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Zinc(II), Aluminum(III), and Gallium(III) Complexes of Schiff Bases of 3-Amino-3-phosphonopropionic Acid and 5′-Deoxypyridoxal

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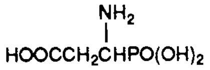
Potentiometric equilibrium studies of Zn(II), Al(III), and Ga(III) complexes of Schiff bases (SB) of 3-amino-3-phosphonopropionic acid (3APP) and 5′-deoxypyridoxal (DPL) are described. Schiff base formation by 3APP and DPL was investigated by measurement of ^{31}P NMR spectra and by potentiometric equilibrium measurements. The logarithmic value for the formation constant $[SB^{4-}]/[DPL^-][APP^{3-}]$ of the completely deprotonated SB species found is 1.11, and the logarithmic values of the protonation constants ($[H_nSB]/[H_{n-1}SB][H^+]$; n = 1–4) are 12.09, 6.24, 5.68, and 3.49 for the mono-, di-, tri-, and tetraprotonated species, respectively. ^{31}P NMR spectroscopy was also useful in helping to assign the protonation sites on the Schiff base. While the major species for Zn^{II}–SB and Al^{III}–SB systems are 1:1 complexes, in the Ga^{III}–SB system the major species are protonated and deprotonated forms of the 1:2 complexes: $Ga(SB)_2^{5-}$, $GaH(SB)_2^{4-}$, $GaH_2(SB)_2^{3-}$, $GaH_3(SB)_2^{2-}$, and $GaH_4(SB)_2^{-}$. Equilibrium constants are reported for all metal chelate and protonated metal chelate species formed in solution over a wide pH range, and distribution curves are presented that show the distribution of all molecular species in solution over the pH range 2–10. This detailed quantitative description of the complex system in solution are required for future kinetic studies, kinetic analysis, and mechanistic interpretation of the DPL and metal ion catalyzed decarboxylation and/or dephosphonylation of 3APP.

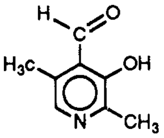
Introduction

A recent report comparing vitamin B$_6$ catalyzed β-decarboxylation and β-dephosphonylation of amino acids in model systems showed only a few detailed publications on these reactions.[1] In this paper, a new substrate, 3-amino-3-phosphonopropionic acid (3APP, **1**) is suggested for the study of decarboxylation and/or dephosphonylation of amino acids. This compound forms a relatively stable Schiff base with 5′-deoxypyridoxal (DPL, **2**), but on the basis of previously suggested mechanisms of dephosphonylation and decarboxylation[2] dephosphonylation is not expected to take place. Instead, rather facile DPL and metal ion or hydrogen ion catalyzed decarboxylation should take place. The use of DPL, an analogue of vitamin B$_6$, is employed because it forms a much higher concentration of Schiff base compared to pyridoxal (which has its carbonyl group tied up in hemiacetal formation), thus providing higher concentrations of protonated or metal ion coordinated reactive intermediates, with consequent higher vitamin B$_6$ catalyzed reaction rates. In addition, DPL does not have the phosphate ester as in pyridoxal 5′-phosphate, which produces a ^{31}P NMR resonance at about the same position as the resonance of inorganic phosphate in neutral and acid solutions, thus interfering with an important technique employed for following the rate of the dephosphonylation reaction. This was the situation in the series of investigations of the dephosphonylation of 2-amino-3-phosphonopropionic acid (**3**), reported previously,[3-6] and would also be true for the vitamin B$_6$ catalyzed reactions of (1-amino-2-phosphonoethyl)phosphonic acid (**4**), which would be expected to undergo dephosphonylation. We plan to carry out similar investigations on this substrate at a later date.



$\mathrm{HOOCCH_2\overset{\displaystyle NH_2}{\overset{|}{C}}HPO(OH)_2}$

1



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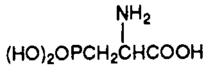
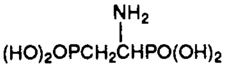


$\mathrm{(HO)_2OPCH_2\overset{\displaystyle NH_2}{\overset{|}{C}}HCOOH}$

3



$\mathrm{(HO)_2OPCH_2\overset{\displaystyle NH_2}{\overset{|}{C}}HPO(OH)_2}$

4

The purpose of this research is to determine the concentrations of the Schiff base species present under varying conditions in the following systems: metal-free DPL–3APP, Zn^{II}–DPL–3APP, Al^{III}–DPL–3APP, and Ga^{III}–DPL–3APP. The results of these equilibrium studies will be employed to select the p[H] ranges and reaction conditions necessary to relate the observed rates of transamination and decarboxylation reactions to the concentrations of specific Schiff base species in solution, to determine the specific rate constants for these molecular species, and possibly to relate catalytic activity to chemical constitution for the reactions studied.

Experimental Section

Materials. 3-Amino-3-phosphonopropionic acid was synthesized as described in the literature.[7] The product was further purified by dissolving it in a small amount of water and crystallizing by the addition of ethanol. Potentiometric titration of a sample gave a purity of 99.3%. 5′-Deoxypyridoxal was also synthesized as described in the literature.[8] The product was further purified by sublimation onto a cold finger (P = 100 mmHg and t = 75–85 °C). Potentiometric titration of a sample gave a purity of 99.4%. Zinc(II) sulfate ($ZnSO_4 \cdot 7H_2O$), aluminum(III) sulfate, and hydrochloric acid were purchased from Fisher Scientific Co. Gallium metal was obtained from Johnson Matthey, Inc. Potassium chloride was purchased from Mallinckrodt, Inc. Potassium deuteroxide (KOD, 40%), deuterium oxide (D_2O, 99.8% D), and deuterium chloride (DCl) were obtained from Aldrich Chemical Co., Inc. CO$_2$-free potassium hydroxide was obtained from J. T. Baker Chemical Co.

Potentiometric Equilibrium Determinations. The standard Zn(II) and Al(III) solutions, having a concentration of about 5.0 × 10^{-3} M, were prepared from reagent grade zinc(II) sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), chloride, and aluminum(III) sulfate octadecahydrate ($Al_2(SO_4)_3 \cdot 18H_2O$), respectively, and both solutions were standardized by titration with the disodium salt of dihydrogen EDTA.[9] Some HCl was added to the Al(III) solution to avoid hydrolysis. A standard gallium(III) solution was prepared by dissolving an accurately weighed quantity of 99.9999% pure gallium metal in concentrated HCl. The exact amount of excess hydrochloric acid in the Al(III) and Ga(III) solutions was determined by a Gran plot of $(V_0 - V_{KOH})10^{-pH}$ vs V_{KOH}, where V_0 = the initial volume of the Al(III) or Ga(III) solution and V_{KOH} is the volume of added standard KOH. The intercept on the abscissa obtained by extrapolating the straight-line portion of the plot is a direct measure of the excess acid present.[10]

Samples of about 0.10 mmol of 3APP and DPL and 0.050 and 0.10 mmol of Zn(II), Al(III), or Ga(III) were diluted with 40 mL of doubly distilled water in a sealed vessel thermostated at 25.00 ± 0.05 °C. The potentiometric titration vessel was equipped with a Sargent silver–silver chloride glass electrode and a calomel reference electrode, N$_2$ inlet and bubbler outlet, and a graduated (Metrohm) microburet. The experi-

