where g_e is the free-electron g value, k_1 and k_2 are the orbital reduction factors, ξ is the spin–orbit coupling constant of the free ion, and

$$\Delta = |E_{xy} - E_{x^2-y^2}|$$

$$\delta = |E_{xy} - E_{yz}| \quad \text{or} \quad |E_{xy} - E_{xz}|$$

where E_{xy} etc. are the one-electron orbital energies.

The d–d bands observed in the electronic spectrum of $N_2H_5VOF_3$ at 16 000 cm^{-1} ($\epsilon = 3.6$) and at 12 000 cm^{-1} ($\epsilon = 0.85$) can be correlated to the transitions $(xy) \leftrightarrow (x^2 - y^2)$, Δ, and to $(xy) \leftrightarrow (xz)$ or (yz), δ, respectively. In order to get an idea regarding the extent of covalency involved in different

planes, we calculated the ratio $\xi k_1/\xi k_2$ by making use of the experimentally observed values for Δ and δ. The value of the ratio was found to be 0.89, indicating that the covalency involved in the xy plane is comparatively more than that in the xz plane.

Thus it is evident that, under suitable conditions, trifluorooxovanadate(IV) complexes can be synthesized from aqueous solutions. The complex may have a polymeric distorted octahedral or pseudooctahedral structure through weak V–F···V and V–O···V interactions.

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Thermodynamic Equilibria in the System Zinc(II)–Pyridoxal 5′-Phosphate–2-Amino-3-phosphonopropionic Acid in Aqueous Solution

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The equilibria in D_2O and H_2O between pyridoxal 5′-phosphate (PLP), 2-amino-3-phosphonopropionic acid (APP), the PLP–APP Schiff base (SB), zinc(II), and the secondary ligand 2,6-pyridinedicarboxylic acid (PDA) have been investigated by potentiometric [H$^+$] or [D$^+$] determination, proton magnetic resonance, and ^{31}P magnetic resonance. The protonation and Zn(II) formation constants of the individual components and of the mixed-ligand, SB–PDA–Zn(II), complex are reported. The logarithms of the protonation constants of the mixed-ligand complex Zn(II)–SB–PDA were found to be 8.38, 6.66, and 5.75, corresponding respectively to protonation of the phosphonate group, the phosphate group, and the pyridine nitrogen. The species distribution curves were calculated for the pH range 2–12. The ^{31}P magnetic resonance of the Zn(II)–SB–PDA complex and the Zn(II)–SB′–PDA complex, where SB′ is the Schiff base formed by 2-(aminoethyl)phosphonic acid (2-AEP) and PLP, showed that at pD values <8.50 zinc(II) is coordinated to the carboxylate group of the amino acid moiety of the SB, while at pD values >8.50 the phosphonate group is coordinated to the Zn(II) ion. Evidence is reported for hydrogen bonding involving the phosphate and phosphonate groups of the monoprotonated Zn(II)–SB–PDA complex.

Introduction

The purpose of this research is to determine the concentrations of zinc–Schiff base species present under varying conditions in equilibrium systems containing Zn(II), pyridoxal 5′-phosphate (PLP), and 2-amino-3-phosphonopropionic acid (APP). In order to avoid hydrolysis of the metal ions[1,2] and precipitation of neutral complexes and to maintain if possible a 1:1 molar ratio of the PLP–APP Schiff base to Zn(II), a terdentate secondary ligand, 2,6-pyridinedicarboxylic acid (PDA), was added in a nearly equimolar ratio (1:1:1:1.2 = Zn(II):PLP:APP:PDA). The measurement of the equilibrium constants in this mixed-ligand system and the determination of the composition and concentrations of the Zn(II) complexes formed are essential for the planning and interpretation of the rates and mechanisms of metal and vitamin B_6-catalyzed reactions of 2-amino-3-phosphonopropionic acid.

Preliminary studies of Schiff base formation between pyridoxal (PL) and (aminomethyl)phosphonic acid,[3] β-(aminoethyl)phosphonic acid,[3] and 2-amino-3-phosphonopropionic acid (APP) have been reported.[3,4] It has also been demonstrated by spectrophotometric measurements that the PL–APP system undergoes metal ion catalyzed transamination and

dephosphonylation.[5–7] The aminophosphonic acid, APP, is of considerable interest because it is found in nature[7–10] and is analogous to aspartic acid, and the reaction pathway suggested for its dephosphonylation reaction[5] appears to resemble the mechanism proposed for the pyridoxal-catalyzed β-decarboxylation of aspartic acid.[11]

Potentiometric data have been employed by Leussing et al.[12,13] and Martell et al.[14] to determine stability constants for the metal ion–vitamin B_6–amino acid Schiff base systems, but

(1) Smith, R. M.; Martell, A. E. "Critical Stability Constants"; Plenum Press: New York, 1974, Vol. 1; 1975, Vol. 2; 1982, Vol. 5.
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†Abstracted in part from a dissertation submitted by Bruno Szpoganicz to the faculty of Texas A&M University in partial fulfillment of the requirements of the degree of Doctor of Philosophy, 1984.

their kinetic studies were limited to a small range of pH or were complicated by the presence of 1:2:2 metal ion–vitamin B_6–amino acid complexes. Thus, the advantage in using a secondary ligand is the availability of a wider pH range for kinetic studies of metal ion–vitamin B_6 catalyzed reactions without precipitation and complications of kinetic measurements that would probably result from the additional contributions of 2:1 Schiff base–metal ion systems to the overall rate of the vitamin B_6-catalyzed reactions to be investigated.

