A Potent Pyridoxal Model Capable of Promoting Transamination and β-Elimination of Amino Acids

Hiroki KONDO,* Jun-ichi KIKUCHI, Shinji UCHIDA, Tatsuya KITAMIKADO, Eiji KOYANAGI, and Junzo SUNAMOTO

Department of Industrial Chemistry, Faculty of Engineering Nagasaki University, Nagasaki 852

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N-Dodecylpyridoxal chloride (**1a**) has been synthesized as a potent pyridoxal 5′-phosphate model. The equilibrium constant for Schiff base formation of **1a** with phenylalanine (21000 M^{-1}) in 3 mM hexadecyltrimethylammonium chloride (CTACl) micelle is over a thousand times larger than that for the *N*-methyl counterpart (**1b**). The Schiff base of **1a** and phenylalanine exists over a broad pH range of 5—11 in two ionic forms, HSB and SB^-, that differ in the protonation state at the azomethine group. These Schiff bases undergo transamination at 30 °C in the absence of a metal ion at considerably different rates; 1.33×10^{-3} and 1.5×10^{-5} s^{-1} for HSB and SB^-, respectively. Likewise, the former species exhibited 11 times higher reactivity in the β-elimination of *O*-phosphonoserine catalyzed by **1a** in 3 mM CTACl at 40 °C. These results indicate unambiguously the importance of a positive charge on the azomethine nitrogen as well as the one residing on the pyridine nitrogen in vitamin B_6 catalysis. The kinetic α-deuterium isotope effect of 5.1 in the transamiantion of phenylalanine with **1a** at pH 7.7 and 30 °C revealed that α-hydrogen abstraction takes place in the transition state.

Pyridoxal 5′-phosphate (PLP)-dependent enzymes play a pivotal role in the metabolism of amino acids.[1,2] Such metabolic reactions comprise transamination, decarboxylation *etc.* It is well known that these reactions can be duplicated in nonenzymatic systems, but rather unphysiological conditions such as high temperature, presence of metal ions[3] and/or high buffer salts[4] are usually needed to promote the reaction. It is, hence, clear that factors responsible for a more efficient catalysis of the coenzyme in enzymatic systems remain to be elucidated. In light of the fact that the PLP catalysis appears to be subject to general acid-base catalysis in the enzyme active site[5,6] as well as in model systems,[4,7] we have envisioned that duplication of this aspect of catalysis would generate a potent pyridoxal model capable of mediating reactions of amino acids under ambient conditions. The present model system consists of a pyridoxal bearing a dodecyl group on the pyridine nitrogen (**1a**) and a hexadecyltrimethylammonium chloride (CTACl) micelle.[8] The positive charge residing on the pyridine ring exerts a strong electron-withdrawing effect on the Schiff base of amino acid, and hydroxide ions concentrated at the cationic micellar surface are expected to serve as a base catalyst.[9] It was found that this system is an excellent catalyst of transamination and β-elimination reactions of ordinary amino acids in the absence of metal ions.

Experimental

Materials. Pyridoxal hydrochloride was obtained from Wako Pure Chemical Ind., Osaka. Pyridoxal methochloride (**1b**) was prepared according to the literature.[10] DL-Phenylalanine and DL-[α-^{2}H]phenylalanine were prepared by the literature method.[11] The deuterium content in the latter was determined as 95.4% by mass spectroscopy.[12] *O*-Phosphonoserine was either prepared[13] or purchased from Sigma. Other L-amino acids, ammonium molybdate and other common chemicals were obtained from Wako. Sodium tetraphenylborate and EDTA were the products of Dojindo, Kumamoto. CTACl was the same as that used previously.[14] Rabbit muscle lactate dehydrogenase and NADH were obtained from Boehringer Mannheim and Kyowa Hakko, Tokyo, respectively.

