protonation and deprotonation of the amide groups are rate-determining steps, similar to what has previously been observed for open-chain ligands. Thus **2** has a predominant macrocyclic character, whereas **1** is controlled by the reactivity of the amide groups.

On the other hand, the attainment of the trivalent state for both nickel and copper complexes, which is greatly favored by the presence of deprotonated amido groups, is mainly dependent on the ring size but practically independent of the more or less rigid structure of the macrocycle.

Contribution from the Department of Chemistry,
Texas A&M University, College Station, Texas 77843

Gallium(III), Aluminum(III), and Zinc(II) Pyridoxal 5′-Phosphate Catalyzed Transamination and Dephosphonylation of 2-Amino-3-phosphonopropionic Acid

Bruno Szpoganicz† and Arthur E. Martell*

Received June 11, 1985

Kinetic studies of reactions of the Schiff bases (SB) formed from pyridoxal 5′-phosphate (PLP) and 2-amino-3-phosphonopropionic acid (APP) and of the 1:1:1 Zn(II)–SB–PDA system (PDA = 2,6-pyridinedicarboxylic acid), the 1:2 Ga(III)–SB system, and the 1:2 Al(III)–SB system have been carried out. Formation and disappearance of a ketimine intermediate and its complexes were followed by proton NMR and ^{31}P NMR. The reaction occurs in two distinct sequential steps: transamination and dephosphonylation. The specific rate constants for individual species of the metal-free systems are $k_{H_4SB} = 1.64 \times 10^{-4}$ s^{-1}, $k_{H_3SB} = 7.56 \times 10^{-5}$ s^{-1}, and $k_{H_2SB} = 2.34 \times 10^{-5}$ s^{-1} for the transamination step. The values for k_{HSB} and k_{SB} are about zero. The corresponding dephosphonylation rate constants are $k'_{H_4SB} = 4.27 \times 10^{-6}$ s^{-1}, $k'_{H_3SB} = 1.26 \times 10^{-6}$ s^{-1}, and $k'_{H_2SB} = 6.84 \times 10^{-7}$ s^{-1}. The values for k'_{HSB} and k'_{SB} are about zero. Transamination and dephosphonylation reactions proceed more rapidly for Ga(III) complexes than for those of Al(III) and Zn(II). The specific transamination rate constants for the individual species of the 1:2 Ga(III)–SB system are $k_{Ga(H_3SB)_2} = 4.66 \times 10^{-4}$ s^{-1}, $k_{GaH_5(SB)_2} = 3.51 \times 10^{-4}$ s^{-1}, $k_{Ga(H_2SB)_2} = 3.13 \times 10^{-4}$ s^{-1}, $k_{GaH_3(SB)_2} = 3.13 \times 10^{-4}$ s^{-1}, $k_{Ga(HSB)_2} = 3.15 \times 10^{-4}$ s^{-1}, $k_{GaH(SB)_2} = 2.20 \times 10^{-4}$ s^{-1}, and $k_{Ga(SB)_2} = 3.12 \times 10^{-5}$ s^{-1}. The specific rate constants for the dephosphonylation step are $k_{GaH_5(SB)_2} = 5.2 \times 10^{-6}$ s^{-1}, $k_{Ga(H_2SB)_2} = 5.20 \times 10^{-6}$ s^{-1}, $k_{GaH_3(SB)_2} = 5.17 \times 10^{-6}$ s^{-1}, $k_{Ga(HSB)_2} = 5.09 \times 10^{-6}$ s^{-1}, $k_{GaH(SB)_2} = 2.53 \times 10^{-6}$ s^{-1}, and $k_{Ga(SB)_2} = 4.92 \times 10^{-7}$ s^{-1}. The results show that the most active species are those in which the carboxylate group of the amino acid moiety of the SB ligand is coordinated to the metal ion and the phosphonate is not coordinated.

Introduction

This paper describes kinetic studies of transamination and dephosphonylation of 2-amino-3-phosphonopropionic acid (APP) catalyzed by pyridoxal 5′-phosphate(PLP) and Zn(II), Al(III), or Ga(III) as the culmination of a series of comprehensive investigations of reactions of the PLP–APP system.[1-3] The extensive equilibrium studies conducted on PLP–APP, Zn(II)–PLP–APP–PDA (PDA = 2,6-pyridinecarboxylic acid), Al(III)–PLP–APP, and Ga(III)–PLP–APP, systems have provided a complete description of molecular species formed as a function of p[H] and of concentrations of the components. Preliminary spectrophotometric studies on APP demonstrated metal ion and pyridoxal catalysis of dephosphonylation, and a general reaction mechanism was proposed.[4,5] The reaction pathway suggested resembles the mechanism proposed for the pyridoxal-catalyzed β-decarboxylation of aspartic acid.[6,7] The purpose of this study is to measure the reaction kinetics, relate the reaction rate constants to the active species in solution, and, if possible, further clarify the reaction mechanism. Another objective of this investigation is to detect any intermediates formed during the reaction to provide additional information characterizing the nature of the reactions that occur with and without metal ions. The detection of intermediates by nuclear magnetic resonance in the metal ion–vitamin B_6 systems has been carried out for other reaction types.[7,8] Recently, the characterization of a general intermediate in the transamination reaction has been described.[9]

There are several advantages in using PLP rather than PL as a catalyst in the reactions being studied. The degree of formation of Schiff base intermediates is much higher with PLP. Also the phosphate ester moiety of vitamin B_6 increases solubility in water for the Schiff base as well as for its complexes. The metal complexes formed with the SB of PLP have less tendency to form neutral compounds, which could precipitate, than do those formed with the Schiff bases of pyridoxal.