Experimental Section

Materials. Pyridoxal 5′-phosphate was obtained from Sigma Chemical Co. The 2-amino-3-phosphonopropionic acid was purchased from Calbiochem-Behring Corp. Zinc chloride, hydrochloric acid, and potassium chloride were obtained from Fisher Scientific Co. Potassium deuteroxide (KOD; 40%), deuterium oxide (D_2O; 99.8% D), deuterium chloride (DCl), and 2,6-pyridinedicarboxylic acid were obtained from Aldrich Chemical Co., Inc.

Potentiometric Equilibrium Determinations. The standard Zn(II) solution, having a concentration of about 10^{-2} M, was prepared from reagent grade zinc(II) chloride and was standardized by direct titration with ethylenediaminetetraacetic acid (EDTA) with Eriochrome schwarz T as indicator.[15] Samples of about 0.10 and 0.20 mmol of APP, PLP, and PDA and 0.10 mmol of Zn(II) were diluted with 50 mL of double-distilled water in a sealed, thermostated (25.00 ± 0.05 °C) potentiometric titration vessel 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 test solution, adjusted to 1.00 M with KCl, was titrated with 0.1000 M standard CO_2-free KOH containing 0.9000 M KCl, while $-\log$ $[H^+]$ was measured with a Corning Model 130 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, pD is defined as $-\log$ $[H^+]$.) Potentiometric measurements were made on solutions of the following composition: the ligands alone; each ligand and Zn(II) in 1:1 and 2:1 molar proportions; a 1:1:1 molar ratio of Zn(II):APP:PDA, Zn(II):APP:PLP, and solutions containing Zn(II), APP, PLP, and PDA in a 1:1:1:1.2 molar ratio. These systems were titrated with 0.1000 M KOH and back-titrated with a 0.2000 M standard solution of HCl.

NMR Measurements. The proton nuclear magnetic resonance spectra were recorded with a Varian EM 390 NMR spectrometer. The chemical shifts are recorded in ppm with respect to the resonance of a chloroform solution of Me_4Si placed in an inner tube. The temperature of the NMR solutions was 35.0 ± 0.5 °C.

The ^{31}P NMR spectra were recorded with a Varian FT80A NMR spectrometer. The chemical shifts are reported in ppm with respect to the resonance of H_3PO_4 (0.30 M in D_2O). A spectral width of 2000 Hz was generally employed, with a pulse of 10 μs and an acquisition time of 1.0 s. The temperature of the solutions was 35.0 ± 0.5 °C.

The purity of APP, PLP, and PDA and the extent of hydration of the solid material were determined by potentiometric measurements. The analytical concentrations of Zn(II), APP, and PLP employed for NMR measurements were 0.100 M, and PDA concentration was 0.100–0.150 M. Experimental solutions of APP and PLP were prepared by direct weighing. Experimental solutions of Zn(II) and PDA were prepared by dilution of stock solutions. The Zn(II) solution of about 0.45 M was prepared from reagent grade zinc chloride and was standardized by the same procedure as described previously.[15] The appropriate amount of base necessary to achieve the desired pD was taken from the corresponding potentiometric titration curve, and the ionic strength was maintained at 1.00 M by addition of KCl.

The pD values of the D_2O solutions were measured with a Metrohm/Brinkman Model 103 pH meter fitted with a Metrohm miniature combination-glass electrode. The instrument was calibrated by standardization with dilute HCl and KOH at 1.00 M (KCl) ionic strength to read $-\log$ $[H^+]$. The pD values were computed by adding 0.40 to the observed reading.[16,17] pD values higher than 12.5 were

(15) Schwarzenbach, G.; Flaschka, H. "Complexometric Titration"; Methuen Co. Ltd.: London, 1969; p 264.
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(17) Schowen, K. B. J. "Transition States of Biochemical Process"; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1970; p 224.

Table I. Protonation Constants[a] of 2-Amino-3-phosphonopropionic acid (APP), Pyridoxal 5′-Phosphate (PLP), 2,6-Pyridinedicarboxylic acid, and APP–PLP Schiff Base ($t = 25.0$ °C, $\mu = 1.00$ M (KCl))
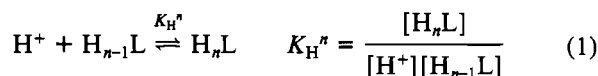
compd	$\log K^3{}_H$	$\log K^2{}_H$	$\log K^3{}_H$	$\log K^4{}_H$
2-amino-3-phosphono-propionic acid	10.406^b	5.928^b	2.317^b	
	10.68^c	6.05^c	2.34^c	
pyridoxal 5′-phosphate	7.915^b	5.756^b	3.579^b	
	8.45^c	6.01^c	3.44^c	
2,6-pyridine-dicarboxylic acid	4.424	2.092		
	$4.69 (1)^c$	$2.09 (1)^c$		
APP–PLP Schiff base	$11.89 (4)^d$	$7.54 (4)^d$	$6.27 (4)^d$	$5.43 (4)^d$

^{a} For log K values determined in this work the standard deviations deviations are less than 1% overall for data points on the potentiometric equilibrium curves. ^{b} Reference 4 ($t = 25.0$ °C, $\mu = 1.00$ M (KCl)). ^{c} Reference 1 ($t = 25.0$ °C, $\mu = 0.10$ M). ^{d} From reference 4 ($t = 35.0$ °C, $\mu = 1.00$ M (KCl) in D_2O) and corrected for aqueous solution with the relationship $pK(D_2O) - pK(H_2O) = 0.41 + 0.020[pK(H_2O)]$.[21]

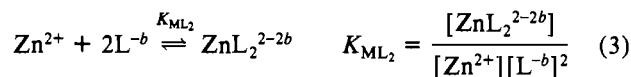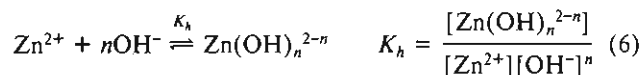determined by comparison with the pD readings of solutions containing 0.100–0.500 M KOD in D_2O.

