computed for the left-handed 3_{10}-helix A form and the right-handed δ helix of poly(L-1-NapAla) are shown in Figure 7. As compared with the previous results (Figure 6 in the previous paper), the present CD curves show a broader ^1L_a band, which makes the theoretical curves more realistic. However, the pattern of the CD curves, especially at the ^1B_b band, is not so affected as to alter the previous conclusion.

Registry No. (±)-2-Naphthylalanine, 14108-60-2; 2-naphthylalanine NCA derivative, 85421-92-7; poly(γ-benzyl DL-glutamate), 25087-28-9; *N*-acetyl L-2-naphthylalanine ethyl ester, 37440-00-9; poly(L-2-naphthylalanine), 85421-91-6.

Transamination Kinetics of Pyridoxal 5′-Phosphate Schiff Bases

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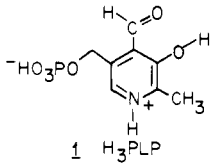
Abstract: A direct transimination pathway for the exchange of ethylamine in H_iPLPEta Schiff bases by alaninate or aspartate has been established. This rate is roughly twice as fast as that for exchange proceeding along an indirect pathway involving the reaction of aminoacidate with PLP freed by the hydrolysis of H_iPLPEta. The rate expression for direct exchange can be written in terms of the simple second-order reactions of aminoacidate with the various protonated forms of the Eta Schiff base. No effects by excess solution components or added buffers consistent with a requirement for catalysis of proton transfer between the intermediate gem diamines were observed, in contrast to results reported for imine exchange between MCB^+ and NH_2OH (ref 16). It is concluded that proton transfers in the PLP imines are internally catalyzed by the PLP phosphate side group or the phenoxide ring substituent. This latter site may also serve as a catalyst in the enzymic reaction.

Pyridoxal 5′-phosphate (PLP), shown as the triprotonated molecule in **1**, is one of the active forms of the vitamin B_6 group

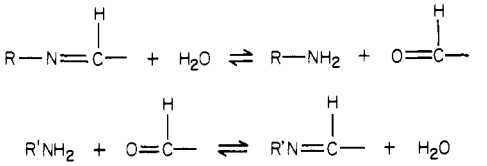


1 H_3PLP

of compounds which are essential enzymatic cofactors in a number of biological reactions.[1,2] An important class of reactions are those involving the aminotransferases where amino acids and α-keto acids are reversibly interconverted. The reactions proceed through a shuttle mechanism in which a PLP Schiff base of the amino acid tautomerizes from an aldimine to a ketimine. Hydrolysis releases the keto derivative of the amino acid and leaves pyridoxamine 5′-phosphate bound to the enzyme. This latter substance is available to convert a reactant keto acid to amino acid in the reverse sequence.[1-7]

Initially PLP is covalently bonded to the enzyme through a Schiff base linkage to a terminal amine group of a protein lysine.[8a-c] In aspartate aminotransferase, one of the most studied of these enzymes, the binding site has been identified as Lys-258.[8c,d,9] The first step in the reaction must therefore be the replacement of the lysine amine group by that of the amino acid. An indirect exchange pathway involves the hydrolysis of the PLP–lysine Schiff base followed by condensation of the PLP with an aminoacidate ion (intermediate carbinolamines[10-12] have been omitted for simplicity) (Scheme I).

Scheme I



Another possible pathway involves the direct displacement of one amine by another in a process termed transimination.[13-15] In a study of the displacement of the pyrrolidine residue by NH_2OH from N–P–methoxybenzylidinepyrrolidinium cation (MCB^+) Hogg, Jencks, and Jencks[16] established the reaction mechanism shown in Scheme II.

A proton situated on the imine nitrogen atom is necessary for attack by the entering amine. To drive the reaction to completion a proton transfer from the entering group of the intermediate gem diamine to the leaving group is required, and as a consequence

(1) (a) A. E. Braunstein, *Enzymes, 2nd Ed.*, **2** (1960). (b) A. E. Braunstein, *Enzymes, 3rd Ed.*, **9**, 379 (1973).
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(8) (a) W. Terry Jenkins and Irwin W. Sizer, *J. Am. Chem. Soc.*, **79**, 2655 (1957). (b) R. C. Hughes, W. T. Jenkins, and E. H. Fischer, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 1615 (1962). (c) Y. A. Ovchinnikov, A. E. Braunstein, C. A. Egorov, O. L. Polyanovsky, N. A. Aldanova, M. Yu Feigina, V. M. Lipkin, N. G. Abdulaev, E. V. Grishin, A. P. Kiselev, N. N. Modyanov, and V. V. Nosikov, *Dokl. Acad. Nauk SSSR*, **207**, 728 (1972). (d) S. V. Shlyapnikov, S. V. Myasnikov, A. N. Severin, E. S. Myagkova, M. A. Torchinsky, M. Yu, and A. E. Braunstein, *FEBS Lett.*, **106**, 385 (1979).
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(11) In PLP Schiff base formation the rate of formation/dissociation of carbinolamine is fast followed by slower dehydration/hydration (ref 12). Therefore, the rate expressions for these reactions are described by the simple equations shown in Table II.
(12) Steve Hershey and Daniel L. Leussing, *J. Am. Chem. Soc.*, **99**, 1992 (1977).
(13) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86**, 2413 (1964). D. Mackay, *Biochim. Biophys. Acta*, **73**, 445 (1963).
(14) William P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, 1969.
(15) Peter S. Tobias and Roland G. Kallen, *J. Am. Chem. Soc.*, **97**, 6530 (1975).