Experimental Section

Materials. Pyridoxal 5′-phosphate was obtained from Sigma Chemical Company. 2-Amino-3-phosphonopropionic acid was purchased from Calbiochem-Behring Corp. Aluminum sulfate, hydrochloric acid, and potassium chloride were obtained from Fisher Scientific Co. Gallium metal was purchased from D. F. Goldsmith Chemical Metal Corp. 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. A standard Al(III) solution, having a concentration of about 10^{-2} M, was prepared from reagent grade aluminum(III) sulfate octadecahydrate ($Al_2(SO_4)_3 \cdot 18H_2O$) and was standardized by titration with the disodium salt of EDTA.[10] Some HCl was added to avoid hydrolysis. Standard gallium(III) solutions were prepared by dissolving an accurately weighted quantity of 99.99% 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's plot of $(V_0 + V_{KOH}) \times 10^{-pH}$ 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.[11] The excess acid was also checked by the procedure of Harris and Martell.[12]

Samples of about 0.10 and 0.20 mmol of APP and PLP and 0.10 mmol of Al(III) or Ga(III) were diluted with 50 mL of double distilled water in a sealed, thermostated (25.0 ± 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 gradu-

† Abstracted in part from a dissertation submitted by Bruno Szpoganicz to the faculty of Texas A&M University in partial fullfillment of the requirements for the degree of Doctor of Philosophy.

(1) Szpoganicz, B.; Martell, A. E. *J. Am. Chem. Soc.* **1984**, *106*, 5513.
(2) Szpoganicz, B.; Martell, A. E. *Inorg. Chem.* **1984**, *23*, 4442.
(3) Szpoganicz, B.; Martell, A. E. *Inorg. Chem.* **1985**, *24*, 2414.
(4) Langohr, M. F.; Martell, A. E. *J. Chem. Soc., Chem. Commun.* **1977**, 343.
(5) Langohr, M. R.; Martell, A. E.; Tatsumoto, K. *Inorg. Chim. Acta* **1985**, *108*, 105.
(6) Sakkab, N. Y.; Martell, A. E. *Bioinorg. Chem.* **1975**, *5*, 67.
(7) Martell, A. E. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1982**, *53*, 163.
(8) Abbott, E. D.; Martell, A. E. *J. Am. Chem. Soc.* **1973**, *95*, 5014.
(9) Taylor, P.; Martell, A. E. *Inorg. Chem.* **1984**, *23*, 2734.
(10) Schwarzenbach, G.; Flaschka, H. "Complexometric Titration"; Methuen Co. Ltd.: London, 1969.
(11) Rossotti, F. J. C.; Rossotti, H. *J. Chem. Educ.* **1965**, *42*, 375.
(12) Harris, W. R.; Martell, A. E. *Inorg. Chem.* **1976**, *15*, 713.

0020-1669/86/1325-0327$01.50/0

ated (Metrohm) microburet. The test solution, adjusted to 1.00 M in KCl, was titrated with 0.2000 M standard CO_2-free KOH containing 0.8000 M KCl while -log [H^+] was measured with a Corning Model 130 pH meter calibrated with dilute standard hydrochloric acid and with dilute standard potassium hydroxide at 1.00 M (KCl) ionic strength to read -log [H^+] directly (for the purposes of this reseach p[H] is defined as -log [H^+]). Potentiometric measurements were made on solutions of the following composition: each ligand and Al(III) in 1:1 and 2:1 molar proportions; each ligand and Ga(III) in 1:1 and 2:1 molar proportions; solutions containing Al(III), APP, and PLP having 1:2:2 molar ratio; solutions containing Ga(III), APP, and PLP in a 1:2:2 molar ratio.

NMR Measurements. The proton nuclear magnetic resonance spectra were recorded on a Varian EM390 NMR spectrometer. The chemical shifts were recorded in ppm with respect to the resonance of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS), an internal reference. Only enough DSS was used to give a small methyl peak, and the diffuse pattern of the CH_2 peaks barely showed on the baseline. The temperature of the NMR solution was 35.0 ± 0.5 °C.

The ^{31}P NMR spectra were recorded with a Varian FT 80A 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 of 1.0 s. The temperature of the solutions was 35.0 ± 0.5 °C.

The purity of APP and PLP and the extent of hydration of the solid material were determined by potentiometric measurements. The analytical concentrations of Al(III), Ga(III), APP, and PLP employed for NMR measurements were 0.0500, 0.0500, 0.100, and 0.100 M, respectively. The experimental solutions were prepared by direct weighing of APP and PLP, and by dilution of stock solutions of Al(III) or Ga(III). A standard Al(III) solution having a concentration of about 0.50 M was prepared from reagent grade Al(III) sulfate octadecahydrate in D_2O. The aluminum salt was dissolved in D_2O and some DCl was added to avoid formation of hydrolysis products. The solution was standardized by the procedure described previously.[11] A Gilmont microburet was employed for the measurement of small volumes. A standard Ga(III) solution having a concentration of 0.500 M was prepared by dissolving an accurately weighed quantity of 99.99% pure gallium metal in concentrated HCl. The solution was evaporated to dryness and redissolved with a small volume of 20% DCl in D_2O, and dilution to the final volume was completed with D_2O.

