Biochimica et Biophysica Acta 965 (1988) 93–96
Elsevier

BBA 20216

BBA Report

Schiff bases of pyridoxal 5′-phosphate with Tris and glycine

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(Received 1 December 1987)

Key words: Tris; Schiff base; Pyridoxal 5′-phosphate

Schiff bases of Tris and of glycine with pyridoxal 5′-phosphate have been studied spectrophotometrically. Formation constants and pK_a values have been measured and graphs have been prepared showing the amount of free pyridoxal phosphate present as a function of pH and of the concentration of amino compound. The electronic and ^{1}H-NMR spectra of the Tris Schiff base at high pH are consistent with its existence as a carbinolamine.

The formation of Schiff bases of pyridoxal 5′-phosphate (PLP) and related aldehydes with a variety of primary amines has been reported and has been reviewed recently [1,2]. However, the Schiff base with the popular buffer Tris has been studied relatively little [3]. Because the interaction of PLP with Tris buffers is of practical importance, we have examined the PLP-Tris system at 25°C, ionic strength 0.2 and have also evaluated spectra of Schiff bases of PLP with glycine, another commonly used buffer component.

Tris base and glycine were purchased from Fisher Scientific and PLP from Sigma. The experimental procedures used are described by Metzler et al. [4]. The ionic strength was maintained at 0.2 when possible using an appropriate buffer and/or sodium perchlorate. The buffers used were: pH 4–5 acetate; pH 5–8 phosphate; pH 8–11 bicarbonate. Higher pH values were obtained using NaOH alone. Over a range near their pK_a values the Tris or glycine themselves served as buffer. A few solutions containing high concentrations of the amine component (up to 0.6 M for Tris) exceeded the ionic strength limit set.

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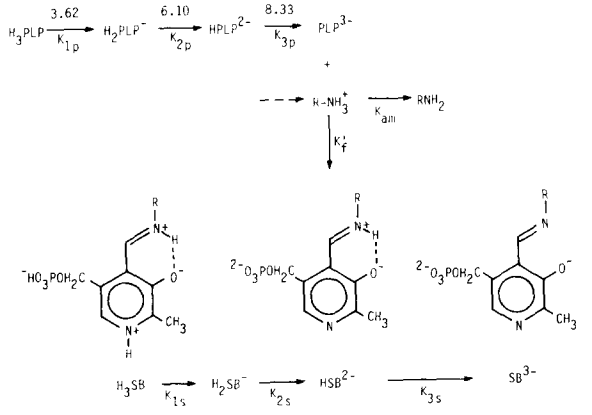
The solutions were allowed to equilibrate after mixing for about 20 min. Complete spectra were recorded using a Cary 219 spectrophotometer coupled to an Apple II-Plus computer. Data were collected from $18.0 \cdot 10^{-3}$ to $38.0 \cdot 10^{3}$ cm^{-1} at intervals of 200 cm^{-1}. A radiometer model PHM64 pH meter was used to record pH values for each solution and corrections were applied at high pH for sodium ion errors.

Data were collected at pH values of approx. 7.5 and approx. 9.0 for Tris concentrations of 0.0006, 0.002, 0.006, 0.01, 0.05, and 0.2 M. Additional data were collected at pH 4.54, 5.62, 7.40, 10.07, 10.31, 10.78, 11.16, 11.51, and 12.28 for 0.2 M Tris, pH 4.62, 5.14, 5.46, 6.57, and 7.70 for 0.4 M Tris and pH 12.09 for 0.6 M Tris.

For the glycine-PLP system data were collected at pH ≈ 9.0 for 0.001, 0.002, 0.005, 0.01, 0.05, 0.1, and 0.2 M glycine. Additional data were collected at pH 4.77, 5.34, 5.68, 6.24, 6.77, 8.01, and 10.45 for 0.2 M glycine and at pH 11.35, 11.47, 12.00, 12.30, 12.52, and 12.63 for 0.6 M glycine.

Equilibrium constants were evaluated by the method of Nagano [4,5] using a computer-assisted least-squares method. The resulting spectra were analyzed with log-normal curves to evaluate tautomeric equilibria [6,7].

The approach that we have taken [1,4] is to calculate apparent formation constants and pK_a values for the Schiff bases in aqueous solutions from absorption spectra using data from a broad range of wavelengths. The procedure also gives us a calculated spectrum for each ionic species of a Schiff base. The equilibria involved can be defined as in Scheme I.



Scheme I.

Here K_{2p} and K_{2s} represent primarily the pK_a of the phosphate group. Since its dissociation is usually accompanied by only a small change in the absorption spectrum, it is possible to ignore it and to omit these two pK_a values from the treatment. This is equivalent to assuming that the pK_a is the same in the Schiff base as in PLP. We have analyzed the Tris system both with the phosphate pK_a included and without. The differences are very small. For glycine we used the simpler analysis in which we ignored this pK_a.