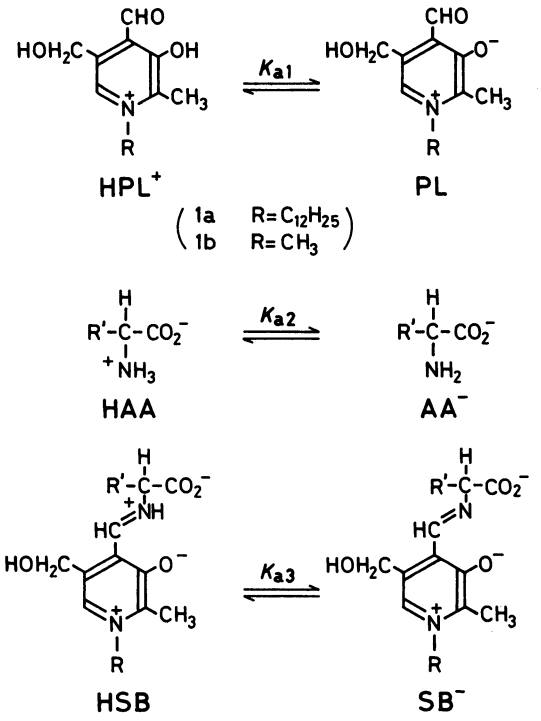
1-Dodecylpyridoxal Chloride (1-dodecyl-4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridinium Chloride, 1a). "Pyridoxal inner monomethyl acetal"[10] (1,3-dihydro-1-methoxy-6-methylfuro [3,4-c]pyridin-7-ol, 0.50 g, 3 mmol) was allowed to react with dodecyl iodide (3.0 g, 10 mmol) in 12 ml of refluxing dry MeOH–benzene (5:1 by vol) for 200 h. The solvents were removed *in vacuo* and 100 ml of ether was added to the residue. The precipitate formed was collected by filtration. It was purified by column chromatography twice on a Sephadex LH-20 (Pharmacia) column (1.2×30 cm) with MeOH as eluant. The product was obtained as a light yellow oil in 20% yield.

An aqueous solution (50 ml) of "1-dodecylpyridoxal iodide inner monomethyl acetal" prepared above was mixed with an aqueous suspension of silver chloride, prepared fresh from silver nitrate and potassium chloride. The mixture was stirred at room temperature for 2 h. After removing the solid by filtration, the pH of the filtrate was brought to 2 with hydrochloric acid. Evaporation of the solvent left a very hygroscopic yellow oil. TLC (silica gel) R_f 0.08 (CH_3CN). NMR (DMSO-d_6) δ 0.86 (t, J=5 Hz, 3H), 1.0—2.0 (m, 20H), 2.71 (s, 3H), 4.61 (t, J=7 Hz, 2H), 5.04 (d, J=14 Hz, 1H), 5.18 (d, J=14 Hz, 1H), 6.81 (s, 1H), 8.72 (br s, 1H).

Apparatus. Electronic absorption spectra were taken on a Hitachi 124 or 200 spectrophotometer. Rapid-scanning spectra were determined with a Union Giken RA-401 spectrophotometer furnished with a rapid scanning device RA-415. Fluorescence spectra were recorded on a Hitachi 650-10 fluorescence spectrophotometer. ^{1}H NMR spectra were obtained on a Jeol JNM-MH-100 spectrometer. Mass spectra were determined with a Jeol JMS-DX 300 mass spectrometer in the chemical ionization mode. The pH values of aqueous solutions were read on a Toa pH meter HM-5A fitted with a combination electrode GS-195C.

Equilibria. For determining equilibrium constants for the formation of a Schiff base of **1a** or **1b** with amino acids, a number of aqueous solutions containing $1-3\times10^{-4}$ M† **1a** or **1b** and 0—1 M amino acid at various pH values were prepared

† 1 M=1 mol dm^{-3}.



Scheme 1.

in the absence or presence of 3 mM CTACl.[15] Equilibria of Schiff base formation were attained within 10 min and absorption spectra were determined at 30.0 °C. The pertinent equilibria are depicted in Scheme 1. With **1b**, the two ionic species of Schiff base, HSB and SB^-, were detected spectroscopically over the pH range 7—11. On the other hand, HSB species was hardly visible with **1a** in 3 mM CTACl in the same pH region, though the equilibrium constant for the Schiff base formation is much greater with this system. A pH jump-rapid scanning spectroscopy, however, allowed one to observe HSB species (see below).