Results and Discussion

Ligand Protonation Constants. The potentiometric equilibrium curves for APP, PLP, and PDA were determined with the equipment described in the Experimental Section. Protonation constants of APP and PLP were taken from the literature,[4] and those of PDA were calculated with the computer program PKAS and are defined by eq 1, where L represents the ligand (APP, PLP, PDA).[18] These constants were

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

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 are compared to the constants reported previously.[1]

Stability Constants. The normal chelate stability constants 1:1 (molar ratio of zinc(II) to ligand) and 1:2 (molar ratio of zinc(II) to ligand) are defined by eq 2 and 3, respectively.

$$Zn^{2+} + L^{-b} \overset{K_{ML}}{\rightleftharpoons} ZnL^{2-b} \qquad K_{ML} = \frac{[ZnL^{2-b}]}{[Zn^{2+}][L^{-b}]} \qquad (2)$$

$$Zn^{2+} + 2L^{-b} \overset{K_{ML_2}}{\rightleftharpoons} ZnL_2{}^{2-2b} \qquad K_{ML_2} = \frac{[ZnL_2{}^{2-2b}]}{[Zn^{2+}][L^{-b}]^2} \qquad (3)$$

ZnL^{2-b} represents the 1:1 unprotonated, unhydrolyzed chelate; $ZnL_2{}^{2-2b}$ represents the 1:2 unprotonated, unhydrolyzed chelate; Zn^{2+} represents the free unhydrolyzed aquozinc(II) ion; and L^{-b} represents the uncomplexed, totally deprotonated form of the ligand.

The chelate protonation constants were expressed in the same form as the ligand protonation constants (eq 4 and 5),

$$K_{MH_nL} = \frac{[ZnH_nL^{2-b+n}]}{[ZnH_{n-1}L^{2-b+n-1}][H^+]} \qquad (4)$$

$$K_{MH_nL_2} = \frac{[ZnH_nL_2{}^{2-2b+n}]}{[ZnH_{n-1}L_2{}^{2-2b+n-1}][H^+]} \qquad (5)$$

and these constants as well as the normal stability constants were determined by iterative nonlinear least-squares fits of the respective potentiometric equilibrium curves through the mass
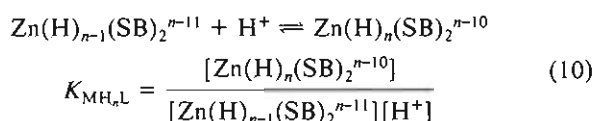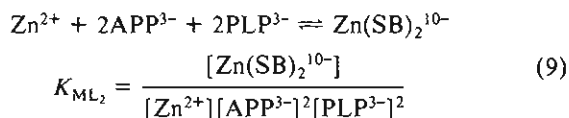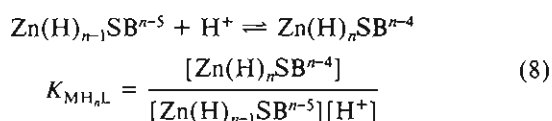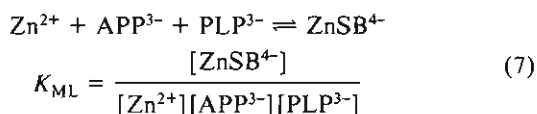
(18) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 168.

balance constraints for all components expressed in terms of known and unknown equilibrium constants using the Fortran computer program BEST.[19] The hydrolysis of zinc(II) (eq 6)
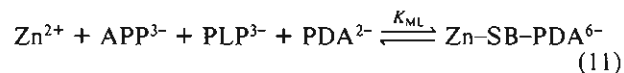
$$Zn^{2+} + nOH^- \overset{K_h}{\rightleftharpoons} Zn(OH)_n{}^{2-n} \qquad K_h = \frac{[Zn(OH)_n{}^{2-n}]}{[Zn^{2+}][OH^-]^n} \quad (6)$$

was considered in the calculation with the hydrolysis constants of Zn^{2+} taken from the literature.[2] The stability constant values determined for the Zn–APP, Zn–PLP, and Zn–PDA systems are reported in Table II. Mohan and Abbott reported an equilibrium study of the metal ion–APP system[20] and discussed the geometry of the complexes formed as well as the stability constants. The logarithmic value of the protonation constant of the 1:2 $Zn(APP)_2$ complex determined in this investigation is almost 2 log units lower than that reported previously (Table II). This difference may be due to precipitation of $Zn(OH)_2$ above pH 8, which could lead to error in the determination. Since the solubility product of $Zn(OH)_2$ is exceeded near pH 8, only points in the potentiometric titration below pH 8 were considered useful for calculation.