The p[D] 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 at 1.00 M ionic strength (adjusted with KCl) to read -log [H^+]. The p[D] values were computed by adding 0.40 to the observed reading.[13,14]

Equilibrium Data. All the equilibrium calculations for the systems APP–PLP, Ga(III)–SB, Al(III)–SB, and Zn–SB–PDA in 1:1, 1:2, 1:2, and 1:1:1 molar ratios, respectively, have been described in previous publications.[1-3]

Kinetic Treatment. The rate measurements were made by monitoring the integrations of the ^{31}P and ^1H M NMR resonances assigned to the individual species present as a function of time. Thus quantitative measurements were made for the disappearance of the metal Schiff base complexes, the appearance of intermediates, the disappearance of intermediates, and, when possible, the appearance of inorganic phosphate. The latter was measured only when its resonance could be distinguished from that of the phosphate ester of PLP. For the metal-free systems the rate measurement was based on the disappearance of Schiff base formed between APP and PLP and also on the appearance and the disappearance of the intermediate and the appearance of inorganic phosphate. The rate equations are based on the following reaction schemes:

for the metal-free system

$$\mathrm{PLP + APP} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SB} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_3} \text{products} \quad (1)$$

for the 1:2 metal–Schiff base systems

$$\mathrm{M^{n+} + 2PLP + 2APP} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{MSB} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_3} \text{products} \quad (2)$$

for the Zn(II)–SB–PDA system

$$\mathrm{Zn^{2+} + PLP + APP + PDA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Zn(II)\text{–}SB\text{–}PDA} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_3} \text{products} \quad (3)$$

(13) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(14) Martell, A. E. In "Chemical and Biological Aspects of Vitamin B_6 Catalysis"; Alan R. Liss, Inc.: New York, 1984; Part A.

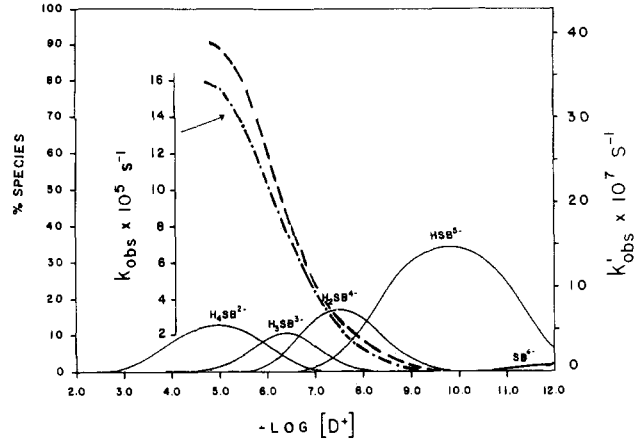


Figure 1. Overlay of species distribution of PLP–APP Schiff base and variation of observed rate constants for (—·—) transamination (k_{obsd}) and (---) dephosphonylation (k'_{obsd}) steps for the PLP–APP system. t = 35.0 °C, and μ = 1.00 M (KCl).

where PLP = pyridoxal 5′-phosphate, APP = 2-amino-3-phosphonopropionic acid, SB = Schiff base, I = intermediate, and PDA = 2,6-pyridinedicarboxylic acid (the secondary ligand used in the Zn(II) system). All of these species exist in several protonated forms in solution, and the terms PLP, APP, SB, etc. in eq 1–3, and subsequently in eq 4–9 are intended to represent the total protonated species. When the individual protonated species are to be distinguished, as in eq 10, the number of protons on each species is specified, as in H_4SB, H_3SB, etc. The following rate equation applies to the metal-free system, in the presence of an appreciable amount of Schiff base, for the formation of the intermediate:

$$d[I]/dt = k_{obsd}[SB] \quad (4)$$

For the dephosphonylation reaction the following equation applies:

$$d[PO_4^{3-}]/dt = k'_{obsd}[I] \quad (5)$$

Thus, the kinetics of the transamination reaction are related directly to the concentration of Schiff base in solution, and dephosphonylation to the concentration of the intermediate. The values of the first-order constants k_{obs} and k'_{obsd} are then found by a plot of $d[I]/dt$ vs. [SB] and $d[PO_4^{3-}]/dt$ vs. [I], respectively.

Similarly, for the metal–Schiff base chelate and its intermediate, the following first-order rate equations were employed:

$$-d[MSB]/dt = k_{obsd}[MSB] \quad (6)$$

and, in the absence of side reactions

$$d[I]/dt = k_{obsd}[MSB] \quad (7)$$

The first step is formation of the Schiff base metal complex, followed by formation of an intermediate. The intermediate is tentatively assumed to be a ketimine metal chelate formed by transamination. Subsequently, dephosphonylation of the ketimine would result in formation of inorganic phosphate. The following equations apply for the dephosphonylation step: disappearance of the intermediate ketimine chelate (8) and appearance of inorganic phosphate (9). In the absence of complicating side reactions, k'_{obsd} would be expected to be equal to k''_{obsd} for the Zn(II)–SB–PDA system and equal to $k''_{obsd}/2$ for the 1:2 Ga(III)–SB and Al(III)–SB systems because each 1 mol of these complexes forms 2 mol of inorganic phosphate. Since the concentrations of the Schiff base metal complexes are known from the equilibrium studies, and may be followed as a function of time by NMR, the first-order rate constants, k_{obsd} and k'_{obsd} may be determined by a plot of log [MSB] vs. time or log [I] vs. time.

$$-d[I]/dt = k'_{obsd}[I] \quad (8)$$

$$d[PO_4^{3-}]/dt = k''_{obsd}[I] \quad (9)$$

Results and Discussion

Reaction Kinetics of the APP–PLP System. The distribution of PLP–APP Schiff base species as a function of p[D] was calculated from the previously determined equilibrium constants[1] and is shown in Figure 1. From this plot, the p[D] values for

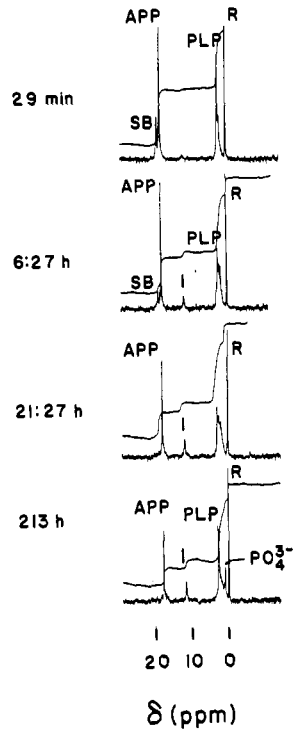


Figure 2. Decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:1 molar ratio of APP and PLP at p[D] 6.51 taken at 29 min, 6.27 h, 21.27 h, and 213 h after mixture of the reagents. I denotes the intermediate and R the reference (0.300 M phosphoric acid in D_2O); t = 35.0 °C, and μ = 1.00 M (adjusted with KCl). $T_{APP} = T_{PLP} = 0.100$ M.