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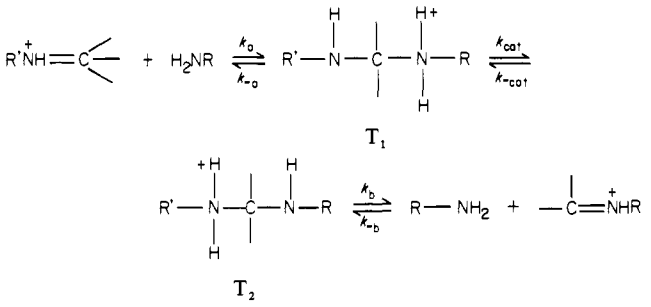
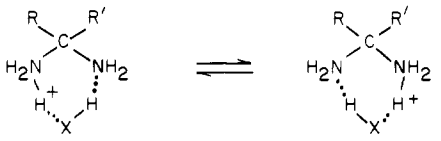
Scheme II



the reaction is subject to acid and base catalysis. High efficacy of carboxylic acids and cacodylic acid as proton transfer catalysts was attributed to their ability to serve as bifunctional proton switch catalysts.[16]



The second-order rate constants for acid- and base-mediated pathways were found to lie in the diffusion-controlled region; however, at low buffer concentrations the interconversion of T_1 and T_2 tautomers of the intermediates in II were found to be slow.

The properties of PLP Schiff bases have been extensively studied but comparatively little is known regarding their transimination behavior. Cyclic gem diamines, which serve as models for the intermediates T_1 and T_2, have been uncovered by Abbott and Martell[17] in mixtures of pyridoxal with 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, and ornithine, by O'Leary[18] in mixtures of PLP and 1,3-propanediamine, by Tobias and Kallen[15] with PLP and ethylenediamine (En), and, recently, by Korpela and his co-workers[19] with PLP and *O*-aminoserine.

The situation with respect to the reaction rates of the gem diamines is confusing. Abbott and Martell[17] found that cyclization was very slow compared to the formation of isomeric imines, and calculated that isomerization proceeded along the indirect path, I. In marked contrast, Tobias and Kallen[15] observed that ring opening and ring closing rates of the PLP–En adducts occurred on the temperature-jump time scale; Korpela[19] reports that cyclization rates are comparable to those for Schiff base formation.

One impediment to studying this reaction using models that are pertinent to the enzyme is the close similarities of the absorption spectra of the reactants and products. In the present investigations of the displacement of ethylamine (Eta) from its Schiff base with PLP by alaninate (Ala^-) or aspartate (Asp^{2-}), we have exploited differences in the complexing abilities of the Schiff bases toward Zn(II) to induce spectral changes that permitted the reaction to be followed. Both aminoacidate ions were found to be able to displace Eta via the direct route, II, but about one-third of the imine exchange was found to procede via the hydrolytic pathway, I. It was also found that the proton transfers required to interconvert PLP analogues of T_1 and T_2 in Scheme II are fast and do not require external buffer catalysis. We propose that the phenoxide oxygen atom and the phosphate group on the PLP residue serve as internal catalysts for proton transfer.

Experimental Section

Experimental. Pyridoxal 5'-phosphate was obtained from the Sigma Chemical Co. The purity determined by titration with standard NaOH was found to be 96.0%. Solutions were freshly prepared by accurate weight and were protected from light to prevent decomposition. Ethylamine hydrochloride (Eastman) was recrystallized from ethanol/water mixtures. 2-(*N*-morpholino)ethanesulfonic acid (Mes, $pK_a = 6.1$) and *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes, $pK_a = 7.5$) were obtained from the Sigma Chemical Co. and employed without further purification. Stock solutions of $ZnCl_2$ were prepared by dissolving accurately weight amounts of Baker analyzed metallic Zn in a slight excess of hydrochloric acid. The excess acid remaining after the dissolution of the metal was determined by passing an aliquot through a cation-exchange column in the acid form and determining the total acid of the effluent by titration. Subtracting 2 equiv of H^+ for each mole of Zn(II) yielded the excess H^+.

The imine nitrogen atoms of PLP Schiff bases are highly basic and become protonated in solutions at pH <11.[17-19] This protonation is accompanied by the development of a spectral absorption band centered at 420 nm.[20-22] While the goal of this investigation was to examine rates in the vicinity of neutral pH for the imine exchange reactions, H_j(PLPEta) + $Aa^{i-} \rightleftarrows H_j$(PLPAa) + Eta,[23] it was not feasible to study the simple reaction mixtures by spectrophotometric means owing to the similarities between the spectra of reactants and products. However, the tridentate aminoacidate Schiff bases form more stable metal ion complexes than do the bidentate ethylamine Schiff base. By adding Zn(II) to the reaction mixtures it was possible to couple complexation,

$$H_i(PLPAa)^{i-3} + Zn^{2+} \overset{fast}{\rightleftharpoons} Zn(H_jPLPAa)^{j-1} + (i-j)H^+$$

to the exchange reactions. Because the aldimine proton of the Schiff base is displaced by Zn(II)[21] the absorption of the Schiff base is shifted to a shorter wavelength on complexation ($\lambda_{max} \sim 360$ nm). This band shift provides a suitable absorption change by which to monitor the exchange. Complexation of the product also helps to drive the reaction to completion.