The data were analyzed in a way similar to those described previously,[16-18] but with some simplifications where possible. The pH-dependent (K_{pH}, Eq. 1) and independent equilibrium constants (K, Eq. 2) for the Schiff base formation are those defined by Metzler.[15] In a micellar **1a** and phenylalanine system, the Schiff base formation is complete at 10 mM amino acid at pH 9—11 and only a single ionic species each is present for **1a**, phenylalanine and Schiff base in that pH region. Hence, the spectrum determined at pH 11 at the highest amino acid concentration represents the intrinsic spectrum of SB^- whose absorption maximum is located at 393 nm. Furthermore, the K_{pH} values obtained in this pH region are set equal to the pH-independent equilibrium constant K. The intrinsic spectrum of HSB was obtained by a pH jump-rapid scanning technique. To determine the pK_a values of Schiff base and phenylalanine in the micelle, steady state spectral data at pH 4.7, 5.6, 6.0, and 11.0 (16 points) were analyzed by iterative computations until a minimum value of the sum of residual squares U is reached (Eq. 3).[16]

$$K_{pH} = \frac{[SB]_T}{[PL]_T \cdot [AA]_T} \quad (1)$$

$$K = \frac{[SB^-]}{[PL] \cdot [AA^-]} \quad (2)$$

$$U = \sum_{i,j,k} (A^{obs}_{ijk} - A^{cal}_{ijk})^2 \quad (3)$$

$$A^{cal}_{ijk} = \sum_{k,l} \varepsilon_{kl} \cdot C_{ijl} \quad (4)$$

Caluculated absorbance A^{cal}_{ijk} at a given pH i, amino acid concentration j and wavelength k is the sum of contributions of HPL^+, PL, HSB, and SB^- (Scheme 1 and Eq. 4).

Rapid Scanning Spectroscopy. Following two stock solutions were prepared: Solution (A) contained 0.40 mM **1a**, 30 mM phenylalanine and 3 mM CTACl in 10 mM borate buffer, pH 12 and Solution (B) contained 3 mM CTACl and dilute hydrochloric acid. Equal volumes of both solutions were mixed to bring the pH of the resulting solution to ≈4 and an electronic absorption spectrum was recorded at 1 ms intervals (Fig. 1). The absorption band centered at 430 nm disappeared gradually with a half life ≈14 s, finally to make the solution colorless. This phenomenon is explained in terms of rapid protonation to SB^- and subsequent slow dissociation of HSB into its constituents.

Kinetics of Transamination. An aqueous solution (3 ml) of 10 mM phenylalanine, 3 mM CTACl and 0.10 mM EDTA in 10 mM phosphate buffer at a specified pH value was placed in a cuvette and thermally equilibrated at 30.0 °C. A solution of **1a** (30 μl) was injected and the whole mixture was agitated quickly. The concentration of **1a** in the mixture was 0.10 mM. A decrease of the Schiff base absorption was followed continuously at its absorption maximum wavelength. The rate of transamination was determined by Eq. 5.

$$-\frac{d[SB]_T}{dt} = k_{obsd}[SB]_T \quad (5)$$

As the Schiff base formation is not always complete, the rate constant k_{obsd} obtained by Eq. 5, was converted to the specific rate constant of the Schiff base (k) with the aid of Eq. 6, which is derived by a combination of Eqs. 1 and 7 as well as an assumption that the amino acid concentration does not change throughout the reaction, *i.e.*, $[AA]_T \simeq [AA]_0$.

$$k_{obsd} = \frac{k \cdot K_{pH} \cdot [AA]_0}{1 + K_{pH} \cdot [AA]_0} \quad (6)$$

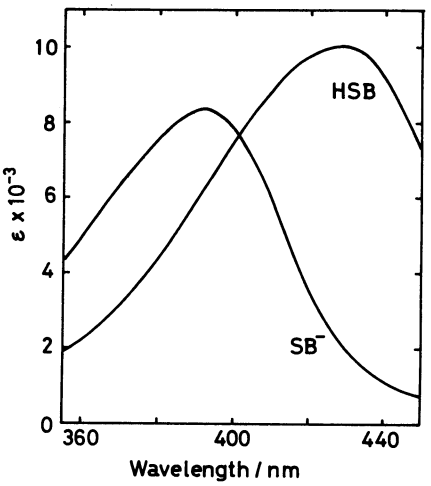


Fig. 1. Electronic absorption spectra of the HSB and SB^- Schiff base of **1a** and phenylalanine in 3 mM CTACl. The trace for HSB was obtained by pH-jump rapid scanning spectroscopy. Details are given under Experimental.

$$\frac{d[PL]_T}{dt} + \frac{d[SB]_T}{dt} + \frac{d[Product]_T}{dt} = 0 \quad (7)$$