The formation constants and the pK_a values of the Schiff bases found are given in Table I along with some data from the literature on the same or related Schiff bases. Agreement with literature values for glycine is not as close as we might have expected. However, ionic strengths and methods of evaluation were both different. The calculated spectra of the three ionic species of the Schiff base of Tris are plotted in Fig. 1. Data on peak positions and band shapes for both Schiff bases are given in Table II.

Tris is a strong Schiff base former with the relative amount of the imine being highest at about pH 8.2. The pH dependence of Schiff base

TABLE I

FORMATION CONSTANTS AND pK_a VALUES FOR SCHIFF BASES FORMED FROM PLP

The pK_a values used for PLP are 3.62, 6.10 and 8.33. The formation constant K_f' is for the reversible reaction: P + HL → HPL. Here P is the unprotonated form of PLP, HL the monoprotonated form of the amine or amino acid and HPL the monoprotonated form of the Schiff base or carbinolamine. Constant K_f is for reaction: P + L → PL.

Amine	pK_a values		formation constants	
	amine	Schiff base	log K_f'	log K_f
Tris	8.24	10.85,6.09,5.33	3.34	0.61
Glycine	2.36,9.78	11.59 5.99	2.93	1.12
		11.33 6.40		1.00 [a]
		11.23 6.70		1.18 [b]
Glutamic acid	4.25,9.67	11.87,6.45,5.85	2.62	0.42 [c]
Valine	2.32,9.62	12.16,6.76,5.61	3.44	0.90 [c]

[a] Ionic strength = 1.0; Tobias et al., as cited in Ref 1.
[b] Motekaitis and Martell [9].
[c] Metzler et al. [4]; ionic strength = 0.2.

formation can be expressed conveniently as the pH-dependent 'constant' K_{pH} (Eqn. 1).

$$K_{pH} = \frac{[\text{Schiff base}]_{total}}{[\text{PLP}]_{total}[\text{amine}]_{total}} = \frac{K'\left(\frac{K_{3s}}{[H^+]} + 1 + \frac{[H^+]}{K_{2s}} + \frac{[H^+]^2}{K_{1s}K_{2s}}\right)}{\left(1 + \frac{[H^+]}{K_{3p}} + \frac{[H^+]^2}{K_{2p}K_{3p}} + \frac{[H^+]^3}{K_{1p}K_{2p}K_{3p}}\right)\left(\frac{K_{am}}{[H^+]^2} + 1\right)} \quad (1)$$

If the pK_a of the phosphate group is omitted, one less term will be present in both numerator and denominator of Eqn. 1. For the glycine-PLP system an additional term involving the pK_a of 2.36 for glycine must be added to the equation. The constants are defined as in Scheme I. The values of log K_{pH} at 25 °C can be calculated from Eqn. 1 and the constants given in Table I.

Investigators may often wish to evaluate the amount of free PLP in a Tris or glycine buffer. This has been done for several concentrations of

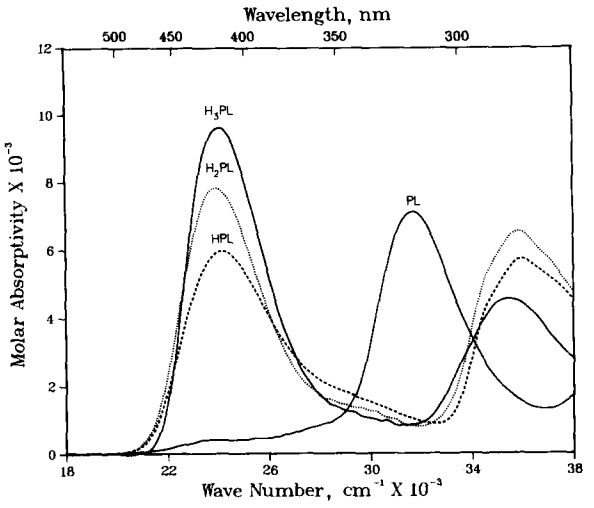


Fig. 1. Absorption spectra calculated for the four ionic forms of the Schiff base of Tris with PLP. PL represents the unprotonated Schiff base, HPL the monoprotonated form, etc.

the buffer amine. The results are shown in Fig. 2.

The spectra, formation constants and pK_a values of the Schiff base of PLP with glycine resemble those of Schiff bases of PLP with valine and glutamate closely (Table I; [4]). In the present case we assumed that the pK_a of the phosphate group in the Schiff base was the same as that in free PLP. This is not necessarily exactly true. We previously found two low pK_a values in the Schiff bases of valine and glutamate (Table I). In the case of glutamate they are separated by the statistical difference of 0.6 unit (log 4). This is the distance expected if the ring nitrogen and phosphate groups each have a pK_a of 6.15 and do not interact. A similar situation appears to hold for the PLP-glycine Schiff base. However, for the PLP-valine Schiff base the vibrational fine structure seen in the 278 nm band when the ring nitrogen becomes deprotonated appears around the pK_a of 5.61. This suggests that the order of dissociation in this Schiff base is first the ring nitrogen, then the phosphate. The same thing is true for the Tris Schiff base. The vibrational structure, clearly seen in the low-wavelength band in Fig. 1, appears as soon as a single proton is dissociated around the pK_a of 5.33. This must be