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Table I. Protonation Constants for 3-Amino-3-phosphonopropionic Acid, 5′-Deoxypryidoxal, and the 3APP–DPL Schiff Base in Water (t = 25.0 °C, μ = 1.00 M (KCl))[a]

compd	$\log K_1^H$	$\log K_2^H$	$\log K_3^H$	$\log K_4^H$
3-amino-3-phosphonopropionic	9.71	5.36	3.37	
acid (3APP)	9.70[b]	5.35[b]	3.4[c]	
5′-deoxypyridoxal (DPL)	8.04	4.18		
	8.03[d]	4.06[d]		
3APP–DPL Schiff base	12.09	6.24	5.68	3.49
	12.05[b]	6.3[c]	5.9[c]	

[a] Absolute errors in log K are less than 0.02 for data from potentiometric equilibrium curves. [b] Protonation constants determined also in water, by change in ^{31}P chemical shift (Figure 1). Absolute errors are less than 0.05. [c] Absolute errors are less than 0.1. [d] Protonation constants at 25.0 °C and μ = 0.100 M, from ref 14.

mental solutions, adjusted to 1.00 M ionic strength with KCl, were titrated with 0.1000 M standard CO_2-free KOH containing 0.9000 M KCl while $-\log [H^+]$ was measured with a Corning Model 250 pH meter calibrated with dilute standard hydrochloric acid at 1.00 M (KCl) ionic strength to read $-\log [H^+]$ directly (for the purposes of this research p[H] is defined as $-\log [H^+]$) and with dilute standard potassium hydroxide at 1.00 M (KCl) ionic strength. Potentiometric measurements were made on solutions of the following composition: each ligand alone; each ligand and Zn(II) in 1:1 and 2:1 molar proportions; each ligand and Al(III) in 1:1 and 2:1 molar proportions; each ligand and Ga(III), also in 1:1 and 2:1 molar ratios; solutions containing Zn(II), 3APP, and DPL having 1:2:2 and 1:1:1 molar ratios; solutions containing Al(III), 3APP, and DPL having 1:2:2 and 1:1:1 molar ratios; solutions containing Ga(III), 3APP, and DPL, also at 1:2:2 and 1:1:1 molar ratios.

Calculations of protonation constants, formation constants, and other equilibrium parameters from potentiometric data, and the corresponding species distribution curves, were carried out by the methods described by Motekaitis and Martell.[11]

NMR Measurements. The phosphorus ^{31}P NMR spectra were recorded with a Varian XL200 NMR spectrometer. The chemical shifts are reported in ppm with respect to the resonance of H_3PO_4 (0.30 M in D_2O) placed in a coaxial inner tube. H_3PO_4 in D_2O was also used as a lock sample.

The purity of 3APP and DPL and the extent of hydration of the solid materials were determined by titration. The analytical concentrations of 3APP and DPL in water in metal-free systems were 0.100 M, prepared by direct weighing. The appropriate amount of base necessary to achieve the desired p[H] value was taken from the corresponding titration curve, and the ionic strength was maintained at 1.00 M by the addition of KCl.

The p[H] values ($-\log [H^+]$) of the solutions were measured with a Corning Model 250 Research pH meter fitted with a thin combination glass electrode purchased from Ingold Electrodes, Inc. This electrode also allowed measurement of the p[H] of solutions directly in the NMR tubes. The temperature of the solutions was in most cases 35.0 ± 0.1 °C, which is the temperature to be employed for kinetic studies.

The proton nuclear magnetic resonance spectra were recorded with a Varian XL200 NMR spectrometer. The chemical shifts are reported in ppm with respect to the resonance of tetramethylsilane (TMS) in chloroform, placed in an inner tube. The temperature of the solutions was 35.0 ± 0.1 °C. The p[D] values of the D_2O solutions were computed by adding 0.40 to the observed reading.[12]

Results and Discussion

Species Formed from 3APP and DPL. The potentiometric equilibrium curves of 3APP and DPL were determined with the equipment described in the Experimental Section, and their protonation constants were calculated by use of the computer program BEST.[13] The protonation constants of 3APP and DPL, defined by eq 1, where L represents ligand (3APP or DPL), were

$$H^+ + H_{n-1}L \overset{K_n^H}{\rightleftharpoons} H_nL \qquad K_n^H = [H_nL]/[H^+][H_{n-1}L] \qquad (1)$$

determined by measuring $-\log [H^+]$ of aqueous solutions of the ligands as a function of the moles of base added per mole of ligand. The protonation constants obtained are given in Table I, and for DPL they are comparable to the constants reported previously for ionic strength 0.100 M.[14] Proton NMR spectra (100 MHz) of 3APP have been reported, and conformations of species formed have been described.[15,16] The 200-MHz proton NMR spectrum of 3APP at p[D] 10.96 obtained in this work consists of three sets of peaks corresponding to the α-methine and β-methylene protons. The two protons of the methylene group are not magnetically equivalent; there is coupling between them and additional coupling with the methine proton and the phosphorus atom. The α-methine proton also couples with the protons of the β-methylene group and with the phosphorus atom.

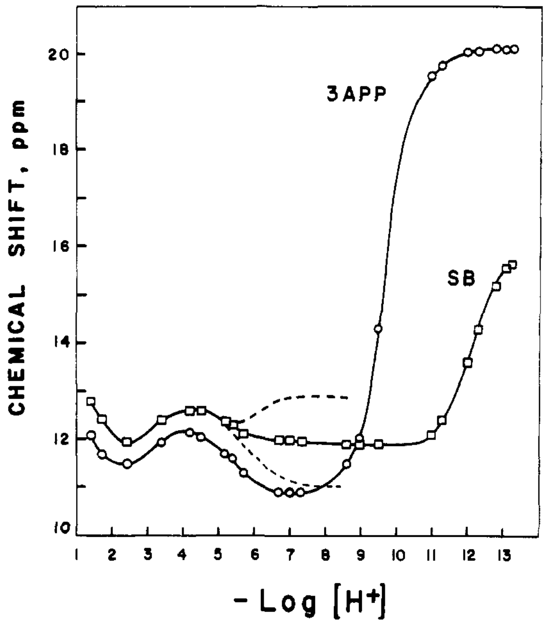
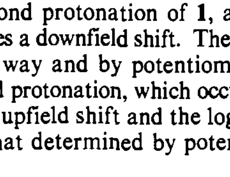


Figure 1. Variation of ^{31}P chemical shifts of the phosphonate group of 3APP (○) and the 3APP–DPL Schiff base (□) as a function of p[H] in water (t = 35.0 ± 0.1 °C; μ = 1.00 M (KCl)). Broken lines represent the possible variation of ^{31}P chemical shifts of the phosphonate group of the Schiff base if the effect of protonation on the phosphonate group could be separated from that on the pyridine nitrogen.

The variation of ^{31}P chemical shifts of the phosphonate group of 3APP as a function of p[H] is shown in Figure 1. The first protonation constant occurs at the amino group, and its value determined by titration (Table I) agrees with the value determined from the change in the ^{31}P chemical shifts. The large change in ^{31}P chemical shift of 3APP on the protonation of the amino group may be explained by hydrogen bonding involving a phosphonate oxygen and a proton covalently bonded to the amino group as in 5. A similar upfield shift of the ^{31}P resonance resulting from hydrogen bonding to the protonated α-amino group of an aminophosphonic acid was also observed for the substrate previously studied, 2-amino-3-phosphonopropionic acid (APP, 3), an analogue of 3APP. The second protonation of 1, assigned to the phosphonate group, causes a downfield shift. The protonation constants determined in this way and by potentiometry are comparable (Table I). The third protonation, which occurs at the carboxylate group, causes a ^{31}P upfield shift and the log K value determined is comparable to that determined by potentiometry.