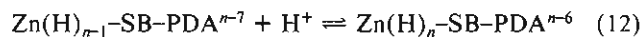**Zn(II)–APP–PLP System.** Potentiometric [H⁺] measurements on 1:1:1 and 1:2:2 molar ratios of Zn(II)–APP–PLP were carried out, and in both systems formation of a precipitate was observed. The precipitate in the 1:1:1 system is probably the neutral Zn(II)–Schiff base species. Also in the 1:2:2 system the formation of a precipitate was observed, but in a lesser amount, and redissolved at about pH 7. As the pH of the solution with the precipitate increases, deprotonation of the reagents (APP and PLP) favors formation of $Zn(SB)_2$, which cannot form a neutral species in the pH range where equilibrium measurements are normally carried out (pH 2–11). Thus, the formation of $Zn(SB)_2$ results in dissolving the original precipitate of the neutral ZnSB complex. The equilibria for normal and protonated chelates are indicated by eq 7–10, and the stability constants determined are presented in Table II.

$$Zn^{2+} + APP^{3-} + PLP^{3-} \rightleftharpoons ZnSB^{4-}$$

$$K_{ML} = \frac{[ZnSB^{4-}]}{[Zn^{2+}][APP^{3-}][PLP^{3-}]} \quad (7)$$

$$Zn(H)_{n-1}SB^{n-5} + H^+ \rightleftharpoons Zn(H)_nSB^{n-4}$$

$$K_{MH_nL} = \frac{[Zn(H)_nSB^{n-4}]}{[Zn(H)_{n-1}SB^{n-5}][H^+]} \quad (8)$$

$$Zn^{2+} + 2APP^{3-} + 2PLP^{3-} \rightleftharpoons Zn(SB)_2{}^{10-}$$

$$K_{ML_2} = \frac{[Zn(SB)_2{}^{10-}]}{[Zn^{2+}][APP^{3-}]^2[PLP^{3-}]^2} \quad (9)$$

$$Zn(H)_{n-1}(SB)_2{}^{n-11} + H^+ \rightleftharpoons Zn(H)_n(SB)_2{}^{n-10}$$

$$K_{MH_nL} = \frac{[Zn(H)_n(SB)_2{}^{n-10}]}{[Zn(H)_{n-1}(SB)_2{}^{n-11}][H^+]} \quad (10)$$

Zn(II)–APP–PLP–PDA System. Potentiometric equilibrium measurements for a system having a 1:1:1:1.2 molar ratio of Zn(II)–APP–PLP–PDA were carried out. The formation of normal and protonated chelates is indicated by eq 11 and 12, and the equilibrium constants determined are indicated

(19) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 2403.
(20) Mohan, M. S.; Abbott, E. H. *J. Coord. Chem.* **1978**, *8*, 175.
(21) Robertson, R. E.; Laughton, P. M. "Solute Solvent Interactions"; Coetzee, J. F.; Ritchie, C. D., Eds.; Marcel Dekker: New York, 1969; pp 400–538.
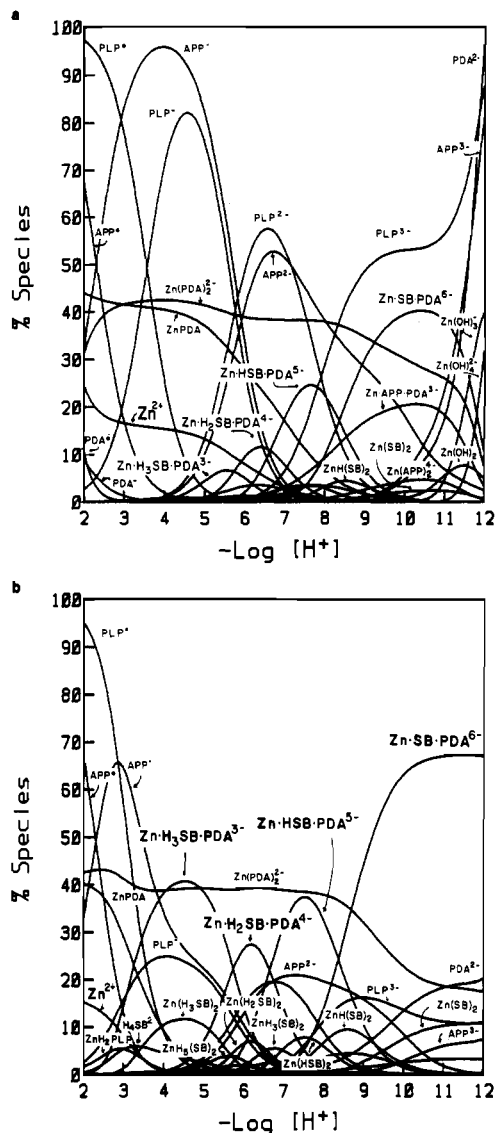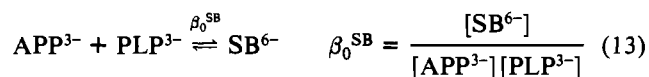
$$Zn^{2+} + APP^{3-} + PLP^{3-} + PDA^{2-} \overset{K_{ML}}{\rightleftharpoons} Zn–SB–PDA^{6-} \quad (11)$$

$$K_{ML} = \frac{[Zn–SB–PDA]^{6-}}{[Zn^{2+}][APP^{3-}][PLP^{3-}][PDA^{2-}]}$$

$$Zn(H)_{n-1}–SB–PDA^{n-7} + H^+ \rightleftharpoons Zn(H)_n–SB–PDA^{n-6} \quad (12)$$

$$K^H{}_{MH_nL} = \frac{[Zn(H)_n–SB–PDA^{n-6}]}{[H^+][Zn(H)_{n-1}–SB–PDA^{n-7}]}$$