β-Elimination of O-Phosphonoserine. Reaction rates were determined by quantitating colorimetrically the inorganic phosphate liberated.[19] A reaction mixture (6 ml) containing 20 mM substrate, 5 mM EDTA and 3 mM CTACl and adjusted to a specified pH value with sodium hydroxide was placed in a constant temperature bath of 40.0°C. The reaction was started by the addition of 300 μl of **1a** or **1b** solution, the final concentration of the catalyst being 0.50 mM. A 1 ml-aliquot was withdrawn at appropriate time intervals and transferred to a flask containing 1 ml of 3.1 mM sodium tetraphenylborate to precipitate most of the surfactant. A 4 ml-aliquot of molybdate solution, prepared according to Taussky and Shorr,[19] was added and the whole mixture swirled for 1 min. The precipitate was removed by ultrafiltration through a 0.05 μm pore size filter paper (Millipore). The absorbance of the filtrate was read at 720 nm.

Reaction rates (v) were linear with time in the initial stage of the reaction. Combination of a rate equation (Eq. 8) and K_{pH} (Eq. 1) yields Eq. 6′, from which the specific rate constant of the Schiff base (k) is obtained.

$$v = k[SB]_T \quad (8)$$

$$v = \frac{k \cdot K_{pH}[AA]_0 \cdot [PL]_0}{1 + K_{pH} \cdot [AA]_0} \quad (6')$$

Product Analysis. Keto acids produced by transamination of phenylalanine and β-elimination of *O*-phosphonoserine were identified spectroscopically after conversion to their 2,4-dinitrophenylhydrazones. Pyruvic acid was also quantitated by the "coupled assay," in which pyruvic acid is reduced with NADH and lactate dehydrogenase and the remaining NADH is determined by fluorescence spectroscopy.[8] Formation of the pyridoxamine form of **1a** upon transamination with phenylalanine was revealed by an increase in fluorescence intensity at ~438 nm of a reaction mixture.[8] This compound was also confirmed by TLC on silica gel, in which a new ninhydrin-positive fluorescent spot became visible with the progress of reaction.

Results

Schiff Base Formation. The acid dissociation constant (pK_{a1}) of the 3-hydroxyl group of **1a** and **1b** was determined by spectroscopic titrations in water at 30.0°C as 3.7 or 4.0, respectively. The acid form of either compound possesses an absorption maximum at 296 nm, while the ionized form at 323.5 nm. Whereas the absorption spectrum of **1b** was not affected by SDS or CTACl micelles, the spectrum of **1a** is red-shifted considerably in either micelles. Thus the λ_{max} value of 323.5 nm is shifted to 330.5 and 326 nm in 3 mM CTACl and SDS micelles, respectively. In addition, the pK_{a1} value of **1a** is altered to 2.6 and 4.9 in the CTACl and SDS micelles, respectively. These data indicate that **1a** forms a mixed micelle with the two surfactants.

The equilibrium constant for the Schiff base formation between **1a** or **1b** and amino acid was determined by spectral measurements of solutions that contain varying amounts of amino acid at different pH values.[15] The Schiff base exists over a pH range of 7—11 in two ionic forms, *i.e.*, protonated or ionized with respect to the azomethine group (Scheme 1). The former Schiff base species of **1b** and phenylalanine has a λ_{max} at 416 nm and the latter at 380 nm. The equilibrium constant K defined by Eq. 2 was also determined as 17 M^{-1}. The formation of Schiff base of phenylalanine was enhanced greatly with **1a** in the presence of 3 mM CTACl, but only a single ionic species that has a λ_{max} at 393 nm was discernible in the neutral to alkaline media. This species is likely to be SB^- and the red-shift by 13 nm from that of the corresponding **1b** species is explicable in terms of its location in the micellar phase. The polarity of the micellar environment of the Schiff base was assessed by determining the Schiff base absorption as a function of dioxane concentration in water. As shown in Fig. 2, the λ_{max} shifts to the blue as the dioxane content in the medium is increased. The value of 393 nm in the CTACl micelle corresponds to the polarity of 46% dioxane in water.