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(12) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

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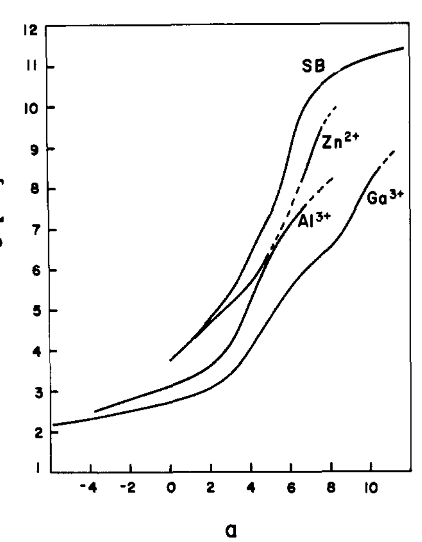


Figure 2. Potentiometric p[H] profiles for solutions containing 2.50 × 10^{-3} M DPL and 3APP, in the absence and in the presence of 1.25 × 10^{-3} M Zn(II), Al(III), or Ga(III) (a = moles of base added per mole of metal ion; t = 25.0 °C; μ = 1.00 M (KCl)).

Schiff Base Formation. A potentiometric equilibrium curve for the 3APP-DPL Schiff base system was determined (Figure 2), and its formation and protonation constants were calculated by using the computer program mentioned above.[13] Equation 2

$$\mathrm{DPL^- + 3APP^{3-} + }n\mathrm{H^+ \rightleftharpoons H_{\mathit{n}}SB^{\mathit{n}-4}}$$

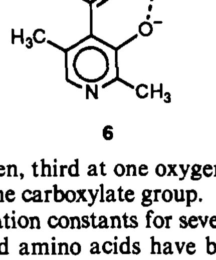
$$\beta_n^{\mathrm{SB}} = \frac{[\mathrm{H}_n\mathrm{SB}^{n-4}]}{[\mathrm{DPL^-}][\mathrm{3APP^{3-}}][\mathrm{H^+}]^n} \qquad (2)$$

$$\beta_n^{\mathrm{SB}} = \beta_0^{\mathrm{SB}}K_1^{\mathrm{SB}}K_2^{\mathrm{SB}}...K_n^{\mathrm{SB}} \qquad (3)$$

$$\mathrm{DPL^- + 3APP^{3-} \rightleftharpoons SB^{4-}} \qquad \beta_0^{\mathrm{SB}} = \frac{[\mathrm{SB^{4-}}]}{[\mathrm{DPL^-}][\mathrm{3APP^{3-}}]} \qquad (4)$$

defines the overall formation constants for the Schiff base. The overall formation constants of the Schiff base and its protonation constants are related to the formation constants of the unprotonated Schiff base, β_0^{SB}, by eq 3, where β_0^{SB} is the formation constant of the completely deprotonated Schiff base defined by eq 4.

K_1^{SB}–K_n^{SB} are the protonation steps defined in the same way as for 3APP and DPL (eq 1). The formation constant was found to be log β_0^{SB} = 1.11, and the protonation constants are reported in Table I. The protonation sequence of the Schiff base is assigned as follows: first protonation at the azomethine nitrogen (**6**), second



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at the pyridine nitrogen, third at one oxygen of the phosphonate group, and fourth at the carboxylate group. Values of Schiff base formation and protonation constants for several systems involving 5′-deoxypyridoxal and amino acids have been described previously.[17] For example, to decide if the second protonation occurs at the pyridine nitrogen or at the phosphonate group, it is helpful to know that values reported for protonation on the pyridine nitrogen in several 5′-deoxypyridoxal–amino acid systems are in the range 6.12–6.59, a strong indication that log K_2^{SB} = 6.24 should correspond to protonation at the heterocyclic nitrogen, rather than at a phosphonate oxygen. Also, if Figure 1 is inspected more closely, it is seen that the variation of the ^{31}P chemical shift of the Schiff base in the p[H] range 5–7 may be interpreted as a combination of two effects on the ^{31}P chemical shift of the phosphonate group: a decrease in the ^{31}P chemical shift due to protonation on pyridine nitrogen and an increase due to protonation of the phosphonate group. They are indicated in Figure 1 by dotted lines, and one effect almost counterbalances the other. The effect due to protonation of the phosphonate group parallels the one observed in the protonation of the same group in 3APP. Thus, the second protonation constant (log K = 6.24) is assigned to the pyridine nitrogen and the third to the phosphonate group (log K = 5.68), with considerable overlap between the two microscopic sites.

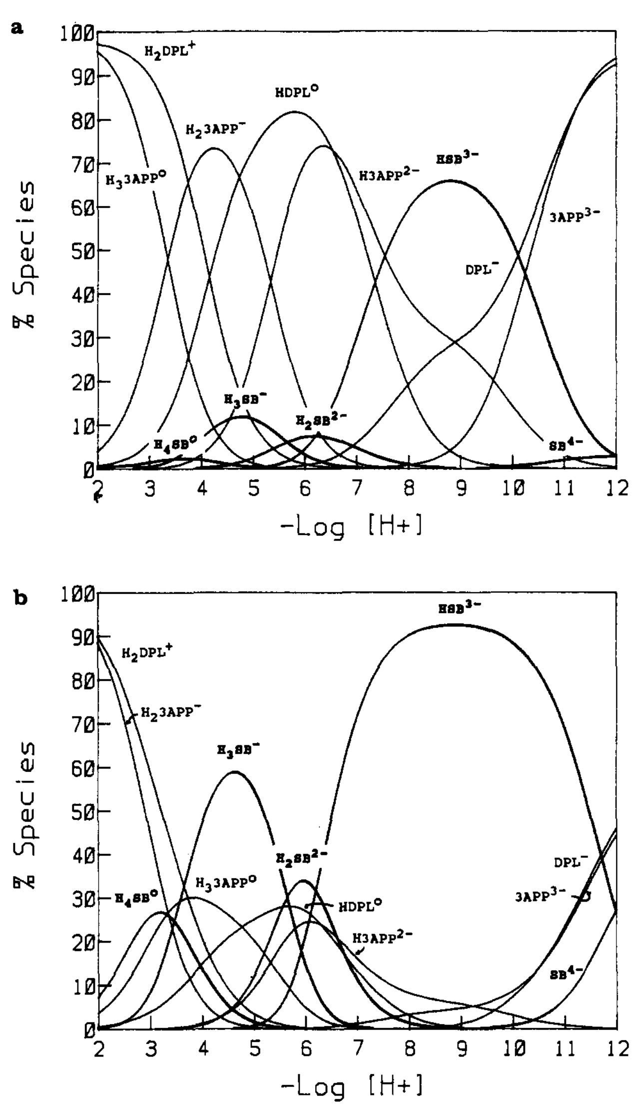

Figure 3. (a) Species distribution curves for a solution containing a 1:1 molar ratio of 3APP to DPL, where $\mathrm{SB^{4-}}$, $\mathrm{HSB^{3-}}$, $\mathrm{H_2SB^{2-}}$, $\mathrm{H_3SB^-}$, and $\mathrm{H_4SB^0}$ are respectively non-, mono-, di-, tri-, and tetraprotonated forms of the Schiff base. $\mathrm{3APP^{3-}}$, $\mathrm{H3APP^{2-}}$, $\mathrm{H_23APP^-}$, and $\mathrm{H_33APP^0}$ represent the ionized and neutral species of 3APP, and $\mathrm{DPL^-}$, $\mathrm{HDPL^0}$, and $\mathrm{H_2DPL^+}$ are the anionic, neutral, and cationic species of DPL, respectively. (b) Species distribution curves for the 1:1 3APP–DPL system with initial concentrations of 3APP and DPL 40.0 times their concentrations in (a).