TABLE II

POSITIONS AND SHAPES OF ABSORPTION BANDS OF TWO PLP SCHIFF BASES (SB) RESOLVED WITH LOG-NORMAL DISTRIBUTION CURVES

Schiff base	Band maximum		Height	Width	Skewness	Area
	nm	$cm^{-1} \times 10^{-3}$	$(M^{-1} \cdot cm^{-1})$	$(cm^{-1} \times 10^{-3})$		$(m/mol \times 10^{-6})$
Tris + PLP						
H_3SB	416	24.03	9.71	3.51	1.53	376.1
	328	30.52	0.68	3.74	1.16	27.3
	281	35.53	4.50	5.01	1.40	244.9
H_2SB^-	418	23.93	7.84	3.69	1.41	314.6
	334	29.90	1.01	4.47	1.16	48.4
	279	35.89	6.47	5.14	1.58	368.1
HSB^{2-}	414	24.16	5.97	4.30	1.47	281.3
	337	29.70	0.92	4.75	1.18	46.7
	305	32.81	0.30	4.00	1.16	12.7
	277	36.06	5.56	5.39	1.80	341.8
SB^{3-}	316	31.60	7.03	3.93	1.57	306.4
Glycine + PLP						
H_2SB^-	416	24.01	9.29	3.70	1.58	381.0
	343	29.12	0.33	3.68	1.16	13.0
	280	35.69	4.91	5.23	1.38	278.6
HSB^{2-}	415	24.11	6.53	4.10	1.39	291.6
	347	28.80	0.53	4.75	1.18	26.9
	319	31.35	0.49	4.60	1.16	24.2
	278	36.00	6.02	5.26	1.76	358.8
SB^{3-}	345	28.95	4.66	6.02	1.30	302.5

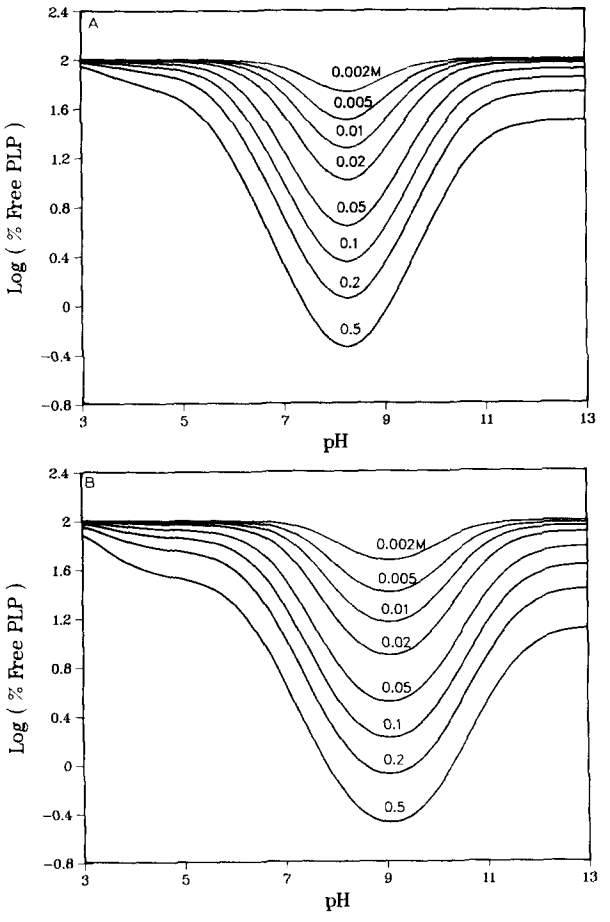


Fig. 2. (A) The logarithm of the percentage of free PLP at various values of pH and various total concentrations of Tris as a function of pH. The formation constants and pK_a values given in Table I were used to evaluate these curves. (B) Similar curves for glycine buffers.

largely the pK_a of the ring nitrogen and the pK_a of 6.09 largely that of the phosphate group.

The shapes of the absorption bands were all analyzed with log-normal curves [4,6]. For both glycine and Tris the shapes were, with one exception, very similar to those of Schiff bases studied previously [4]. However, the high-pH deprotonated form of the Tris Schiff base has an absorption maximum at 316 nm whereas the glycine and valine Schiff bases peak at 345 and 349 nm, respectively. It also has an unusually narrow band width of $3.9 \cdot 10^3$ cm^{-1} ($5.1 \cdot 10^3$ and $6.0 \cdot 10^3$ cm^{-1} for the Schiff bases of valine and glycine, respectively). This suggests strongly that the Schiff bases of Tris forms some kind of adduct at high pH. Kobayashi and Makino [3] suggested on the basis of a proton NMR resonance at 6.22 ppm and the loss of the 9.2 ppm resonance of the 4'-H of the Schiff base at high pH that a carbinolamine is formed. An alternative possibility is that one of the three -OH groups of the Tris adds to the double bond. We verified the reported NMR observations.

We wish to express our appreciation to John York, who assisted in the experimental work, to Carol Metzler for advice and consultation, and to Pierre Robitaille for NMR measurements.

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