The presence of Zn(II) introduces complications into the data analysis owing to an increase in the number of species present in the reaction mixture. Not only were Schiff base complexes formed but also complexes of the free aminoacidate ions were formed. In order to interpret the rate data a thorough knowledge of the equilibrium properties of the separate amine–PLP–Zn(II) ternary systems was necessary. Because exchange through the pathways shown in Scheme I was anticipated, it was also necessary to determine accurate rate laws for Schiff base formation and hydrolysis for each of the ternary systems. The details of these separate studies will be provided elsewhere,[24] but the equilibrium rate constants which were employed here are summarized in Tables I and II, respectively.

In the exchange studies the PLPEta Schiff base was formed prior to transimination by allowing solutions containing 2×10^{-4}–5×10^{-4} M PLP and 0.10–0.16 M Eta·HCl to equilibrate at pH 8.5 for 30 min at 25.0 °C. KCl was added to bring the ionic strength to 0.50. This aging time was found to be sufficient to achieve an equilibrium condition in which more than 97% of the PLP was converted to Schiff base. These solutions were freshly prepared for each series of experiments to obviate problems arising from decomposition.

A series of solutions containing $ZnCl_2$ and amino acid at accurately known concentrations and adjusted to an ionic strength of 0.50 with KCl were also prepared. In the alanine experiments the concentration of amino acid was varied from 0.10 to 0.40 M and the $ZnCl_2$ was varied from 0.010 to 0.020 M. The pH values were adjusted with NaOH so that on mixing with equal volumes of the PLPEta solutions the pH of the mixed reaction solutions lay in the 5.2–7.6. The asparatate solutions were 0.08 to 0.40 M in amino acid and 0.012–0.020 M in $ZnCl_2$. Reaction rates were determined in the pH range 5.0–7.4.

Transimination rates were determined at 25.0 °C by following the absorbance at 420 nm after equal volumes of the PLPEta and Zn(II)–amino acid solutions were mixed. The pH of each of the reaction solutions was measured at the completion of the reaction. Proton transfers and Zn(II) complex fomation and dissociation rates are sufficiently fast that the reaction pH is established within a few milliseconds after mixing. Therefore, these reactions could be safely assumed to have come to equilibrium with respect to slower imine exchange.

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(20) D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 188 (1963).
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(22) Carol M. Metzler, Allen Cahill, and David E. Metzler, *J. Am. Chem. Soc.*, **102**, 6075 (1980).
(23) The Schiff bases are abbreviated as H_iPLPX where *i* is the number of protons on the Schiff base and X is ethylamine (Eta), alaninate (Ala^-), or aspartate (Asp^{2-}). Aa^{i-} refers to either of the aminoacidate ions, in general. En is ethylenediamine.
(24) Shu-Huan Weng, Steve Hershey, and Daniel L. Leussing, to be published.

Table I. Equilibrium Constants for the Formation of Complexes and Adducts (25 °C, $I = 0.5$)

Amines[a]			
	log K for A =		
reaction	Eta	Ala^{-} [b]	Asp^{2-}
$A + H^+ \rightleftharpoons HA$	10.81	9.81	9.68
$HA + H^+ \rightleftharpoons H_2A$		2.45	3.71
$Zn^{2+} + A \rightleftharpoons ZnA$	2.30	4.57	5.54
$ZnA + A \rightleftharpoons ZnA_2$	2.03	4.0	4.4
$ZnA_2 + A \rightleftharpoons ZnA_3$		2.0	

Pyridoxal 5'-Phosphate[b]	
reaction	log K
$PLP^{3-} + H^+ \rightleftharpoons HPLP^{2-}$	8.14
$HPLP^{2-} + H^+ \rightleftharpoons H_2PLP^-$	5.86
$H_2PLP^- + H^+ \rightleftharpoons H_3PLP$	3.75
$H_3PLP + H^+ \rightleftharpoons H_4PLP^+$	1.6
$PLP^{3-} + Zn^{2+} \rightleftharpoons ZnPLP^-$	3.6
$ZnPLP^- + H^+ \rightleftharpoons ZnHPLP$	6.3
$ZnHPLP + H^+ \rightleftharpoons ZnH_2PLP^+$	5.6