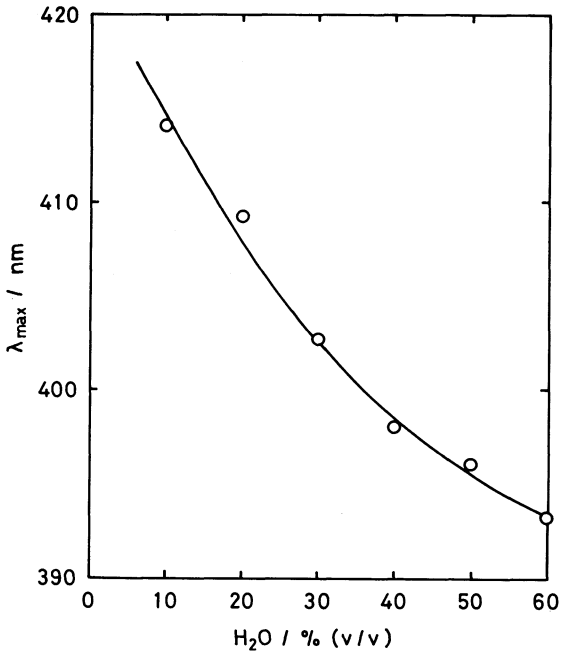


Fig. 2. Relationship of the absorption maximum of SB^- of **1a** and phenylalanine with the water content in dioxane.

The HSB species of the Schiff base of **1a** and phenylalanine in 3 mM CTACl was observed at lower pH values of ≈5. This species possesses an absorption maximum at 430 nm, again shifted to the red by 14 nm from the corresponding Schiff base species of **1b**. Analysis of the spectroscopic data in this pH region yielded the results summarized in Table 1. The equilibrium constant of 21000 M^{-1} is over 1000 times greater than the corresponding value for the **1b** system (17 M^{-1}). Based on the former value, molar distribution of the two Schiff base species of 0.10 mM **1a** and 10 mM phenylalanine is depicted over a broad pH

TABLE 1. EQUILIBRIUM CONSTANTS AND SPECTROSCOPIC DATA FOR THE SCHIFF BASES OF PHENYLALANINE WITH **1a** OR **1b**

Aldehyde	K[a)]/M^{-1}	pK_{a3}[b)]	λ_{max}/nm (ε_{max}/$M^{-1}\cdot cm^{-1}$)	
			HSB	SB^-
1a	21,000	5.0	430(10,000)	393(8,300)
1b	17	8.0	416(8,400)	380(7,900)

a) Defined by Eq. 2. b) For definition see Scheme 1.

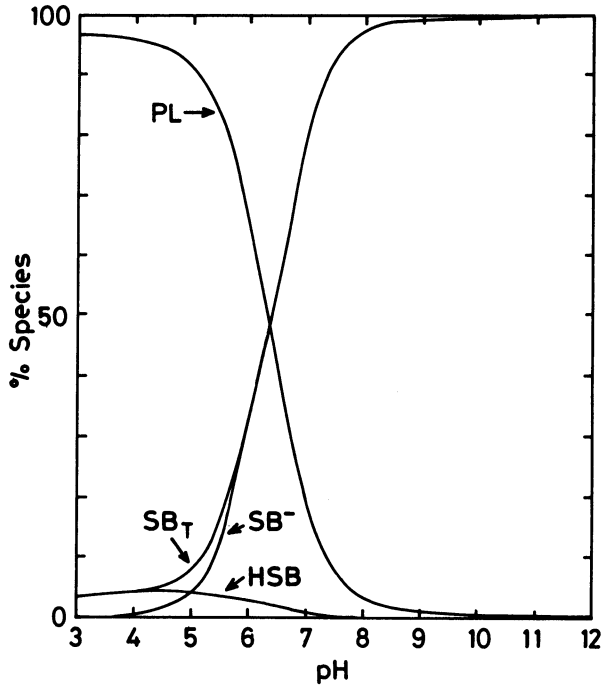


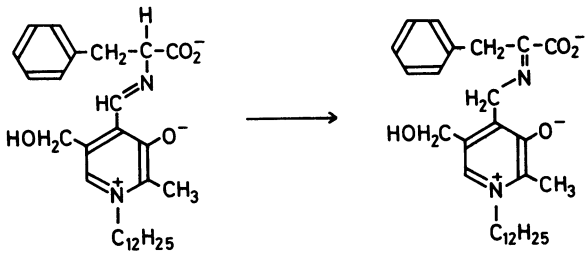
Fig. 3. pH-Distribution profile for HSB and SB^- species of the Schiff base of 0.10 mM **1a** and 10 mM phenylalanine in 3 mM CTACl. The following numerical values are employed: K 21000 M^{-1}; pK_{a1} 2.6; pK_{a2} 8.7; pK_{a3} 5.0.

range in the micelle (Fig. 3). The origin of favorable Schiff base formation in this system stems from the hydrophobic interaction of the apolar side chain of amino acid with the micelle. Owing to this interaction a large portion of phenylalanine is partitioned in the micellar phase to form a Schiff base with **1a** residing there. As stated previously,[8] the equilibrium constant is highly dependent on the hydrophobicity of amino acids; the more hydrophobic, the greater is the equilibrium constant. This view is further supported by the fact that equilibrium constants for hydrophilic amino acids such as glutamic acid and *O*-phosphonoserine are comparable with the corresponding values for **1b** or pyridoxal (see below).