in Table II. Two possible bonding arrangements for the chelate are represented by **1** and **2**. Formula **1** has the
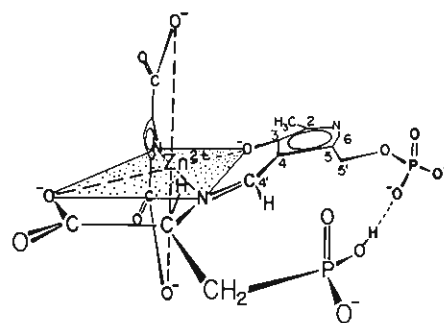
1, Zn–HSB–PDA⁵⁻
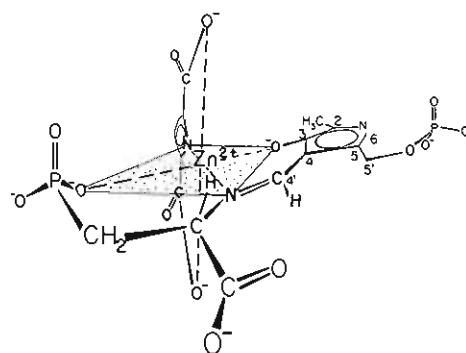
2, Zn–SB–PDA⁶⁻

carboxylate oxygen of the amino acid part of the complex coorindated to the metal ion, and the other has the phosphonate oxygen coordinated. The first protonation of the chelate occurs at the phosphonate oxygen of the amino acid part of the complex. The second protonation of the chelate occurs at the phosphate oxygen, and the third one is at the pyridine nitrogen. An NMR study of APP–PLP Schiff base, SB, clearly showed the protonation sites of that system.[4]

The Fortran computer program SPECIES, which employs the same algorithm as BEST, was used to calculate the species distribution curves shown in Figure 1. Figure 1a shows all the protonated species of the Zn(II)–SB–PDA chelate system. The formation of the unprotonated mixed-ligand chelate (Zn–SB–PDA⁶⁻) has its maximum concentration at pH 10.40. The concentrations of the protonated forms of this chelate initially increase, and then drop, as the pH decreases, and become zero at pH values lower than 3.0. The initial concentrations of the reagents in Figure 1b are 50 times higher than those in Figure 1a. This increase of the concentration reflects an increasing amount of Zn–SB–PDA complex relative to the free components and other complexes present in the system. Since the initial concentrations of the components in the metal ion–vitamin B_6–amino acid systems generally employed for kinetic studies by NMR are usually about 0.10 M, Figure 1b reflects more closely than Figure 1a the distribution of species that will be employed. The completely deprotonated Schiff base formed between APP and PLP was considered in

Figure 2. Proton magnetic resonance spectra (90 MHz): (a) a D_2O solution containing a 1:1:1 molar ratio of Zn(II), PLP–APP Schiff base, and PDA at pD 6.60 (μ = 1.00 M (completed with KCl); t = 35.0 °C); (b) Zn·SB·PDA and $Zn(SB)_2$ complexes at pD 10.16 under the same conditions as in (a).

Figure 1. (a) Species distribution curves for the system Zn–PLP–APP–PDA in 1:1:1:1.2 molar ratio, where Zn·SB·PDA, Zn·HSB·PDA, $Zn·H_2SB·PDA$, and $Zn·H_3SB·PDA$ are nonprotonated, mono-protonated, diprotonated, and triprotonated, respectively, forms of zinc(II)–Schiff base–PDA complexes. $Zn(SB)_2$, $ZnH(SB)_2$, $Zn(H-SB)_2$, $ZnH_3(SB)_2$, $Zn(H_2SB)_2$, $ZnH_5(SB)_2$, and $Zn(H_3SB)_2$ are nonprotonated and protonated species of Zn(II)–Schiff base 1:2 complexes. PLP^0, PLP^-, PLP^{2-}, and PLP^{3-} represent species of PLP; APP^0, APP^-, APP^{2-}, and APP^{3-} are the APP species; and PDA^0, PDA^-, and PDA^{2-} are the PDA species. Zn^{2+} is free aquozinc(II); ZnPDA and $Zn(PDA)_2{}^{2-}$ are 1:1 and 1:2 complexes of Zn and PDA; ZnAPP$^-$ and $Zn(APP)_2{}^{4-}$ are 1:1 and 1:2 Zn–APP complexes; and Zn·APP·PDA^{3-} and Zn·HAPP·PDA^{2-} are mixed-ligand Zn–APP–PDA complexes. Conditions: t = 25.0 °C; concentrations of PLP, APP, and metal ion equal 2.00 × 10^{-3} M and that of PDA is 2.40 × 10^{-3} M; μ = 1.00 M (KCl). (b) Species distribution curves for the Zn–PLP–APP–PDA system with initial concentrations of all reagents 50.0 times their concentrations in (a).

the calculation of these species distributions. The formation constant of completely deprotonated Schiff base ($\beta_0{}^{SB}$; eq 13)

$$APP^{3-} + PLP^{3-} \overset{\beta_0{}^{SB}}{\rightleftharpoons} SB^{6-} \qquad \beta_0{}^{SB} = \frac{[SB^{6-}]}{[APP^{3-}][PLP^{3-}]} \quad (13)$$