Table I. Observed Rate Constants for Transamination and Dephosphonylation Reactions in the Metal-Free PLP–APP System

pD	k_{obsd}(transamination), s^{-1}	k'_{obsd}(dephosphonylation), s^{-1}
4.99	1.55×10^{-4}	3.97×10^{-6}
6.51	7.08×10^{-5}	1.50×10^{-6}
7.51	2.51×10^{-5}	6.27×10^{-7}
9.99	no reaction or too slow	no reaction or too slow

kinetic runs were chosen. Disappearance of APP–PLP Schiff base, formation and disappearance of an intermediate, and formation of inorganic phosphate are illustrated in Figure 2 by a set of decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:1 molar ratio of APP and PLP at p[D] 6.51. The spectra were taken at the following intervals of time: 29 min, 7.27 h, 21.27 h, 213 h. The resonance due to the phosphonate group of the intermediate appears at 11.70 ppm to higher field than the resonance of the free APP, which appears at 17.15 ppm.

Table I shows the values of the first-order rate constants determined by NMR for the first step of the reaction, which is formation of the intermediate. This initial step is believed to be transamination, which is then followed by dephosphonylation to give inorganic phosphate. The rate constants determined show considerable p[D] dependence. The observed rate constant for transamination and dephosphonylation reactions at any p[D] value may be expressed as a function of the contribution of each species (eq 10). As the p[D] decreases from 7.0 to 5.0 the concentration

$$k_{obsd}[SB_T] = k_{H_4SB}[H_4SB] + k_{H_3SB}[H_3SB] + k_{H_2SB}[H_2SB] + k_{HSB}[HSB] + k_{SB}[SB] \quad (10)$$

of H_4SB^{2-} increases, corresponding to increasing relative concentrations of the more highly protonated Schiff base species.

The observed rate constants for the transamination and dephosphonylation reactions, k_{obsd} and k'_{obsd}, respectively, are shown in Table I. The results indicate that when the transamination step is faster, the dephosphonylation rate constant also increases. Thus transamination is a required step for dephosphonylation. The specific rate constant of each protonated species may now be calculated with eq 10 by solving a set of the appropriate simultaneous equations. The values found were $k_{H_4SB} = 1.64 \times 10^{-4}$ s^{-1}, $k_{H_3SB} = 7.56 \times 10^{-5}$ s^{-1}, and $k_{H_2SB} = 2.34 \times 10^{-5}$ s^{-1} for the tranamination step. The values of k_{HSB} and k_{SB} are 0. Values of $k'_{H_4SB} = 4.27 \times 10^{-6}$ s^{-1}, $k'_{H_3SB} = 1.26 \times 10^{-6}$ s^{-1}, and $k'_{H_2SB} = 6.84 \times 10^{-7}$ s^{-1} were found for the dephosphonylation step. The values for k'_{HSB} and k'_{SB} are about zero.

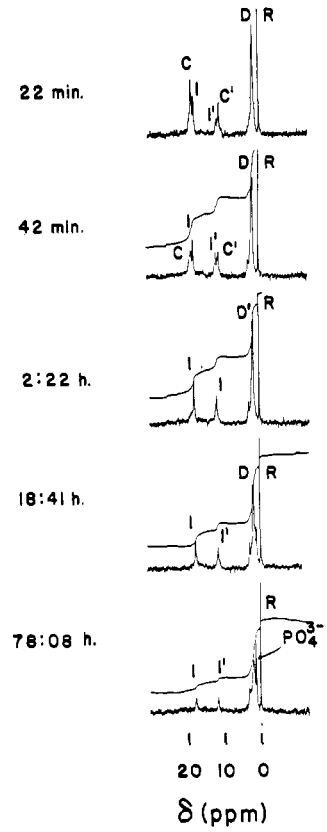


Figure 3. Decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:2:2 molar ratio of Ga(III), PLP, and APP at p[D] 6.52 taken at 22 min, 42 min, 2.22 h, 18.41 h, and 78.08 h after mixing of the reagents. C and C′ denote resonances due to the two phosphonate groups of the 1:2 Ga(III)–SB complex, and I and I′ denote resonances of the same groups of the intermediate. D and D′ denote resonance due to the two phosphonate groups of the 1:2 Ga(III)–SB complex. The resonance due to the two phosphate groups of the intermediate is at the same position as those due to the 1:2 Ga(III)–SB complex. R is the reference (0.300 M phosphoric acid in D_2O); t = 35.0 °C, and μ = 1.00 M (adjusted with KCl). T_{Ga} = 0.050 M.