One peculiarity encountered in the Schiff base of *O*-phosphonoserine with **1a** in 3 mM CTACl at pH 9 should be worth mentioning. At low amino acid concentrations, the Schiff base formation is observed with a λ_{max} at 387 nm, but further addition of amino acid was not accompanied by a steady build-up of that absorption. Analysis of these spectroscopic data led to a molar extinction coefficient of ≈2000 for SB^- species. The value is quite untenable, as all Schiff bases of pyridoxal and related compounds share a common chromophore which is responsible for the absorption in the visible region with a molar extinction coefficient of 6000—10000.[15-18,20] It is speculated that highly anionic *O*-phosphonoserine may change the micellar structure of CTACl significantly and/or the amino acid may not be partitioned in the micelle to a large extent for some reasons which we are not aware at present. Hence, an extinction coefficient of 7000 was assumed for the SB^- species of *O*-phosphonoserine and this value was used for the evaluation of K_{pH} in the micellar system. The K value for this amino acid is estimated as 7 M^{-1}.

Another point to note is the low pK_a value (5.0) of the Schiff base of **1a** and phenylalanine in the micelle. It is well known that acid dissociation is enhanced at a cationic micellar surface typically by one pH unit (see above), but the change as large as ≈3 pH units cannot be explained by the micellar effect of concentrating hydroxide ions at its surface alone. Since the micellar environment of the Schiff base is less polar (see above), ionization of the iminium proton may be facilitated to generate SB^- species which is electrically neutral with respect to the azomethine group.

Transamination of Phenylalanine. The Schiff base of **1a** formed in 3 mM CTACl micelle undergoes a slow spectral change from 393 to 335 nm.[21] This phenomenon is compatible with transamination of an aldimine into a ketimine, and eventually into its components (Eq. 9). The product analysis supported



(9)

this interpretation. The rate of transamination was determined from the decay of Schiff base absorption at 30.0°C. It should be noted here that as this reaction, like most other nonenzymatic PLP-dependent reactions of amino acids,[3] is accelerated drastically by traces of a metal ion, an appropriate amount of EDTA was supplemented to all reaction solutions to suppress this metal ion effect. The amount of EDTA was decided by the following criteria. The rate obtained in the absence of EDTA was considerably greater than that in its presence because of a metal ion(s) contaminating the reagents used in these experiments, but it was decreased by an incremental addition of the chelator. There is a certain concentration of EDTA beyond which further addition of the reagent brought about no more rate-suppressing effect. At this concentration, addition of 1×10^{-5} M copper(II) or iron(III) ion did not give out any rate acceleration. Hence, the rates

Table 2. Rate constants for the transamination of **1a** with phenylalanine in 3 mM CTACl at 30.0 °C in the presence of 0.10 mM EDTA[a)]

pH	$k_{obsd}\times10^5/s^{-1}$	$k\times10^4/s^{-1}$
4.8	4.07	6.22
5.3	6.63	5.77
5.9	7.10	2.63
8.0	1.39	0.15

a) Rates were determined as described under Experimental and were analyzed with the aid of Eqs. 5 and 6.

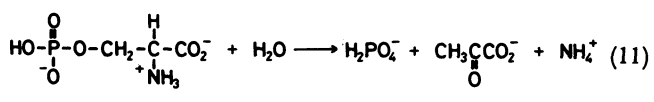
determined in the presence of EDTA represent true rates of the Schiff base, free from metal contamination.