The species present in the 3APP–DPL–SB system illustrated in Scheme I are related to each other by formation constants and protonation constants listed in Table I. The determination of the equilibrium constants makes possible the calculation of the concentration of all species that are formed in appreciable concen-

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[17] Kallen, R. G.; et al. In *Transaminases Chemical and Spectroscopic Properties of Pyridoxal and Pyridoxamine Phosphate*; Christen, P., Metzler, D. E., Eds.; Wiley: New York, 1985; pp 37–108.

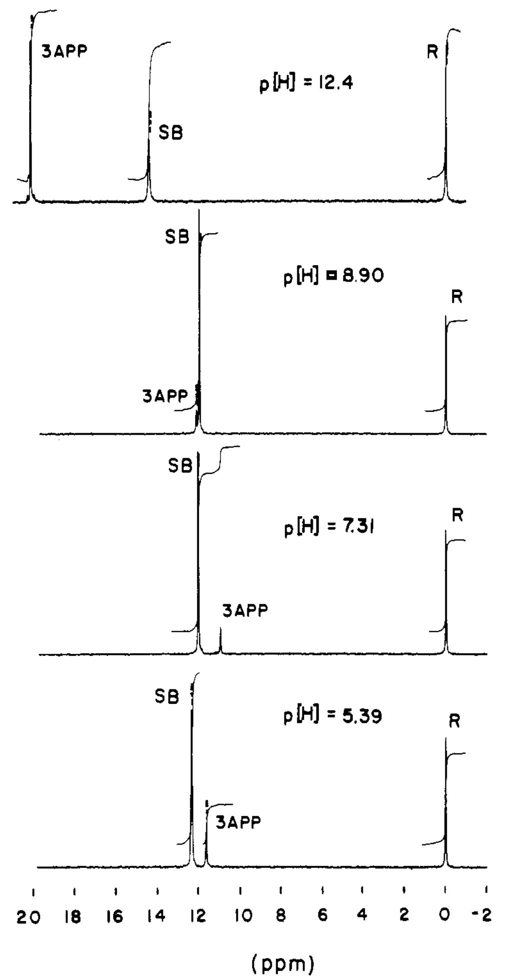

Figure 4. Decoupled ^{31}P magnetic resonance spectra of an H_2O solution containing a 1:1 molar ratio of 3APP and DPL at p[H] values of 12.4, 8.90, 7.31, and 5.39. R is the reference (0.300 M phosphoric acid in D_2O); t = 35.0 °C; μ = 1.00 M (KCl).

trations as a function of p[H] or of other solution conditions. The distribution curves of species concentrations vs p[H] (Figure 3) show that the concentration of Schiff base is highest at p[H] = 8.9, and it is monoprotonated. At this p[H] value, DPL is almost completely deprotonated and the amino group of 3APP is nearly completely protonated. Apparently the proton coordinated to the azomethine nitrogen is strongly hydrogen bonded to the carboxylate and phenolate oxygens, and it is this trifurcated hydrogen bond[2,18] that is responsible for the high stability of HSB^{3-} relative to all other forms of the Schiff base, SB^{4-}, H_2SB^{2-}, and H_3SB^-. The drop in the Schiff base concentration at higher p[H] values is due to the formation of SB^{4-}, which is much less stable due to the loss of the proton on the azomethine nitrogen. The species distribution curves are useful for indicating the conditions necessary to achieve maximum Schiff base formation. It is these conditions that will ensure the maximum catalytic effect of the coenzyme analogue on the reaction of the aminophosphonate substrate, such as decarboxylation, transamination, dephosphonylation, and others. Figure 3b reflects more closely the conditions to be employed for kinetic studies by means of the NMR technique, which requires much higher concentrations of reagents than those that are usually employed in potentiometric studies. The species distribution curves for 0.100 M amino acid and coenzyme, the concentrations usually employed when reaction kinetics are followed by NMR spectroscopy, are illustrated in Figure 3b. It is seen that, at the higher concentration, Schiff base formation is much more extensive and is almost complete in the p[H] range

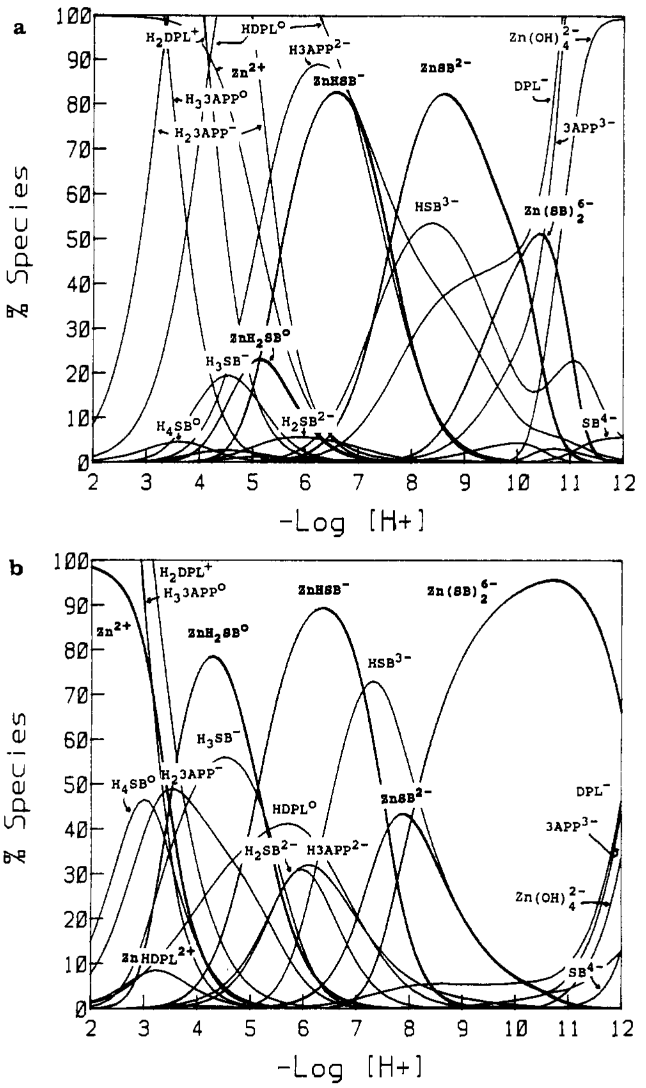

Figure 5. (a) Species distribution curves forr the system Zn^{II}–3APP–DPL at 1:2:2 molar ratio, supersaturated, where $Zn(SB)_2^{6-}$ is the 1:2 Zn(II) Schiff base complex. $ZnSB^{2-}$, $ZnHSB^-$, and ZnH_2SB^0 are respectively non-, mono-, and diprotonated forms of the 1:1 Zn(II) Schiff base complex. ZnHDPL is the monoprotonated form of the 1:1 Zn^{II}–DPL complex. Zn(II) is the free (aquo) zinc(II) ion, and $Zn(OH)_4^{2-}$ is the zincate anion. SB^{4-}, HSB^{3-}, H_2SB^{2-}, H_3SB^-, and H_4SB^0 are respectively non-, mono-, di-, tri-, and tetraprotonated forms of the Schiff base. $3APP^{3-}$, $H3APP^{2-}$, H_23APP^-, and H_33APP^0 represent the ionized and neutral species of 3APP, and DPL^-, $HDPL^0$, and H_2DPL^+ are respectively the anionic, neutral, and cationic species of DPL. (b) Species distribution curves for the 1:2:2 Zn^{II}–3APP–DPL system with initial concentrations of Zn(II) ion, 3APP, and DPL 40.0 times their concentrations in (a).