Metal Ion Catalysis. The distribution of species as a function of p[D] have been determined for the 1:1:1:1 Zn(II)–APP–PLP–PDA system[2], the 1:2:2 Ga(III)–APP–PLP system[3], and the 1:2:2 Al(III)–APP–PLP system[3]. From these plots, the p[D] values for the kinetic runs were chosen. The p[D] values chosen for individual kinetic runs were guided by the species distributions originally determined for aqueous systems. Figure 3 shows a sequence of ^{31}P NMR spectra for the Ga(III) system at p[D] 6.52. Appearance of intermediate I is fast compared to its rate of disappearance with the formation of inorganic phosphate. The NMR resonance of the latter appears close to that of the phosphate ester group of the 1:2 Ga(III)–SB complex and the reference R (0.3 M phosphoric acid in D_2O). For this reason each step was studied separately. The intermediate has two resonances due to the phosphate group, one at 17.80 ppm and the other at 11.57 ppm. Both resonances are close to the two phosphonate resonances of the Ga(III)–SB 1:2 complex, which are at 18.32 and 10.85 ppm. The resonances due to the phosphate groups of the intermediate are at the same position as those due to the same group in the 1:2 Ga(III)–SB complex at 1.80 and 1.56 ppm. Proton NMR of the same system at p[D] 7.08 shows (Figure 4) that the intermediate has two nonequivalent 2′-CH_3 protons as was also observed for the metal–SB complexes.[3] The results from proton NMR (Figure 4) and ^{31}P NMR (Figure 3) suggest that the

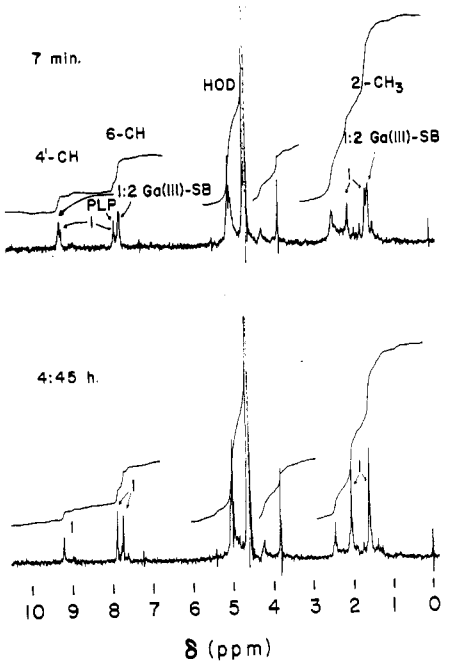


Figure 4. Proton magnetic resonance spectra (90 MHz) of a D_2O solution containing a 1:2 molar ratio of Ga(III) and PLP–APP Schiff base at P[D] 7.08 taken at 13 min and 4.49 h after mixing. The initial concentration of Ga(III) ion was 0.050 M, and the initial concentrations of PLP and APP were 0.10 M; t = 35.0 °C, and μ = 1.00 M (adjusted with KCl). T_{Ga} = 0.050 M. For numbering see formula **4**.

Table II. Observed Rate Constants

	k_{obsd}, s^{-1}			
	disappearance of metal ion complex	I		formn of inorganic phosphate
p[D]		formn	disappearance	
		1:2 Ga(III)–SB Complex		
3.92	4.14×10^{-4}	3.97×10^{-4}	5.22×10^{-6}	
4.77	3.47×10^{-4}	3.31×10^{-4}	5.2×10^{-6}	
5.78		3.2×10^{-4}	5.17×10^{-6}	
6.52	3.13×10^{-4}	3.07×10^{-4}	5.17×10^{-6}	
7.05	2.64×10^{-4}		3.93×10^{-6}	
7.83	1.40×10^{-4}		1.83×10^{-6}	
9.52	3.14×10^{-5}	3.11×10^{-5}	4.89×10^{-7}	9.90×10^{-7}
		Ga(III)–SB′ Complex		
6.02	1.47×10^{-4}	1.42×10^{-4}	7.54×10^{-6}	1.52×10^{-5}
		1:2 Al(III)–SB Complex		
7.85	6.98×10^{-5}		4.28×10^{-6}	8.39×10^{-6}
		Zn(II)–SB–PDA Complex		
6.50	7.68×10^{-5}	7.37×10^{-5}	1.18×10^{-6}	
7.91	1.45×10^{-5}	1.45×10^{-5}	3.1×10^{-7}	

intermediate has the ligands around the metal ion in the same arrangement as the 1:2 metal ion–SB complex.

In order to distinguish the ^{31}P resonance of the phosphate ester of the Ga(III) complex from the resonance due to inorganic phosphate (and also to see if $d[PO_4^{3-}]/dt$ is equal to $-d[I]/dt$ for the dephosphonylation step) pyridoxal (PL) was used in place of PLP in the 1:2 Ga(III)–SB′ system at p[D] 6.02, where SB′ is the Schiff base formed from PL and APP (Figure 5). In this system inorganic phosphate can easily be detected as a resonance close to that of R. Figure 6 shows the disappearance of the 1:2 Ga(III)–SB complex and variation of the concentration of the intermediate as a function of time. The observed rate constants for the dephosphonylation step determined from the appearance of inorganic phosphate is, as expected, 2 times that determined from disappearnce of the intermediate, and they are reported in Table II. Thus most of the calculations of the observed rate constants for the dephosphonylation reaction were based on the disappearance of the intermediate.

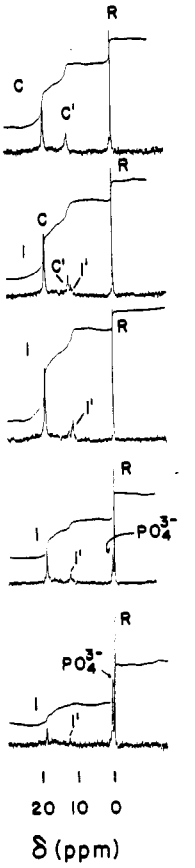


Figure 5. Decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:2:2 molar ratio of Ga(III), PL, and APP at p[D] 6.06 taken at 45 min, 2.05 h, 7.37 h, 47.30 h, and 71 h after mixture of the reagents. C and C′ denote resonances due to the two phosphonate groups of the 1:2 Ga(III)–SB′ complex (SB′ is the Schiff base formed between PL and APP), and I and I′ denote resonances of the same groups of the intermediate. R is the reference (0.300 M phosphoric acid in D_2O); t = 35.0 °C, and μ = 1.00 M (adjusted with KCl). T_{GA} = 0.050 M.