As the Schiff base formation is virtually complete under the present conditions over the pH range 9—11 (Fig. 3), kinetic data were analyzed in terms of a first-order equation with respect to the Schiff base. In cases where the Schiff base formation is incomplete, the determined rate was corrected for with the aid of Eq. 6. The rate constants thus obtained are listed in Table 2. The data were analyzed in terms of different reactivities of the two ionic species of Schiff base involved in the reaction (Eq. 10), where X_{HSB} and X_{SB^-} stand for the

$$k = k_1 \cdot X_{HSB} + k_2 \cdot X_{SB^-} \quad (10)$$

molar fraction of each species. The more acidic species (HSB) has a rate constant $1.33\times10^{-3}\,s^{-1}$ and the other (SB^-) $1.5\times10^{-5}\,s^{-1}$, thus the former is 89 times more reactive. To gain a further insight into the reaction mechanism of this nonenzymatic transamination, kinetic α-deuterium isotope effects of Schiff base have been investigated. The k_H/k_D of 5.1 was obtained for DL-phenylalanine and its α-deuterated counterpart at 30.0 °C and pH 7.7. This value compares with the isotope effect of 9.2 for the transamination of alanine with 4-formyl-3-hydroxy-1-methylpyridinium chloride under similar conditions,[21] suggesting that α-hydrogen abstraction takes place in the transition state in both reactions.

β-Elimination of O-Phosphonoserine. *O*-Phosphonoserine undergoes β-elimination in the presence of **1b** or **1a**-CTACl (Eq. 11).[11] The spontaneous reac-



(11)

tion and its CTACl catalysis were negligible under the present reaction conditions, indicating that the β-elimination proceeds via a Schiff base. First, the apparent catalytic efficiency of **1a** and **1b** was compared. The initial rate with **1a** and 3 mM CTACl in the presence of 5 mM EDTA was $6.3\times10^{-8}\,M\cdot s^{-1}$ at pH 9.0 and 40.0 °C, whereas the corresponding value with **1b** was $4.3\times10^{-9}\,M\cdot s^{-1}$, thus the former catalyst being 15 times more effective. The K_{pH} values for **1a** and **1b** at pH 9.0 were 3.8 and 0.5, respectively. Based on these figures, the above rate constants were converted to the specific rate constants of the Schiff base (mainly SB^- species). The numerical values were $1.0\times10^{-3}\,s^{-1}$ for

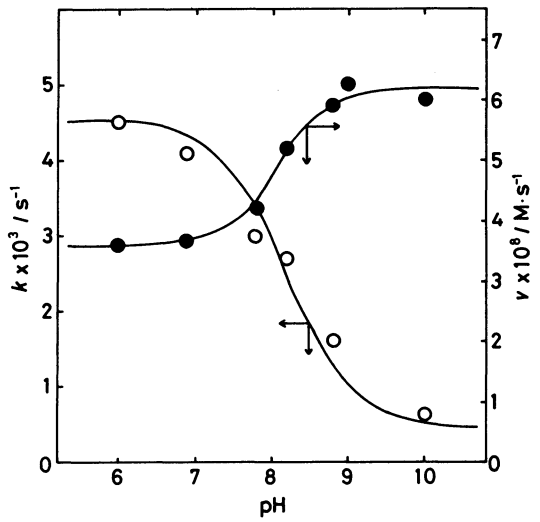


Fig. 4. pH-Rate profiles for the β-elimination of *O*-phosphonoserine in the presence of 0.50 mM **1a** and 5 mM EDTA in 3 mM CTACl at 40.0 °C. Closed circles represent the observed rates and they were converted to the rate constants of the Schiff base with the aid of Eq. 6′ (open circles). The theoretical curve is drawn based on the rate constants 4.6×10^{-3} and $4.2\times10^{-4}\,s^{-1}$ and the acid dissociation constant (pK_{a3}) 8.0.

1a and $0.9\times10^{-3}\,s^{-1}$ for **1b**, demonstrating that both Schiff bases possess a roughly identical reactivity.

The pH-dependence of the reaction rates in the presence of **1a** and CTACl is shown with closed circles in Fig. 4. By using the known K_{pH} values, a new pH-rate profile is constructed for the specific rate constant of the Schiff base (open circles and the sigmoid curve connecting them). The sigmoidal curve is interpreted in terms of different reactivities of the two Schiff base species (Eq. 10). The rate constants k_1 and k_2 obtained by this analysis were 4.6×10^{-3} and $4.2\times10^{-4}\,s^{-1}$, respectively. This result of the higher reactivity of HSB is compatible with the following metal ion effect on the elimination. Addition of 0.50 mM copper(II) ion, equimolar to **1a**, brought about a rate increase to $3.0\times10^{-6}\,M\cdot s^{-1}$ at pH 9.0 and 40.0 °C. Supposing that the copper(II) ion forms only a 1:1 Schiff base complex, the specific rate constant of the Cu^{2+}-SB^- species is $7.9\times10^{-2}\,s^{-1}$, 190 times as large as that of SB^-. Since the metal is divalent, it should exert a stronger electron-withdrawing effect, among other effects, than does a proton, thus rendering the α-proton of the Schiff base more labile.