4–11. This is confirmed by ^{31}P NMR spectra of the Schiff base at several p[H] values (Figure 4). Of the three ^{31}P signals at p[H] 12.4, the resonance at 20.02 ppm is due to the phosphonate group of free 3APP and that at 14.30 ppm is due to the Schiff base. The Schiff base is about 50% formed at this p[H] value, with 16% monoprotonated and 34% nonprotonated Schiff base (Figure 3b). As the p[H] value of the solution decreases to p[H] = 8.90, the resonance due to free 3APP moves upfield to 12.03 ppm and the intensity of the peak decreases as shown by the integral. The resonance due to Schiff base also moves upfield to 11.88 ppm, and the intensity of the peak increases, indicating that more Schiff base is formed. At this p[H] value the Schiff base is 93.5% formed and it is monoprotonated (Figure 3b). At p[H] 7.31 the SB resonance is found at 11.95 ppm and that for free 3APP appears at 10.88 ppm. Schiff base species at p[H] 5.39 are mostly diprotonated and triprotonated. The SB resonance is found at 12.32 ppm, and the free 3APP resonance appears at 11.60 ppm.

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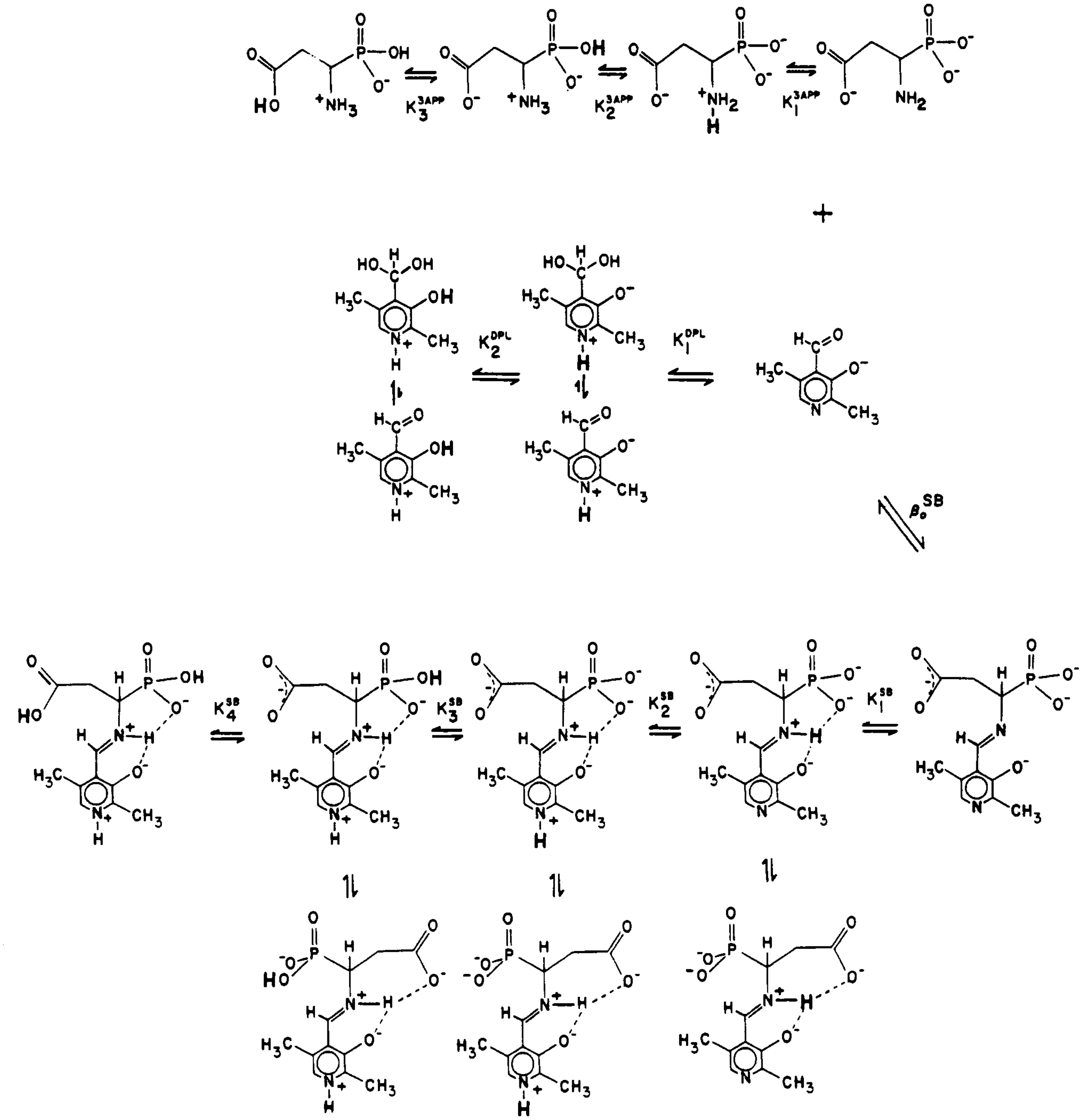
[18] Martell, A. E. In *Chemical and Biological Aspects of Pyridoxal Catalysis*; Snell, E. E., Fasella, P. M., Braunstein, A., Rossi Fanelli, A., Eds.; Pergamon Press: New York, 1963; pp 27, 28.

Scheme I. Microscopic Species Present in the 3APP-DL System



The Metal Ion-3APP and -DPL Complex Systems. The stability constants of normal (deprotonated) chelates having 1:1 and 1:2 molar ratios of metal ion (Zn(II), Ga(III), Al(III)) to ligand (3APP, DPL) are defined by eq 5 and 6, respectively, where *b*

$$M^{m+} + L^{b-} \overset{\beta_{ML}}{\rightleftharpoons} ML^{m-b} \qquad \beta_{ML} = \frac{[ML^{m-b}]}{[M^{m+}][L^{b-}]} \tag{5}$$

$$M^{m+} + 2L^{b-} \overset{\beta_{ML_2}}{\rightleftharpoons} ML_2{}^{M-2b} \qquad \beta_{ML_2} = \frac{[ML_2{}^{m-2b}]}{[M^{m+}][L^{b-}]^2} \tag{6}$$

is unity for DPL, 3 for 3APP, and 4 for SB and *m* is the charge on the metal ion. ML^{m-b} represents the 1:1 unprotonated, unhydrolyzed Schiff base chelate, and $ML_2{}^{m-2b}$ represents the 1:2 unprotonated, unhydrolyzed Schiff base chelate. Chloride was used as supporting electrolyte in this work at a concentration of 1.00 M.