PLP–Amine Adducts[a]			
	log K for A =		
reaction	Eta	Ala^{-} [b]	Asp^{2-}
$A + PLP^{3-} \rightleftharpoons PLPA$	1.40	0.53	0.43
$PLPA + H^+ \rightleftharpoons HPLPA$	12.08	11.78	11.71
$HPLPA + H^+ \rightleftharpoons H_2PLPA$	6.55	6.57	6.70
$H_2PLPA + H^+ \rightleftharpoons H_3PLPA$	5.52	5.44	5.76
$H_3PLPA + H^+ \rightleftharpoons H_4PLPA$	3.05	3.02	3.82
$Zn^{2+} + PLPA \rightleftharpoons Zn(PLPA)$		9.73	9.28
$Zn(PLPA) + A \rightleftharpoons Zn(PLPA)(A)$		3.56	
$Zn(PLPA) + PLPA \rightleftharpoons Zn(PLPA)_2$		7.9	6.7
$Zn(PLPA) + H^+ \rightleftharpoons ZnH(PLPA)$	13.78[c]	7.4	7.9
$ZnH(PLPA) + H^+ \rightleftharpoons ZnH_2(PLPA)$	5.5	5.45	6.1
$Zn(PLPA)_2 + H^+ \rightleftharpoons ZnH(PLPA)_2$		8.6	7.5
$ZnH(PLPA)_2 + H^+ \rightleftharpoons ZnH_2(PLPA)_2$		7.2	7.5

[a] Charges on the amines have been omitted for simplicity. [b] Reference 21. [c] $Zn^{2+} + PLPEta^{3-} + H^+ \rightleftharpoons ZnH(PLPEta)$.

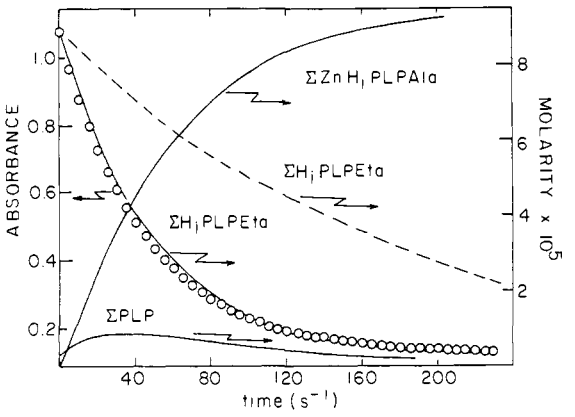


Figure 1. Absorbance–time and theoretical concentration–time curves for transimination. $PLP_{tot} = 9.6 \times 10^{-5}$ M; $Eta_{tot} = 5.25 \times 10^{-2}$ M; $Ala_{tot} = 0.100$ M; $Zn(II)_{tot} = 5.00 \times 10^{-3}$ M; pH = 6.617. (○) Experimentally determined absorbencies (scale on the left). (—) Calculated concentration sums for the proposed reaction model (scale on the right). (---) Calculated concentration sum assuming exchange proceeds only along the known hydrolysis/condensation pathway (scale on the right).

Computations. In order to aid in an understanding of the numerical procedures employed in the analysis of the data, the results of the calculations of a random experiment are described in detail in the sections below. The details of the equilibrium calculations are presented in the Appendix. The calculated distribution of the major components for the selected reaction mixture shortly after mixing (but before appreciable imine exchange has occurred) is presented in Table III, column 2. It is seen here that very little of the PLPEta reacts with Zn(II), and the major portion is present as protonated species. The distribution of the components at the completion of amine exchange for this solution mixture is shown in Table III, column 3. The overall reaction is seen to be the

Table II. Forward Rate Constants[b] for the Formation of PLP Schiff Bases (25 °C, $I = 0.5$)

reaction[a]	ethylamine, $i = 0$	aspartate, $i = -2$	alaninate, $i = -1$
$PLP^{3-} + A^{i-} \rightarrow PLPA^{(3+i)-}$	53	2.8	4.1
$PLP^{3-} + A^{i-} + H^+ \rightarrow HPLPA^{(2+i)-}$	6.6×10^{10}	6×10^{8}	3.8×10^{9}
$PLP^{3-} + A^{i-} + 2H^+ \rightarrow H_2PLPA^{(1+i)-}$	1.05×10^{18}	2.9×10^{17}	1.9×10^{17}
$PLP^{3-} + A^{i-} + 3H^+ \rightarrow H_3PLPA^{i-}$	1.1×10^{23}	1.08×10^{23}	4.3×10^{22}
$PLP^{3-} + A^{i-} + 4H^+ \rightarrow$	3.0×10^{27}	6.2×10^{27}	1.3×10^{26}
$H_4PLPA^{(1-i)+}$	3.0×10^{27}	6.2×10^{27}	1.3×10^{26}
$PLP^{3-} + A^{i-} + Zn^{2+} \rightarrow ZnPLPA^{i-}$	9.0×10^{6}		2.2×10^{5}
$PLP^{3-} + A^{i-} + H^+ + Zn^{2+} \rightarrow ZnHPLPA^{(1-i)+}$	9.1×10^{13}		3.1×10^{13}
$PLP^{3-} + A^{i-} + 2H^+ + Zn^{2+} \rightarrow$	4×10^{19}		1.9×10^{19}

[a] The rate expression is broken down into terms for the kinds and number of species present in the transition state. Preequilibria between various reactants surely are present but have not been taken into account. No implication is intended that pathways with reaction orders higher than second are operative. [b] The rate constants have been calculated by using concentration units of moles per liter and time units of seconds.