It is also noted that the pK_a of the Schiff base of *O*-phosphonoserine with **1a** (8.0) is much higher than that of phenylalanine, but rather is close to that of phenylalanine and **1b**. This fact and the band location of 387 nm suggest that the Schiff base of *O*-phosphonoserine resides in a micellar domain more polar than that for the Schiff base of phenylalanine.

Discussion

As described above, the present pyridoxal model is

capable of promoting transamination and β-elimination of ordinary amino acids in the absence of metal ions. The reactions are presumed to take place in a mixed micelle of CTACl and **1a**. This view is substantiated by the following observations. The critical micelle concentration of CTACl was estimated as 1.2 mM under the present reaction conditions from an increase in the fluorescence intensity of 8-anilino-1-naphthalenesulfonic acid (ANS). A similar transition in the fluorescence intensity of ANS was found to occur at 0.16 mM CTACl in the presence of 0.10 mM **1a**, suggesting that the latter enhances the micellar aggregation of the former. The notion that **1a** and its Schiff base reside in the apolar micellar phase is supported by the large red-shift of their absorption bands and by the change in their pK_a (see Results). A large proportion of hydrophobic amino acids such as phenylalanine is partitioned in the micelle, thereby enhancing the Schiff base formation with **1a** residing there. This in turn is responsible for the facile transformation of the resulting Schiff base.

In addition to the enhanced Schiff base formation, this system has one more advantage in exploring pyridoxal-dependent reactions. Owing to the *N*-substitution in **1a**, the number of Schiff base species derived therefrom is limited (usually two, if we ignore acid dissociation of an amino acid side chain) over the wide pH range. These two are present in fair amounts under the condition of kinetic experiments, provide distinctly different absorption spectra and their reactivities can be assessed precisely. Based on the kinetic analysis of the transamination of phenylalanine and β-elimination of *O*-phosphonoserine, it is concluded that HSB, protonated at the azomethine group, is more reactive than SB^-.[22] This result is understandable in view of the fact that the primary role of PLP is to play as an electron sink[1-3] and that an electron-withdrawing ability of the Schiff base is greater when it bears more net positive charges. Others, however, dealing with similar data provided an opposite interpretation.[23] The reason for this discrepancy is not known.

This finding may be relevant to the enzymatic PLP catalysis. Absorption bands similar to that for the HSB species of **1a** and amino acid (430 nm) are obtained for some of the PLP-dependent enzymes.[24-26] This band location is characteristic for a Schiff base carrying a proton on the azomethine nitrogen. Whether or not the Schiff base bears a proton on the pyridine nitrogen cannot be decided, as the two forms of the Schiff base provide quite similar spectra.[16] Nonetheless, the fact that the *N*-substituted form is more reactive than the unsubstituted Schiff base[21,22] suggests that enzyme-bound PLP might carry a proton on the nitrogen, which is donated by an acidic group of the active site. This idea is supported by the recent crystallographic data on aspartate aminotransferase, in which the nitrogen of enzyme-bound PLP is hydrogen-bonded by an aspartate residue from the active site.[27,28] It is, hence, tempting to conclude that both the ring and azomethine nitrogen of the Schiff base need be protonated for PLP to perform its function of facilitating an electronic shift from the amino acid portion.

A general base catalysis as well as the general acid catalysis discussed above may be responsible for the high efficiency of the coenzyme in the enzymatic systems. The group involved in this function is the lysine amino moiety that binds PLP in the absence of a substrate.[5,6] A similar mode of base catalysis was observed in nonenzymatic systems also.[4,7] By analogy, hydroxide ions concentrated on the cationic micellar surface are a potential candidate to serve as a base catalyst.[8,9] Whether such a mechanism is working or not in the present system remains to be proven in the future. The fact that the α-deuterium isotope effect obtained in the micellar system is smaller than that for the nonmicellar reaction may provide a clue to solve this problem. At any rate, incorporation of a "powerful" base in our system should enhance the reactivity of the catalyst and further work is being continued along this line in this laboratory.

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