The metal Schiff base chelate protonation constants are expressed in the same form as the ligand protonation constants, and these parameters, defined by (7) and (8), as well as the stability

$$K_{MH_nL} = \frac{[MH_nL^{m-b+n}]}{[MH_{n-1}L^{m-b+(n-1)}][H^+]} \tag{7}$$

$$K_{MH_nL_2} = \frac{[MH_nL_2{}^{m-2b+n}]}{[MH_{n-1}L]_2{}^{m-2b+(n-1)}][H^+]} \tag{8}$$

$$M^{m+} + yOH^- \overset{\beta_y}{\rightleftharpoons} M(OH)_y \qquad \beta_h = \frac{[M(OH)_y{}^{m-y}]}{[M^{m+}][OH^-]^y} \tag{9}$$

constants defined by (5) and (6) were determined by iterative nonlinear least-squares fits of the respective potentiometric equilibrium curves with the Fortran computer program BEST.[13] The hydrolysis of metal ions (eq 9) was considered in the calculations, and the appropriate hydrolysis constants were obtained from the literature.[14,19]

Table II. Stability Constants and Protonation Constants of Zn(II), Al(III), and Ga(III) Chelates of 3-Amino-3-phosphonopropionic Acid (3APP), 5′-Deoxypyridoxal (DPL), and 1:1 and 1:2 Complexes of the 3APP–DPL Schiff Base (t = 25.0 °C; μ = 1.00 M (KCl))[a]

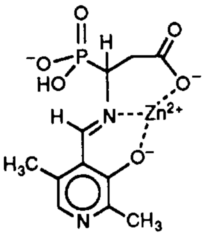
ligand	metal ion	log quotient				
		[ML]/[M][L]	[MHL]/[ML][H⁺]	[MH$_2$L]/[MHL][H⁺]	[ML$_2$]/[M][L]	[MHL$_2$]/[ML$_2$][H⁺]
3APP	Zn(II)	5.02	6.30		9.81	6.89
	Al(III)	10.93	4.19			
	Ga(III)	13.37	2.91			
DPL	Zn(II)	2.33	7.10			
	Al(III)	6.15			12.19	
	Ga(III)	8.54				
SB[b]	Zn(II)	10.58	7.64	5.17	17.26	
	Al(III)	18.68	4.86	3.23	25.10	
	Ga(III)	22.60	3.64	2.85	36.28	5.99[b]

[a] The standard deviations are less than 0.02 pH unit overall for data points on the potentiometric equilibrium curves. [b] Additional protonation constants of Ga^{III}–SB 1:2 species and their definitions: $[MH_2L_2]/[MHL_2][H^+] = 10^{5.83}$; $[MH_3L_2]/[MH_2L_2][H^+] = 10^{5.07}$; $[MH_4L_2]/[MH_3L_2][H^+] = 10^{4.37}$.

In making allowances for the possible hydrolysis of the aquo metal ions, we considered the following hydrolytic species: $Zn(OH)^+$, $Zn(OH)_2$, $Zn(OH)_4^{2-}$, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$, $Al(OH)_4^-$, $Ga(OH)^{2+}$, $Ga(OH)_2^+$, $Ga(OH)_3$, and $Ga(OH)_4^-$. The stability constant values that were determined for Zn–3APP and Zn–DLP systems are reported in Table II and are comparable to those of 2-amino-3-phosphonopropionic acid (APP, **3**), an analogue of 3APP, which has been previously reported.[4,5]

The stability constants determined for the Zn(II), Al(III), and Ga(III) DPL and 3APP complexes, listed in Table II, are comparable to those of 2-amino-3-phosphonopropionic acid and pyridoxal 5′-phosphate described previously.[6]

Zn^{II}–3APP–DPL Schiff Base System. Potentiometric [H⁺] measurements on Zn^{II}–3APP–DPL mixtures with 1:1:1 and 1:2:2 molar ratios were carried out (Figure 2), and in both systems formation of a precipitate was observed. The precipitate in the 1:1:1 system is probably the neutral protonated Zn^{II}–SB species **7**, in which the carboxylate group is coordinated with zinc(II) ion and the protonated phosphonate is not (i.e., protonation of the phosphonate group weakens its coordination tendency, probably to the point where it may be a weaker donor than the carboxylate group, as has been suggested previously[3]).



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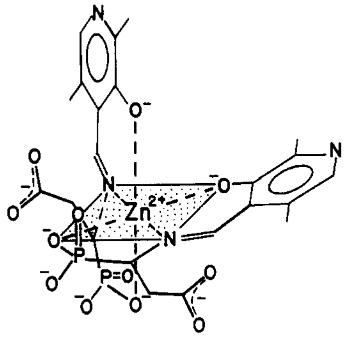
Also in the 1:2:2 system the formation of a precipitate was observed, but in a lesser amount and it redissolved at about p[H] = 8. As the p[H] of the solution with the precipitate increases, deprotonation of the reagents (3APP and DPL favors a higher degree of formation of $Zn(SB)_2^{6-}$, which is highly soluble. Thus, conversion to $ZnH_n(SB)_2^{n-6}$ results in the dissolving of the original precipitate consisting of the neutral ZnH_2SB complex. The equilibria for formation of normal and protonated chelates are indicated by eq 6–8, with m = 2 and with appropriate values of n. The stability constants determined are presented in Table II.

The first protonation site for ZnSB species occurs at the pyridine nitrogen, and the second is assigned to a noncoordinated phosphonate oxygen. The protonation constant involving the pyridine nitrogen of $ZnSB^{2-}$ is roughly 1 log unit higher than that of the aldimine SB itself at the same site. One might expect that the positive metal center should lower this protonation constant. However, it was demonstrated some time ago[18] that a proton coordinated to the azomethine nitrogen of a pyridoxal–amino acid Schiff base has a stronger electron-withdrawing (–I) effect than divalent transition-metal ions, as well as Zn(II). Thus, the monoprotonated Schiff base should have a somewhat less basic pyridine nitrogen than the corresponding Schiff base in which the azomethine proton is replaced by the Zn(II) ion. Protonation reactions of the 2:1 Schiff base Zn(II) chelate **8** were not observed, primarily because it is formed only at relatively high pH, as indicated by the species distribution curves illustrated in Figure 6.