Table III. Molarities of the Important Species before and after Imine Exchange for the Experiment Described in Figure 1

species	before exchange[a]	after exchange
Zn^{2+}	1.08×10^{-3}	1.06×10^{-3}
PLP^{3-}	(2.7×10^{-6})[b]	1.08×10^{-8} (5.1×10^{-7})[b]
Ala^-	6.1×10^{-5}	6.1×10^{-5}
ETa	3.4×10^{-6}	3.4×10^{-6}
$H_2PLPEta^-$	2.4×10^{-5}	2.2×10^{-7}
$HPLPETa^{2-}$	6.5×10^{-5}	2.6×10^{-7}
Zn(PLPEtaH)	negligible	1.4×10^{-8}
$HPLPAla^{3-}$	0	3.2×10^{-7}
$H_2PLPAla^{2-}$	0	2.9×10^{-7}
$Zn(PLPAla)^{2-}$	0	1.3×10^{-5}
$Zn(HPLPAla)^-$	0	7.6×10^{-5}
$Zn(H_2PLPAla)$	0	5.2×10^{-6}
$Zn(Ala)^+$	2.4×10^{-3}	2.4×10^{-3}
$Zn(Ala)_2$	1.5×10^{-3}	1.4×10^{-3}
HALa	9.5×10^{-2}	9.5×10^{-2}
HEt	5.2×10^{-2}	5.2×10^{-2}

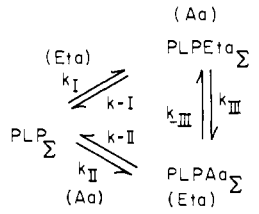
[a] Calculated for the period after reactant solutions were mixed and proton and Zn(II) equilibria were established. [b] The figure in parentheses represents the sum $[PLP^{3-}] + [HPLP^{2-}] + [H_2PLP^-] + [H_3PLP]$.

almost complete conversion of a mixture of $HPLPEta^{2-}$ and $H_2PLPEta^-$ to $Zn(HPLPAla)^-$ and $Zn(PLPAla)^{2-}$. Product formation was less complete with Asp^{2-}; nevertheless, this did not cause a serious degradation in the amplitudes of the absorbance changes, and incomplete reaction due to reversibility is incorporated into the data analysis algolrithm.

The absorption changes measured at 420 nm for the reaction mixture described in Table III are shown in Figure 1 as open circles. The kinetics are pseudo first order owing to buffering in the concentrations of H^+, Ala^-, and Eta by high concentrations of components with which they are in rapid equilibrium: $Zn^{2+} + HAla^{\pm} \rightleftharpoons Zn(Ala)^+ + H^+$, $Zn(ala)^+ + Ala^- \rightleftharpoons Zn(Ala)_2$, and $HEta^+ \rightleftharpoons H^+ + Eta$.

In general, exchange will proceed along both direct and indirect pathways. If an appreciable fraction of the PLP in the system appears as either uncombined PLP or gem diamine during the course of the reaction, biphasic reaction rates will result. The absorption–time curves found for the transimination experiments were, indeed, found to be biphasic: for example, the curve shown in Figure 1 is resolvable into a major and a minor exponential component (see below). The hydrolysis rates are known, and in all cases the amplitude of one of the components was found to be consistent with the amount of PLP expected to be released via hydrolysis. In no case was evidence obtained for the conversion of an appreciable fraction of the PLP to gem diamine. Therefore, it appears safe to assume that this last species is formed at only a low steady-state concentration, if at all. Because the reaction conditions are pseudo first order and only three PLP species need be considered, rate

Scheme III



III

equations were set up according to the pseudo-first-order reactions defined in Scheme III.

The subscript Σ indicates the summation over the concentrations of all protonated and complexed forms of the species so designated.

The rate constants, k_1, k_{-1}, k_{II}, ..., etc. are pseudo-first-order rate constants for the interconversion of the three forms of PLP in the reaction system. Except for k_{III} and k_{-III}, which are unknown, these constants can be calculated for a given set of reaction conditions by using the values given in Tables I and II. In principle it is then possible with the additional use of the experimentally determined decay constants to obtain k_{III} (and k_{-III}) from the relaxation rate equations based on Scheme III. Decay constants from experimental absorbance time curves are often obtained by fitting an equation, such as eq 1, directly to the observed data

$$\mathrm{Abs}_t = \mathrm{Abs}_\infty + A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} \quad (1)$$

points. Unfortunately, in the present case, this approach was found not to give reliable results owing to close-lying values of λ_1 and λ_2.

To circumvent this difficulty the degrees of freedom in the fitting function were reduced by directly incorporating the calculated values of k_1, k_{-1}, k_{II}, and k_{-II} into the function and finding the value of k_{III} that gives the best fit to the experimental absorbance-time curve. The procedure is outlined as follows.