was determined with the computer program SPECIES mentioned above, by varying the magnitude of $\beta_0{}^{SB}$ at the pH of maximum formation (pH 9.1), minimizing the difference between the total amount of calculated Schiff base and the total amount of Schiff base determined from the conditional constant de-
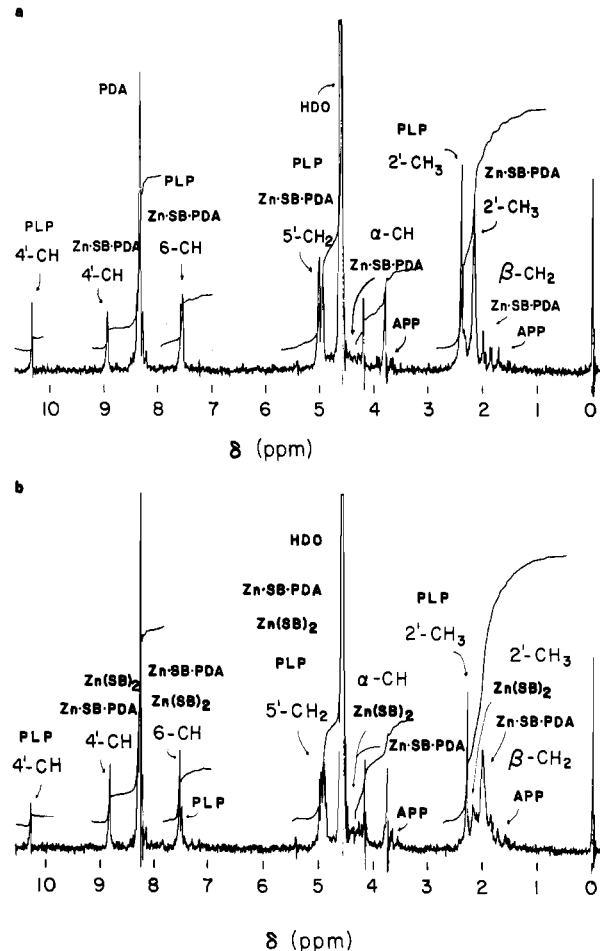
termined in previous work.[2] Where [APP^{3-}], [PLP^{3-}], and [SB^{6-}] are the concentrations of the completely deprotonated forms of APP, PLP, and APP–PLP Schiff base. The value thus found for $\beta_0{}^{SB}$ is 0.69. The most favorable conditions for studying the kinetics of Zn(II)–PLP-catalyzed transamination and dephosphonylation reactions of APP can now be selected. Kinetic studies are in progress in order to determine the relative effect of each species toward transamination and dephosphonylation reactions.

The formation of a zinc(II)–Schiff base 1:1 complex was not observed, but a small amount of the 1:2 complex is formed, as is more readily seen when concentrations of the reagents are increased (Figure 1b). The deprotonation of the Schiff base increases its basicity and results in more effective competition with the mixed-ligand SB–PDA complex.

In order to verify if there is only one complex species containing Zn(II), SB, and PDA as indicated by the potentiometric data and to determine which donor groups are coordinated with the metal ion, proton NMR and ^{31}P NMR measurements were made. Figure 2a shows the ^1H NMR spectrum of Zn(II)–SB–PDA system with a 1:1:1.1 molar ratio, at pD 6.60. The numbering system used to identify the protons of the complex are illustrated in formula **1**. The $2'-CH_3$ protons are found at 2.16 ppm, upfield with respect to the $2'-CH_3$ protons of free PLP (2.40 ppm), and the $4'-CH$ proton appears at 8.86 ppm. The 6-CH proton is found at 7.55 ppm, at about the same position as the 6-CH proton of free PLP (7.52 ppm). The $5'-CH_2$ protons are found at 4.98 ppm together with the $5'-CH_2$ protons of free PLP. The resonance
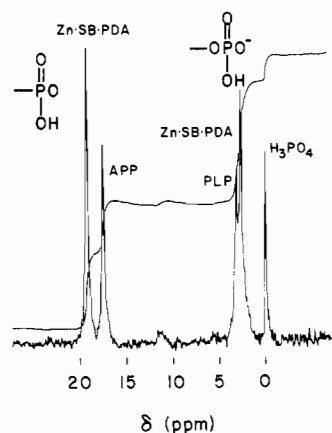
Figure 3. Decoupled ^{31}P magnetic resonance spectrum of a D$_2$O solution containing a 1:1:1 molar ratio of Zn(II), PLP–APP Schiff base, and PDA at pD 6.93 (μ = 1.00 M (completed with KCl); t = 35.0 °C).

due to the α-CH proton (of the amino acid part of the complex) is not clearly observed because it appears near the HDO peak. The β-CH$_2$ proton resonances are also not observed because they are obscured by the 2′-CH$_3$ proton resonances. The protons of the secondary ligand (PDA) appear at 8.32 ppm, at about the same position as in ZnPDA and Zn(PDA)$_2$ complexes. At pD 10.16, in addition to the 2′-CH$_3$ resonance of Zn–SB–PDA, a second methyl resonance appears (Figure 1b) that is assigned to the 2′-CH$_3$ of the Zn(SB)$_2$ complex.

The decoupled ^{31}P NMR spectrum of Zn(II)–SB–PDA complex at pD 6.93 is shown in Figure 3. The resonance due to the phosphonate group appears at 19.30 ppm, downfield with respect to the phosphonate group of free APP (17.54 ppm). The resonance due to the phosphate group appears at 2.66 ppm, upfield with respect to the phosphate group of free PLP. The variations of the chemical shift vs. pD of the resonance of the phosphonate group of APP, APP–PLP Schiff base, and Zn(II)–SB–PDA complex (Figure 4a,b) indicate that the first protonation of Zn(II)–SB–PDA complex takes place at the phosphonate group. The logarithmic value determined for this protonation constant is 8.50 ± 0.05 in D$_2$O, which is comparable with the constant determined in H$_2$O by potentiometric titration (Table II).