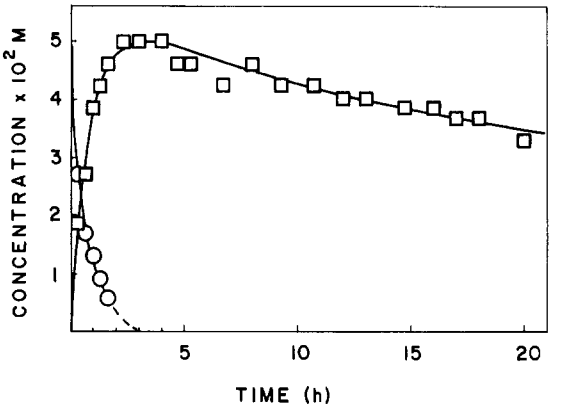


Figure 6. Disappearance of 1:2 Ga(III)–SB complex (○), and variation of the concentration of the intermediate as a function of time (□). MSB ⇌ I → products.

Protonation constants of weak acids in D_2O have been observed to be related to those measured in H_2O at 25.0 °C by the following empirical linear equation:[15]

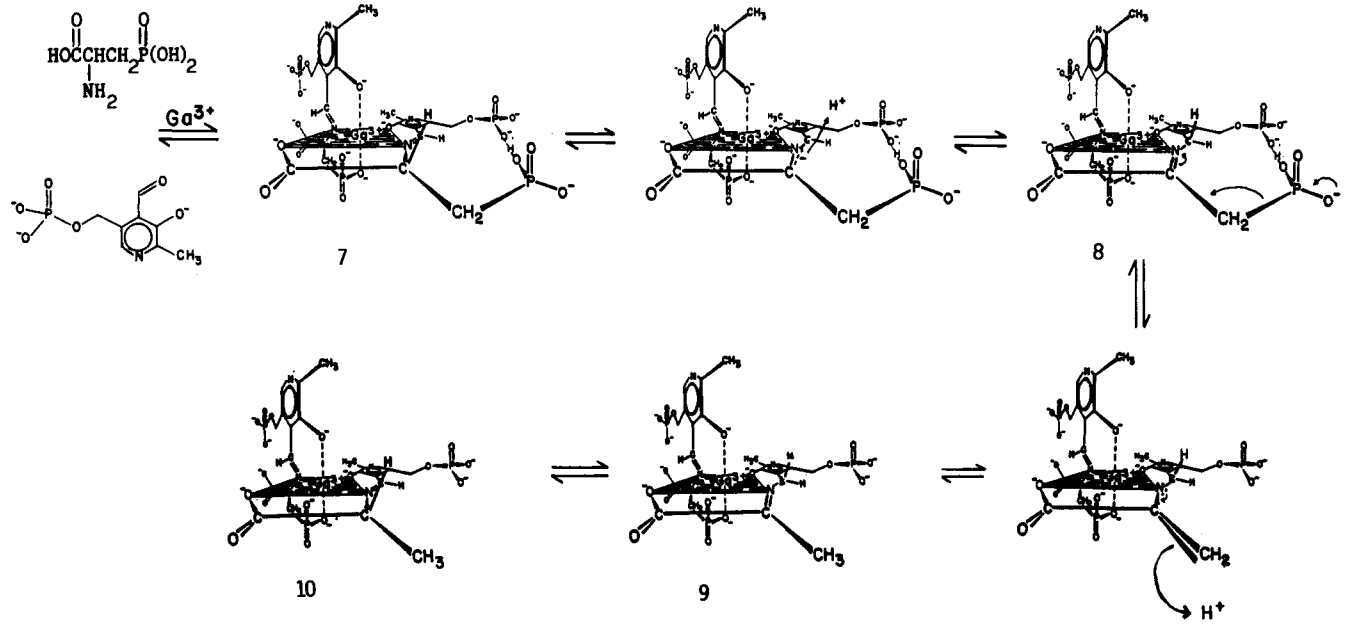
$$\log K(D_2O) = 1.02 \log K(H_2O) + 0.41 \qquad (11)$$

This relationship was employed to determine the speciation in D_2O for the kinetic runs from the speciation in H_2O determined potentiometrically as described above.

As with the metal-free system, the observed rate constants for the transamination and dephosphonylation steps are considered

(15) Schowen, K. B. J. "Transition States of Biochemical Process"; Gandour, R. D., Schowen, R. L., Ed.; Plenum Press: New York, 1970; p 244.

Scheme I. Proposed Mechanism for the Metal Ion and Pyridoxal Catalyzed Dephosphonylation Reaction



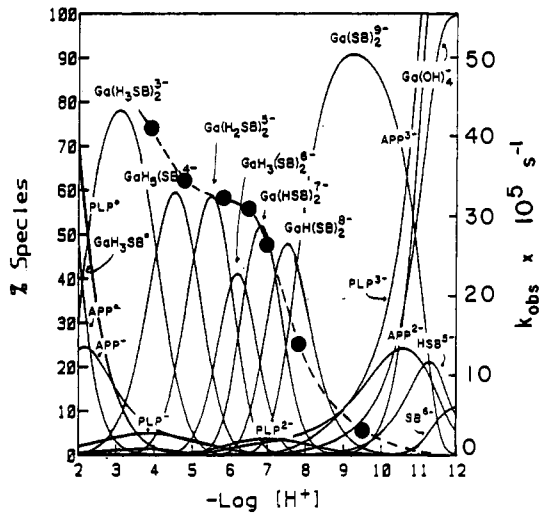


Figure 7. Variation of observed rate constant (---) for transamination reaction as a function of p[H] and species (—) of 1:2 Ga(III)–SB complexes. $Ga(SB)_2^{9-}$, $GaH(SH)_2^{8-}$, $Ga(HSB)_2^{7-}$, $GaH_3(SB)_2^{6-}$, $Ga(H_2SB)_2^{5-}$, $GaH_5(SB)_2^{4-}$, and $Ga(H_3SB)_2^{3-}$ are the various deprotonated and protonated species, respectively, of 1:2 Ga(III)–SB complexes. GaH_3SB^0 is the neutral protonated species of 1:1 Ga(III)–SB complex. SB^{6-} and HSB^{5-} are completely deprotonated and monoprotonated species of PLP–APP Schiff base. $Ga(OH)_4^-$ is the gallate anion. PLP^{3-}, PLP^{2-}, PLP^-, and PLP^0 are the completely deprotonated and successively less protonated species, respectively, of pyridoxal 5′-phosphate, and APP^{3-}, APP^{2-}, APP^- and APP^0 are the corresponding deprotonated and protonated species of 2-amino-3-phosphonopropionic acid. $T_{Ga} = 0.050$ M.

