ± 4	67 ± 6	−89 ± 15	−62 ± 20
2	38 ± 3	69 ± 12	−77 ± 10	−64 ± 40
3	20 ± 7	71 ± 12	−155 ± 23	4 ± 40

$\Delta H^{\neq}$ in kJ mol^{-1}, $\Delta S^{\neq}$ in J mol^{-1} K^{-1}

Table 7. Thermodynamic Parameters for the Elemental Processes and Equilibrium Constants Obtained from Kinetic (Tables 3 and 4) and Activation Parameters (Table 6)

i	ΔH^0	ΔS^0	ΔG^0	$\log K_{\mathrm{eq}}$	$\log (K_{\mathrm{pH}}{}^i = (k_1{}^i/k_2{}^i))$
0	−49 ± 16	−17 ± 54	−44 ± 30	7.56	7.52
1	−42 ± 10	−27 ± 35	−34 ± 20	5.96	5.82
2	−31 ± 15	−13 ± 50	−27 ± 30	4.70	4.49
3	−51 ± 19	−159 ± 63	−3 ± 38	0.52	0.47

ΔH^0 and ΔG^0 in kJ mol^{-1}, ΔS^0 in J mol^{-1} K^{-1}

It should be noted that the thermodynamic parameter values calculated for the elemental processes can be used to examine the formation of a covalent bond between PLP and GABA in aqueous media.

This work was funded by Spain's DGI (Projects BQ2000-0646 and BQU2000-0787) and the Programa para la Cooperación con Iberoamérica.

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