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The tentative structure proposed for the 2:1 Schiff base Zn(II) chelate **8** indicates octahedral coordination with one terdentate Schiff base ligand coordinated to the metal ion at three equatorial positions, while the second SB ligand occupies one equatorial and two axial positions. Each ligand is considered to have an approximately planar orientation of the three donor atoms, with the planes at right angles to each other. This type of arrangement of ligands conforms to crystal structures of 2:1 Schiff base chelates (of pyridoxal and amino acids) that have been determined.[20-22]

Species distribution curves shown in Figure 5 were calculated with the aid of the Fortran computer program SPE, which employs the same algorithm as BEST.[13] At p[H] values lower than 5.5, less than 50% of Zn(II) is coordinated and the Schiff base is dissociated to form protonated species of 3APP and DPL (Figure 5a). Figure 5b is plotted for initial concentrations of all reagents 40.0 times higher than those in Figure 5a and reflects the conditions employed for NMR studies, for which the usual concentrations are ~0.050 M in metal ion and 0.100 M in the ligands. At these higher concentrations the degree of formation of metal complexes increases considerably. Figure 5b shows Zn(II) completely coordinated from p[H] = 5 to near p[H] = 11, where $Zn(OH)_4^{2-}$ species begin to form. At p[H] = 10.8 $Zn(SB)_2$ is 95.5% formed. The concentration of $Zn(SB)_2^{4-}$ decreases as the

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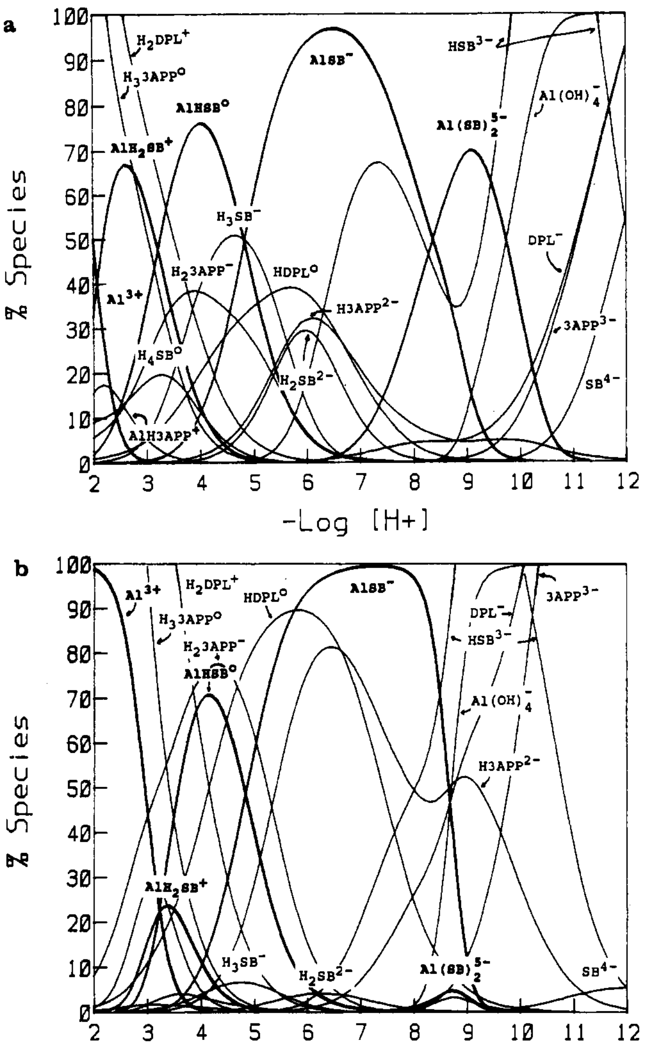


Figure 6. (a) Species distribution curves for a solution containing a 1:2:2 molar ratio of Al(III) 3APP, and DPL, where $Al(SB)_2^{5-}$ is the 1:2 Al(III) Schiff base complex. $AlSB^-$, $AlHSB^0$, and AlH_2SB^+ are respectively non-, mono-, and diprotonated forms of the 1:1 Al^{III}–SB complexes. AlH3APP is the monoprotonated forms of the 1:1 Al^{III}–3APP complex. Al^{3+} is the free (aquo) aluminum(III) ion, and $Al(OH)_4^-$ is the aluminate anion. SB^{4-}, HSB^{3-}, H_2SB^{2-}, H_3SB^-, and H_4SB^0 are respectively non-, mono-, di-, tri, and tetraprotonated forms of the Schiff base. $3APP^{3-}$, $H3APP^{2-}$, H_23APP^-, and H_33APP^0 represent the ionized and neutral species of 3APP, and DPL^-, $HDPL^0$ and H_2DPL^+ are the anionic, neutral, and cationic forms of DPL. (b) Species distribution curves for the 1:2:2 Al^{III}–3APP–DPL system with initial concentrations of Al(III) ion, 3APP, and DPL 40.0 times their concentrations in (a).

p[H] decreases, because of the formation of competing species, $ZnSB^{2-}$, $ZnHSB^-$, and HSB^{3-}. Formation of the monoprotonated Schiff base HSB^{3-} occurs because of the partial breakdown of $Zn(SB)_2^{4-}$ to form $ZnSB,^{2-}$ in the p[H] range where the free SB ligand is monoprotonated as HSB^{3-}. The 1:1 Zn(II)–Schiff base chelate has its maximum degree of formation (43.3%) at p[H] = 7.9. Protonation of this species to form $ZnHSB^-$ reaches a maximum at p[H] = 6.4, at which $ZnHSB^-$ is 89.3% formed. The diprotonated chelate ZnH_2SB species has a maximum degree of formation (78.6%) at p[H] = 4.3. At lower p[H] values the Zn^{II}–SB complexes and the Schiff base itself dissociate, and the major species are triprotonated 3APP, diprotonated DPL, and free (aquo) Zn(II) ion.

Al^{III}–3APP–DPL and Ga^{III}–3APP–DPL Systems. Potentiometric p[H] measurements on Al^{III}–3APP–DPL and Ga^{III}–3APP–DPL mixtures with 1:1:1 and 1:2:2 molar ratios were carried out (Figure 2), and in both systems formation of a precipitate was observed. However, in the 1:2:2 systems precipitation occurred to a lesser extent and at higher p[H] values. In the Al(III) system the precipitate is considered to be the neutral products of hydrolysis of the cations and/or neutral metal ion ligand species $AlHSB^0$ (**9**).

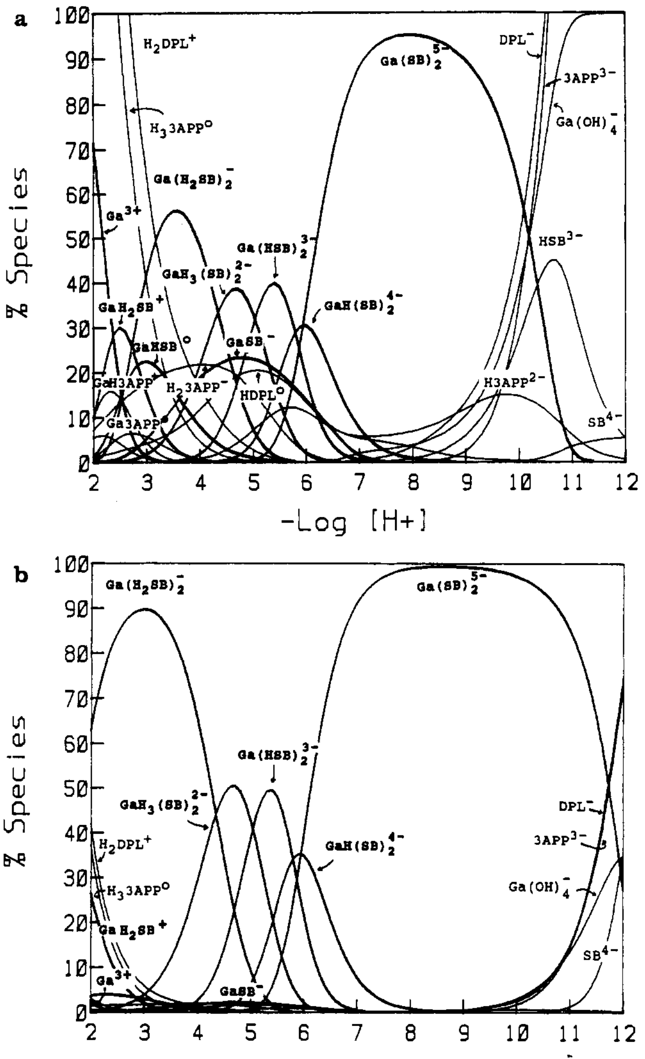
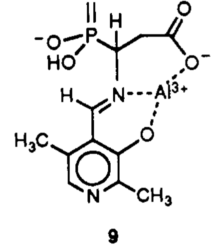