Table III. Specific Rate Constants for the 1:2 Ga(III)–SB System

species	transamination step k, s^{-1}	dephosphonylation step k, s^{-1}
$Ga(H_3SB)_2$	4.66×10^{-4}	
$GaH_5(SB)_2$	3.51×10^{-4}	5.2×10^{-6}
$Ga(H_2SB)_2$	3.13×10^{-4}	5.20×10^{-6}
$GaH_3(SB)_2$	3.13×10^{-4}	5.17×10^{-6}
$Ga(HSB)_2$	3.15×10^{-4}	5.09×10^{-6}
$GaH(SB)_2$	2.20×10^{-4}	2.53×10^{-6}
$Ga(SB)_2$	3.12×10^{-5}	4.92×10^{-7}

to be the summation of the specific rates of each 1:2 Ga(III)–SB complex species (eq 12 and Figures 7 and 8, respectively). The

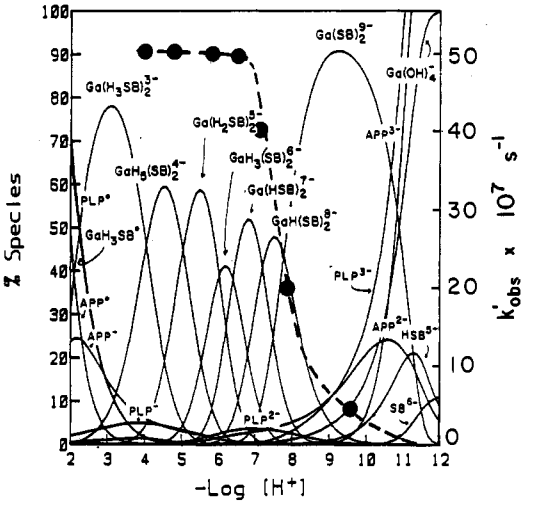


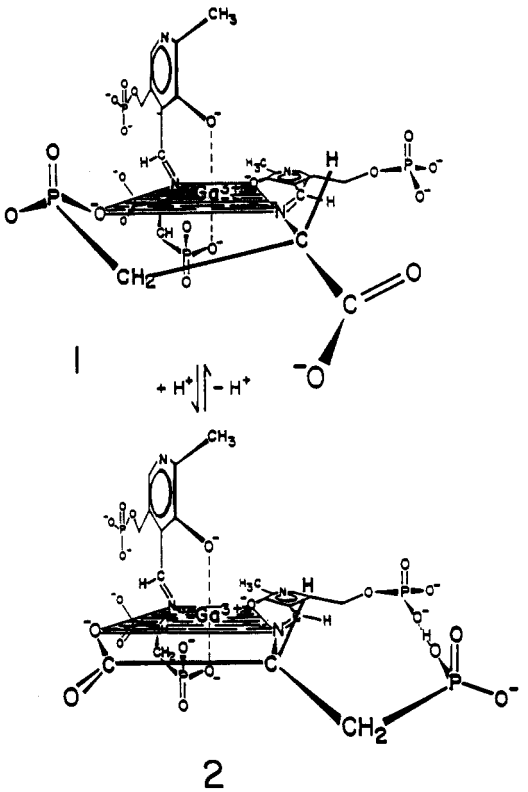
Figure 8. Variation of observed rate constant (---) for the dephosphonylation reaction as a function of p[H] and species (—) of 1:2 Ga(III)–SB complexes. See Figure 7 for species description.

values for the specific rate constants determined in this way are reported in Table III.

$$k_{obsd}[\text{Ga(III)–SB}_T] = k_{Ga(H3SB)_2}[Ga(H_3SB)_2] + k_{GaH_5(SB)_2}[GaH_5(SB)_2] + k_{Ga(H_2SB)_2}[Ga(H_2SB)_2] + k[GaH_3(SB)_2]_{GaH_3(SB)_2} + k_{Ga(HSB)_2}[Ga(HSB)_3] + k_{GaH(SB)_2}[GaH(SB)_2] + k_{Ga(SB)_2}[Ga(SB)_2] \quad (12)$$

The most important factors that probably influence the rates in the absence of metal ion are (1) the extent of the Schiff base formation, (2) the degree of protonation of the Schiff base, (3) the ability to labilize the α-proton of the amino acid moiety, and (4) the influence of the positive azomethine group in shifting negative charge from the phosphonate group. The protonated pyridine species H_4SB is the most active, followed by H_3SB and H_2SB (Table I, Figure 1). The monoprotonated and the completely deprotonated SB are relatively inactive and too slow to be measured. A discussion of the activities of mono- and diprotonated Schiff bases in pyridoxal-catalyzed reactions of the α-amino acids has been reported.[14]

The specific rate constant of each species is important, since it makes possible comparisons of its catalytic effects, with direct implications concerning the reaction mechanisms proposed for the transamination and dephosphonylation steps. There is a moderate