From Scheme III, two independent rate equations, eq 2 and 3, and one mass balance equation, eq 4, may be written

$$\frac{-\mathrm{d}[\mathrm{PLPEta}]_\Sigma}{\mathrm{d}t} = (k_{III} + k_{-1})[\mathrm{PLPEta}]_\Sigma - k_1[\mathrm{PLP}]_\Sigma - k_{III}[\mathrm{PLPAa}]_\Sigma \quad (2)$$

$$\frac{-\mathrm{d}[\mathrm{PLP}]_\Sigma}{\mathrm{d}t} = (k_1 + k_{II})[\mathrm{PLP}]_\Sigma - k_{-1}[\mathrm{PLPEta}]_\Sigma - k_{-II}[\mathrm{PLPAa}]_\Sigma \quad (3)$$

$$[\mathrm{PLP}]_{tot} = [\mathrm{PLP}]_\Sigma + [\mathrm{PLPEta}]_\Sigma + [\mathrm{PLPAa}]_\Sigma \quad (4)$$

where

$$[\mathrm{PLP}]_\Sigma = [\mathrm{PLP}^{3-}] + [\mathrm{HPLP}^{2-}] + [\mathrm{H_2PLP}^-] + [\mathrm{H_3PLP}] + [\mathrm{ZnPLP}^-] + [\mathrm{ZnHPLP}] + [\mathrm{ZnH_2PLP}^+] \quad (5)$$

$$[\mathrm{PLPEta}]_\Sigma = [\mathrm{HPLPEta}^{2-}] + [\mathrm{H_2PLPEta}^-] + [\mathrm{H_3PLPEta}] + [\mathrm{ZnHPLPEta}] + [\mathrm{ZnH_2PLPEta}^+] \quad (6)$$

$$[\mathrm{PLPAa}]_\Sigma = [\mathrm{HPLPAa}] + [\mathrm{H_2PLPAa}] + [\mathrm{H_3PLPAa}] + [\mathrm{ZnPLPAa}] + [\mathrm{ZnHPLPAa}] + [\mathrm{ZnH_2PLPAa}] \quad (7)$$

The distribution of species at the completion of exchange is calculated for each reaction mixture as illustrated in Table III, column 3, and from these results the conditional equilibrium constants for the three paths and their pertinent rate constants were calculated by using the relationships

$$\mathrm{K_I} = [\mathrm{PLPEta}]_\Sigma/[\mathrm{PLP}]_\Sigma \quad (8)$$

$$\mathrm{K_{II}} = [\mathrm{PLPAa}]_\Sigma/[\mathrm{PLP}]_\Sigma \quad (9)$$

$$K_{III} = [\mathrm{PLPAa}]_\Sigma/[\mathrm{PLPEta}]_\Sigma = K_{II}/K_I \quad (10)$$

$$k_1 = (k_0^{\mathrm{E}} + k_1^{\mathrm{E}}[\mathrm{H^+}] + k_2^{\mathrm{E}}[\mathrm{H^+}]^2 + \cdots)[\mathrm{Eta}]\alpha_{\mathrm{PLP}} \quad (11)$$

$$k_{II} = (k_0^{\mathrm{A}} + k_1^{\mathrm{A}}[\mathrm{H^+}] + k_2^{\mathrm{A}}[\mathrm{H^+}]^2 + \cdots)[\mathrm{Aa^{i-}}]\alpha_{\mathrm{PLP}} \quad (12)$$

$$k_{-1} = k_1/K_1 \quad (13)$$

$$k_{-II} = k_1/K_{II} \quad (14)$$

$$k_{-III} = k_{III}/K_{III} \quad (15)$$

$$\alpha_{\mathrm{PLP}} = [\mathrm{PLP^{3-}}]/[\mathrm{PLP}]_\Sigma \quad (16)$$

By differentiating eq 2-4 with respect to $[\mathrm{PLP}]_\Sigma$ and $[\mathrm{PLPEta}]_\Sigma$, the secular array, eq 17, which relates the rate constants of Scheme III to the relaxation constants of eq 1, may be derived.

$$\begin{vmatrix} k_{III} + k_{-I} + k_{-III} - \lambda & k_{-III} - k_I \\ k_{-II} - k_{-I} & k_I + k_{II} + k_{-II} - \lambda \end{vmatrix} = 0 \quad (17)$$

Solving the determinant for λ yields

$$\lambda_1, \lambda_2 = [-b \pm (b^2 - 4c)^{1/2}]/2 \quad (18)$$

where

$$b = -(k_1 + k_{-1} + k_{II} + k_{-II} + k_{III} + k_{-III}) \quad (19)$$

and

$$c = (k_{-1} + k_{III} + k_{-III})(k_1 + k_{II} + k_{-II}) - (k_{-III} - k_1)(k_{-II} - k_{-1}) \quad (20)$$

Owing to the relationship expressed by eq 15, k_{III} becomes the only remaining unknown in eq 20–22. By relating the values of both λ_1 and λ_2 to k_{III} in this manner, it was then possible with the aid of the Marquardt nonlinear curve fitting algorithm[25] to find for each experiment the value of k_{III} that gives the best least-squares fit of eq 1 to the absorbance-time data points.

As an example of these calculations, the following results were obtained for the sample experiment described in Figure 1 and Table III. From the concentrations of the solution components and the pH the following values were calculated: $K_I = 1.03$, $K_{II} = 1.97$, $K_{III} = 191$, $k_1 = 0.0084\ \mathrm{s^{-1}}$, $k_{-1} = 0.0082\ \mathrm{s^{-1}}$, $k_{II} = 0.0311\ \mathrm{s^{-1}}$, and $k_{-II} = 1.58 \times 10^{-4}\ \mathrm{s^{-1}}$. Using these relationships the best value of k_{III} was found to be $0.0167\ \mathrm{s^{-1}}$. This fit corresponds to a decay curve given by

$$\mathrm{Abs}_t = 0.135 + 0.088e^{-0.043t} + 0.867e^{-0.021t} \quad (21)$$

To gain insight into the nature of the biphasic behavior, eq 2-4 were integrated numerically by using the calculated values of k_1, k_{-1}, k_{II}, and k_{-II} and that found for k_{III}. The calculated concentration-time curves are drawn as solid lines in Figure 1.