While the chemical shift of the resonance of the phosphonate group of APP and APP–PLP Schiff base increases at pD values higher than 10, that of the Zn(II) complex remains constant, indicating there is no change in the electronic environment of the phosphonate group of the Zn–SB–PDA complex in this pD range. On the other hand, changes in the chemical shift of the same group in APP and in the APP–PLP Schiff base are due to deprotonation steps:[2] the deprotonation of the amino group of APP, hydrogen bonded to the phosphonate oxygen (**3a–c**), and to the azomethine nitrogen of the Schiff base (SB), hydrogen bonded to the phenolate oxygen and the phosphonate oxygen (**4a,b**).
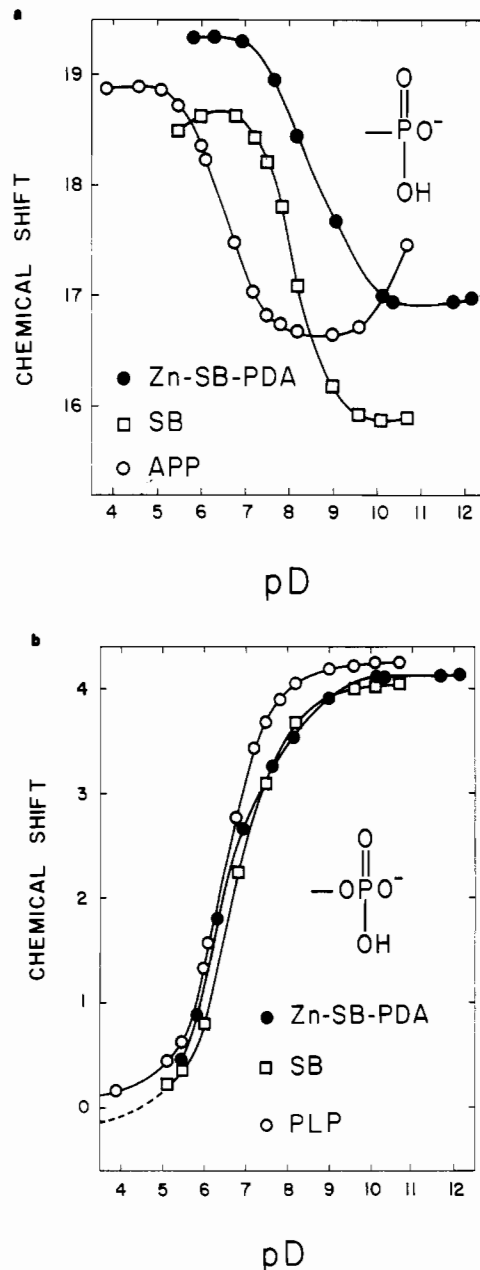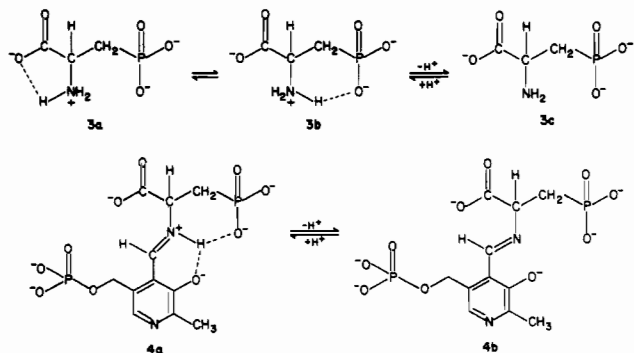

Figure 4. Variation of ^{31}P NMR chemical shifts: (a) of the phosphonate group of APP, the APP–PLP Schiff base (SB), and the Zn–SB–PDA complex as a function of pD in D$_2$O (t = 35.0 °C; μ = 1.00 M (KCl)); (b) of the phosphonate group of PLP, the APP–PLP Schiff base, and the Zn–SB–PDA complex as a function of pD in D$_2$O (t = 35.0 °C; μ = 1.00 M (KCl)).

It is interesting to note that the protonation constant of the phosphonate group of Zn(II)–SB–PDA, 8.38, complex is roughly 2 log units higher than the protonation constant, 5.928, of the phosphonate group of APP in water and in D$_2$O (Tables I and II). These results provide evidence for hydrogen bonding involving the phosphonate group of the Zn(II) complex. With the carboxylate oxygen coordinated to the metal ion, the phosphonate proton could be hydrogen bonded to the phosphate oxygen as indicated by **1**.

In order to confirm this interpretation and to see if a structural change occurs after deprotonation of the phosphonate group of the Schiff base chelate, ^{31}P NMR of 2-aminoethylphosphonic acid (2-AEP), 2-AEP–PLP Schiff base (SB′), and Zn(II)–SB′–PDA complex were measured. 2-AEP was studied because its Schiff base does not have the complication of having an alternative amino acid functional group (the carboxylate group) that may become coordinated to the Zn(II)

Table II. Logarithm of Stability Constants[a] of Zn(II) Chelates ($t = 25.0$ °C, $\mu = 1.00$ M (KCl))

	$\dfrac{[ML]}{[M][L]}$	$\dfrac{[MHL]}{[ML][H^+]}$	$\dfrac{[MH_2L]}{[MHL][H^+]}$	$\dfrac{[MH_3L]}{[MH_2L][H^+]}$	$\dfrac{[ML_2]}{[M][L]^2}$	$\dfrac{[MHL_2]}{[ML_2][H^+]}$
2-amino-3-phosphonopropionic	6.48	6.05			11.42	6.09
acid (APP)	6.63 (4)[b]	6.21 (4)[b]			11.79 (4)[b]	7.97 (7)[b]
pyridoxal 5′-phosphate (PLP)	3.47	6.71	5.17			
	3.6[c]	6.3[c]	5.6[c]			
2,6-pyridinedicarboxylic	6.43				12.46	
acid (PDA)	6.0[d]				11.5[d]	
APP–PDA	12.18	6.98				
APP–PLP (SB)[f,g]	9.42	8.31	6.21	5.48	17.12	9.50
SB–PDA	15.47	8.38	6.66	5.75		
		8.50 (5)[h]				