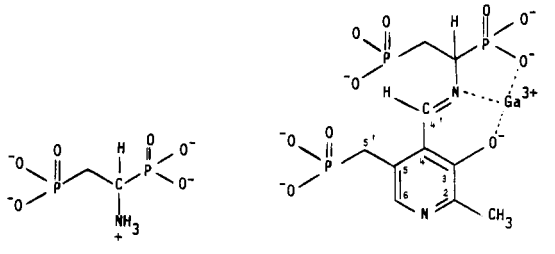


2

increment in the rate constants for the transamination reaction but only a slight increment for the dephosphonylation reaction when the pyridine nitrogen is protonated. However, the rate constant is smaller for the monoprotonated and completely deprotonated species. The completely deprotonated form of the Ga(III)–SB 1:2 complex has the two phosphonate groups preferentially coordinated to the metal ion as shown in **1**.[3] Protonation of one of the two phosphonate groups displaces it from the coordination sphere of the metal ion as in **2**. Thus with both phosphonate groups coordinated to the metal ion, the azomethine nitrogen and the phosphonate oxygen form a six-membered ring. Because of CH_2 groups between the α-carbon and the phosphonate group, the labilization of the α-proton is more difficult, and the specific rate constant of this species for the transamination step is 1 order of magnitude lower than those species in which the carboxylate group is coordinated to the metal ion. For the three metal ions studied, transamination and dephosphonylation proceeded faster for the Ga(III) complexes than for those of Al(III) and Zn(II).

Abstraction of the α-hydrogen from the amino acid moiety of the Schiff base complex for either the metal complex or the metal-free system is an essential first step. The metal ion with a higher charge should labilize the proton more effectively and would be expected to catalyze the transamination step more effectively than one with a lower charge.

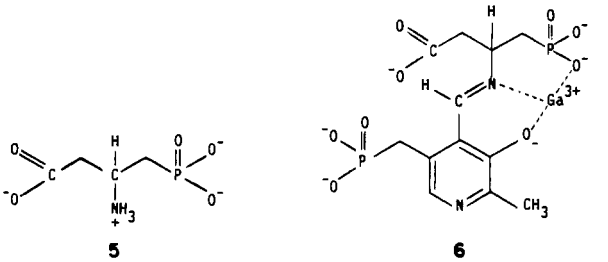
The electron shift for breaking of the bond between the C and P atoms is in the direction of the metal ion. Since APP has two coordinating groups, carboxylate and phosphonate, with the phosphonate coordinated to the metal ion as in **1**, there is a Coulombic interaction that works against the electron shift leading to the dephosphonylation reaction. However with the carboxylate group coordinated to the metal ion as in **2**, where the phosphonate group is protonated, there is a proton stabilizing one of the two negative charges on that group, but the shift of one electron toward the direction of the metal ion may occur more easily. Thus there are two effects that work against the dephosphonylation reaction after the transamination step: (1) the stabilization of the phosphonate group coordinated to the metal ion and (2) the stabilization of one of the negative charges of the phosphonate group by protonation. Since it is not possible to have both uncoordinated and completely deprotonated phosphonate group moieties in the SB ligand of the 1:2 Ga(III)–SB system, (1-amino-2-phosphonoethyl)phosphonate (**3**) would be expected to have a dephosphonylation rate faster at high pH values where the β-phosphonate group will be completely deprotonated and not coordinated to the metal ion because the α-phosphonate would be coordinated, as in **4**.



3 4

Although Martell and Langohr suggested a mechanism for pyridoxal and metal ion catalyzed dephosphonylation of aminophosphonic acids, neither reaction kinetics nor identification of reaction species was reported. The conclusions drawn were based on formation of inorganic phosphate with the use of catalytic amounts of pyridoxal and metal ions. That metal ions are required for catalysis of the pyridoxal (PL)–APP system as reported by them is due to the fact that because the concentration of PL–APP Schiff bases in metal-free, slightly acid solution is very small, the metal ions were necessary for combination of the reagents to give Schiff bases as the corresponding chelates. In this work, PLP was employed in place of PL, in order to achieve a much higher degree of Schiff base formation. Thus metal ions are not required for the occurrence of dephosphonylation in the PLP–APP system, but they increase the reaction rate in a number of ways, as indicated above.

On the other hand, metal ions may stabilize the phosphonate group by coordination and exert an electronic effect on the phosphonate moiety that inhibits dephosphonylation. On this basis one may predict that a substrate such as 3-amino-4-phosphonobutyric acid (**5**) at p[H] values higher than 8 or 9 would undergo decarboxylation, rather than dephosphonylation, since the phosphonate group would be stabilized by coordination with the metal ion as in **6**.



5 6

Ga(III)–PLP-catalyzed decarboxylation of APP is also expected at p[H] values above 7.78 where the completely deprotonated phosphonate group is coordinated to Ga(III) ion rather than to the carboxylate group, which would be free so that its negative charge would shift toward the direction of the metal in leading to the decarboxylation reaction. Kinetic studies are in progress in order to determine if this reaction occurs as predicted.

The proposed mechanism for the pyridoxal phosphate and metal ion catalyzed dephosphonylation of APP is shown in Scheme I. According to this mechanism, the 1:2 Ga(III)–SB chelate is readily formed with a 1:2:2 molar ratio of metal ion:APP:PLP. The transamination step **7** → **8** is followed by loss of the phosphonate group from the ketimine to form the pyruvate ketimine (**9**). Formation of alanine requires reverse transamination to the aldimine **10**. While the C–P bond of aminophosphonic acids is normally very stable, in the ketimine with a β-phosphonate function as in **8** there is a metal ion activated electronic pathway, which, with a negative charge on the phosphonate group, leads to C–P bond fission and release of metaphosphate.

Acknowledgment. This research was supported by Grant AM-11694 from the National Institutes of Health, U.S. Public Health Service.

Registry No. APP, 5652-28-8.