The 420-nm absorbance is a measure of the concentrations of protonated imine nitrogen atom Schiff bases, and it is seen that the curve representing the concentration sum of these species closely follows the course of the absorbance data points. According to the calculations described in Table III, the free PLP in the reaction solution is lower at the end of the reaction than at the beginning. However, in Figure 1 it is seen that during the early stages of the reaction the uncombined PLP concentration increases (from hydrolysis). The increase amounts to about 10% of the total PLP in the system and accounts for the faster component appearing in eq 21.

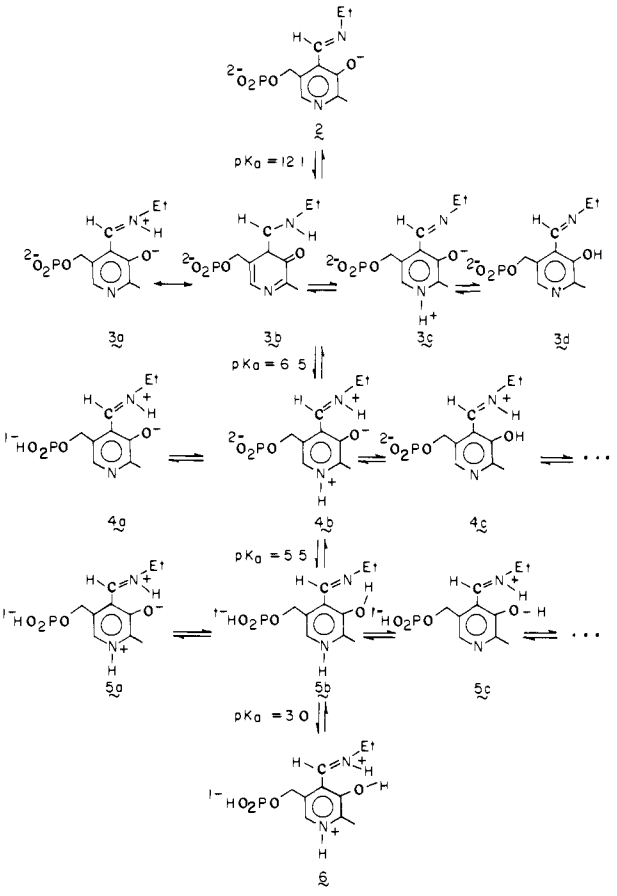
A second integration was performed setting the value of k_{III} equal to 0, i.e., assuming that exchange occurs only via the indirect hydrolysis route, I. The result, shown only for the protonated Schiff bases, is plotted as the dashed line in Figure 1. The rate of change is seen to be in serious disagreement with the observed data points, demonstrating convincingly that hydrolysis alone is not able to account for the experimental rates.

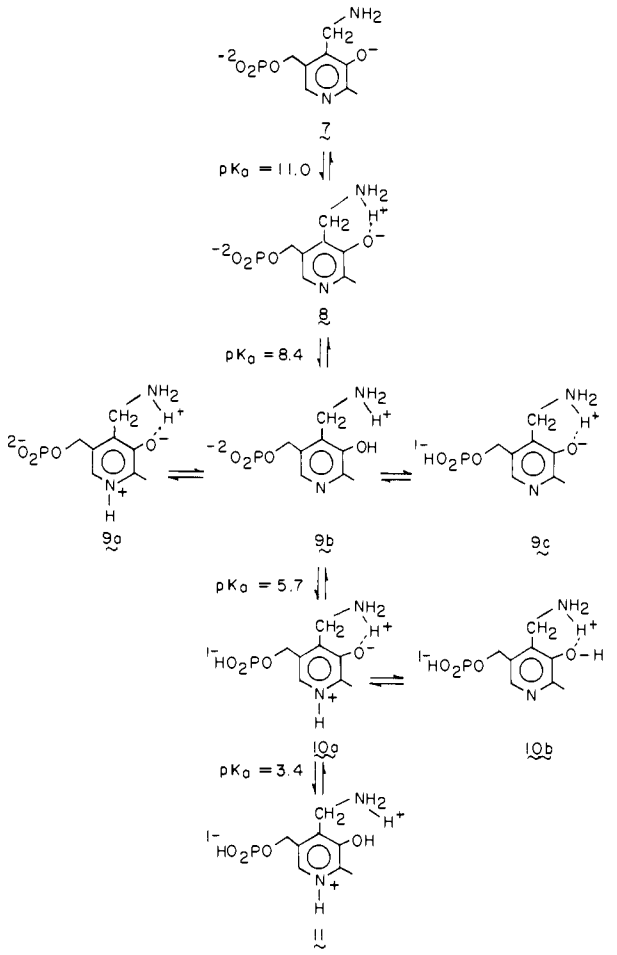
Protonation of PLP Schiff Bases and PMP. In order to devise a reaction mechanism that accounts for the rate behavior, it is important to understand how protons are ordered on the Schiff base. While this subject has been investigated for many years, recent intensive studies on the pH dependencies of UV absorption[22] and ^{13}C magnetic resonance band positions[10,26] have helped to clarify the situation with respect to the microscopic species distribution. The structures of the unprotonated Schiff base is shown by **2**. The first step in protonation mostly occurs at the site at the aldimine nitrogen atom, **3a**. The high pK_a exhibited by this proton (Table I) arises from extensive transfer of electron density from the phenolate oxygen atom to the nitrogen atom, and the mesomeric structure **3b** is often written[20,22] to describe the electron shift and the spectral properties of this species. Metzler et al.[22] present spectral evidence for the presence of small amounts of the pyridinium isomer, **3c** (4-12%), and of the phenol isomer, **3d** (7-15%).