Figure 7. (a) Species distribution curves for a solution containing a 1:2:2 molar ratio of Ga(III), 3APP, and DPL, where $Ga(SB)_2^{5-}$, $GaH(SB)_2^{4-}$, $Ga(HSB)_2^{3-}$, $GaH_3(SB)_2^{2-}$, and $Ga(H_2SB)_2^-$ are respectively non-, mono-, di-, tri-, and tetraprotonated forms of the 1:2 Ga^{III}–SB complexes. $GaSB^-$, $GaHSB^0$, and GaH_2SB^+ are non-, mono-, and diprotonated forms of the 1:1 Ga^{III}–SB complexes. Ga3APP and GaH3APP are non- and monoprotonated forms of the 1:1 Ga^{III}–3APP complexes. Ga^{3+} is the free (aquo) gallium(III) ion, and $Ga(OH)_4^-$ is the gallate anion. SB^{4-} and HSB^{3-} are non- and monoprotonated forms of the Schiff base. $3APP^{3-}$, $H3APP^{2-}$, H_23APP^-, and H_33APP^0 represent the ionized and neutral species of 3APP, and DPL^-, $HDPL^0$, and H_2DPL^+ are the anionic, neutral, and cationic species of DPL, respectively. (b) Species distribution curves for the 1:2:2 Ga^{III}–3APP–DPL system with initial concentrations of Ga(III) ion, 3APP, and DPL 40.0 times their concentrations in (a).



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The equilibria for normal and protonated chelates are indicated by eq 6–8, with $m = 3$, $b = 4$, and appropriate values of n. The stability constants determined are presented in Table II.

Molecular Species in the Al^{III}–SB System. The species distribution curves shown in Figure 6, calculated with the algorithm previously described,[13] indicate concentrations of all molecular species present in the 1:2:2 Al^{III}–3APP–DPL system as a function of p[H]. The completely deprotonated 1:1 Al(III)–Schiff base species, $AlSB^-$, is the predominant form in the p[H] range 5–8.5, reaching its maximum concentration at p[H] = 7.4, where it is 99.5% formed. The complex breaks down to $Al(OH)_4^-$ and the monoprotonated Schiff base, HSB^{3-}, at p[H] values above 8.5. The protonated species $AlHSB^0$ and AlH_2SB^+ predominate at p[H] values below 5, reaching their maximum concentrations at p[H] values 4.2 and 3.4 for the mono- and diprotonated species, respectively, with $AlHSB^0$ 70.8% formed and AlH_2SB^+ 23.7% formed. Figure 6b reflects more closely the conditions employed for NMR studies, where the concentrations are 0.050 M metal ion and 0.100 M ligands. Under these conditions the $Al(SB)_2^{5-}$ species is 70.0% formed at p[H] = 9.2. Above p[H] = 11, all Al(III) is converted to $Al(OH)_4^-$ and the Schiff base ligand is metal free and is a mixture of the monoprotonated and completely deprotonated forms, HSB^{3-} and SB^{4-}, respectively. The 1:1 Al(III) Schiff base chelate is the major species, and it is 96.8% formed at p[H] = 6.6. The mono- and diprotonated species, $AlHSB^0$ and AlH_2SB^+, are 76.0% and 67.0% formed, respectively, at p[H] values of 4.1 and 2.6.

Molecular Species in the Ga^{III}–SB System. The species distribution curves for the 1:2:2 Ga^{III}–3APP–DPL system are shown in Figure 7. They were calculated by the same procedure that was employed for the corresponding Zn(II) and Al(III) systems.[13] The 1:2 Ga(III) Schiff base $Ga(SB)_2^{5-}$ is the major species formed in this system, reaching a maximum concentration of 95.1% at p[H] = 8.0. The mono-, di-, tri-, and tetraprotonated forms of this Schiff base complex are also formed as the p[H] is lowered. $GaH(SB)_2^{4-}$ is 30.5% formed at p[H] = 6.0, $Ga(HSB)_2^{3-}$ is 39% formed at p[H] = 5.4, $GaH_3(SB)_2^{2-}$ is 38.7% formed at p[H] = 4.7, and $Ga(H_2SB)_2^-$ is 56.2% formed at p[H] = 3.6. The 1:1 Ga(III) Schiff base complex appears at p[H] values around 7.0 and below. The $GaSB^-$ species is 23.3% formed at p[H] = 4.80, $GaHSB^0$ is 22.5% formed at p[H] = 3.0, and GaH_2SB^+ is 30.0% formed at p[H] = 2.5.

When the initial concentrations of all reagents are increased 40 fold, the formation of the 1:2 Ga(III) Schiff base complex dominates over most p[H] values. Only above p[H] = 10 does the $Ga(SB)_2^{5-}$ complex break down, forming $Ga(OH)_4^-$, HSB^{3-}, and SB^{4-}. Below p[H] = 4 $Ga(H_2SB)_2^-$ dissociates to form protonated species of 3APP and DPL and low concentrations of the 1:1 Ga(III) Schiff base chelate. Even at p[H] = 2.0, Ga(III) is only 2.5% free as the aquo ion, and the 1:2 Schiff base chelate is still the major constituent. At the higher concentrations of components, $Ga(SB)_2^{5-}$ predominates at p[H] values from 6.0 to 11.5, reaching a maximum at p[H] = 8.70, where it is 99.4% formed. $GaH(SB)_2^{4-}$ is 35.2% formed at p[H] = 6.0, $Ga(HSB)_2^{3-}$ is 49.7% formed at p[H] = 5.4, $GaH_3(SB)_2^{2-}$ is 50.5% formed at p[H] = 4.7, and the most protonated species, $Ga(H_2SB)_2$, is 89.8% formed at p[H] = 3.0.

Of the three metal ions investigated, Ga(III) forms the most stable Schiff base chelates, which persist in moderately acid solutions in various protonated forms under conditions which other Schiff base chelates break down to aquo ions and protonated forms of the amino acid and coenzyme.

Now that the Zn(II), Al(III), and Ga(III) Schiff base systems have been completely elucidated by potentiometric and NMR equilibrium studies, it will be possible, with the aid of the species distribution curves, to select the most favorable conditions for kinetic studies of decarboxylation and dephosphonylation reactions. The kinetic measurements can be correlated with the concentrations of the various metal-containing and metal-free Schiff base species, and this can be done as a function of pH, so the vitamin B_6 catalysis observed can be studied under the influence of additional catalytic effects of hydrogen ions and metal ions and, through the observation of systems containing protonated metal Schiff base chelates, the combined catalytic effects of metal ions and hydrogen ions can be determined.

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