[a] For the stability constants determined in this work, the standard deviations are less than 0.01 overall for data points on the potentiometric equilibrium curves. The numbers in parentheses express the error in the last significant figure. [b] Reference 20 ($t = 25.0$ °C, $\mu = 0.2$ (KNO$_3$)). [c] Reference 18 ($t = 25.0$ °C, $\mu = 0.5$). [d] Reference 12 ($t = 25.0$ °C, $\mu = 1.0$). [e] Reference 18 ($t = 25.0$ °C, $\mu = 0.1$). [f] For a more detailed definition of these constants see eq 7 and 8. [g] Protonation constants of minor 2:1 species and their definitions: $[MH_2L_2]/[MHL_2][H^+] = 10^{7.96}$; $[MH_3L_2]/[MH_2L_2][H^+] = 10^{7.0(1)}$; $[MH_4L_2]/[MH_3L_2][H^+] = 10^{6.6(1)}$; $[MH_5L_2]/[MH_4L_2][H^+] = 10^{5.7(1)}$; $[MH_6L_2]/[MH_5L_2][H^+] = 10^{5.7(1)}$. [h] This work; constant determined in D$_2$O ($t = 35.0$ °C, $\mu = 1.0$).

ion. The results are shown in the Figure 5. The protonation reactions of the amino group and the phosphonate group of 2-AEP are indicated by the change of chemical shift vs. pD. The protonation constants determined from the variation of chemical shift are 11.5 ± 0.1 and 6.77 ± 0.05, for the amino and phosphonate groups, respectively. The protonation constants of the azomethine nitrogen and of the phosphonate group in the 2-AEP–PLP Schiff base (SB′) determined in the same way are found to be 12.5 ± 0.1 and 7.40 ± 0.05. The protonation constants of the phosphonate group of the AEP–PLP Schiff base is higher than the protonation constant of the same group in 2-AEP alone. Since the phosphonate group is coordinated to the azomethine proton as in **5**, this protonation
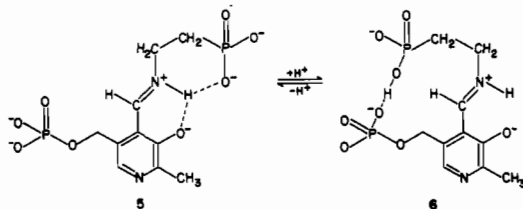
step was expected to be lower than for 2-AEP. The explanation is that probably there is a contribution of species **6**, which has the phosphonate group hydrogen bonded to the phosphate group in the same molecule. On the other hand, the chemical shift of the resonance due to the phosphonate group of the Zn–SB′–PDA complex is constant in the range pH 4.7–12, indicating that the protonation step now occurs at much lower pD than for protonation of the same group in 2-AEP. This lower value for the protonation constant reflects the effect of coordination by the metal ion, and the protonation of phosphonate group therefore occurs only at much lower pD values. However, for the Zn(II)–SB–PDA system the protonation constant of the phosphonate group is 2 log units higher than for the same group in the APP, indicating that it cannot be coordinated to the metal ion at values lower than pH 8.50 and could be hydrogen bonded to the phosphate group of the same molecule, as indicated by structure **1**.

After deprotonation of the phosphonate group, it may displace the carboxylate group from the coordination sphere of
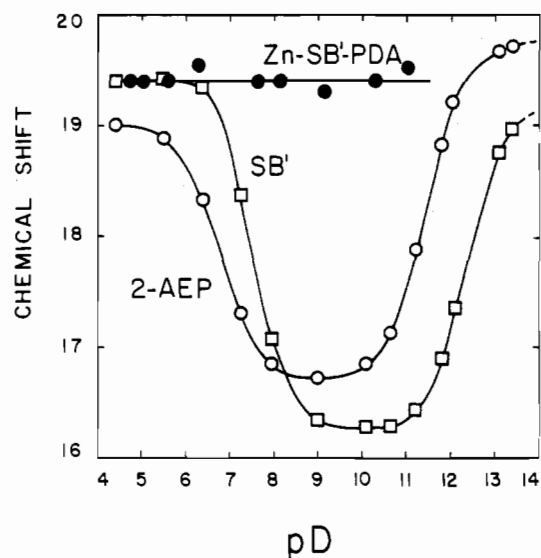

Figure 5. Variation of ^{31}P NMR chemical shifts of the phosphonate group of 2-AEP, the 2-AEP–PLP Schiff base (SB′), and the Zn–SB′–PDA complex as a function of pD in D$_2$O ($t = 35.0$ °C; $\mu = 1.00$ M (KCl)).

the metal ion, as indicated by **2**, or it may not. Since the affinity of Zn^{2+} for the phosphonate group is higher than for the carboxylate group, as may be seen by comparing log K_{ML} for 2-AEP (6.16) and glycine[10] (4.90 ± 0.02), it is concluded that **2** represents the structure of this Schiff base chelate at high pH. A similar conclusion was reached for the APP–PLP Schiff base (APP–PLP system without metal ion), and it is interesting to observe that the protonation constant of the azomethine nitrogen of 2-AEP–PLP Schiff base (12.5 ± 0.1) is very close to that of APP–PLP Schiff base (12.54 ± 0.02).

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