The second proton is acquired predominantly by the phosphate group,

(25) Philip R. Bevington, "Data Reduction and Error Analysis for the Physical Sciences", McGraw-Hill, New York, 1969.

(26) (a) Ming-Daw Tsai, Stephen R. Byrn, Ching-jer Chang, Heinz Floss, and Herschel J. R. Weintraub, *Biochemistry*, **17**, 3177 (1978). (b) Richard C. Harruff and W. T. Jenkins, *Org. Magn. Reson.*, **8**, 548 (1976). (c) Thomas H. Witherup and Edwin H. Abbott, *J. Org. Chem.*, **40**, 2229 (1975).

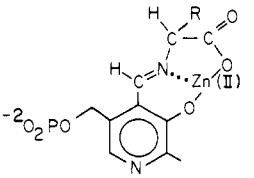




4a. Because this group is not conjugated to the ring π system, its protonation is expected to have little effect on the presence of mesomeric and isomeric structures analogous to **3b**, **3c**, and **3d**. The pyridinium proton has a pK_a about 1 unit lower than that of the phosphate proton, so isomers **4b** and **4c** are expected to comprise roughly 10% of $H_2PLPEta^-$. The predominant form of $H_3PLPEta$ is shown by **5a**. Metzler[22] estimates the ratio **5a**/**5b** to be about 10. The phenol isomer **5c** is also expected to be present to the extent of about 10% of **5a**. The proton ordering of $H_4PLPEta^+$ is shown in **6**. The one remaining site of the phosphate group is too weakly basic to compete appreciably for the protons situated at the other sites, so additional isomers may be neglected.

In the *gem*-diamine the 4′-C atom is saturated and transfer of negative charge from the ring to the 4″-atom is no longer possible. Thus, a reordering of the pK_as of the protonated ring groups is expected. Tobias and Kallen[15] determined two protonation constants for the cyclic gem diamine formed between PLP and En. The larger, $10^{11.29}$ M^{-1}, was assigned to a nitrogen atom of the en residue, and the smaller, $10^{8.61}$ M^{-1}, to the pyridine nitrogen. They concluded that the protonation pattern of the *gem*-diamine closely resembles that of pyridoxamine 5′-phosphate,[27] PMP. PMP^{3-}, **7** acquires a proton at the 4″-N to give **8**. The high pK_a of **8** has been attributed to a hydrogen bond involving the phenolate oxygen atom.[27b] Owing to the higher charge density on the PMP ring compared to that on PLP the second proton is bound chiefly at the pyridine nitrogen atom, **9a**; however, judging from the extensive isomerization of 3-hydroxypyridine,[28] a substantial proportion of the phenol isomer, **9b**, is expected. 3-Hydroxypyridine, in aqueous solutions, shows four electronic absorption bands. The bands at 315 and 245 nm are assigned to the pyridinium–phenolate zwitterion, while those at 278 and 211 nm are assigned to the neutral phenol isomer.[28] The zwitterion bands disappear in ethanol solutions. The absorption spectra of H_2PMP^- show three strong bands at 330, 245, and 218 nm with a broad shoulder at 295 nm (Figure 3, ref 27b). These bands indicate that both of the isomeric forms **9a** and **9b** are present, and a rough estimate made by comparing the relative intensities at 330 and 295 nm suggests that about 20–25% is present as **9b**. About 10% of the phosphate protonated isomer may also be present. The dominant isomer of H_3PMP is shown by **10a**, but here again evidence for a band at 290 nm[27b] suggests the presence of at least 20% of the phenol, **10b**. The structure of H_4PMP^+ is given by **11**.

The Zn(II) complex of a PLP–aminoacidate Schiff base is represented by **12**. The metal ion blocks protonation at the phenolate and aldimine



sites but drains off less negative charge from the ring than does a proton. As a result the pyridine ring nitrogen atom is relatively basic and is protonated before the phosphate group.[21]

Protonation constants for the nitrogen atoms at the gem diamine center have been estimated from the pK_a values of the parent aliphatic amines according to Hogg et al.[16] The values are shown in Scheme IV. Because it is of interest to predict whether the proton is located on the amine nitrogen atom or on the phenolate oxygen atom (see below), the influence on the pK_a of hydrogen bonding between these groups has been ignored. An external base competing for a proton bound to the amine group of T_1 or T_2 would sense pK_a values at least 1 unit larger than those given.

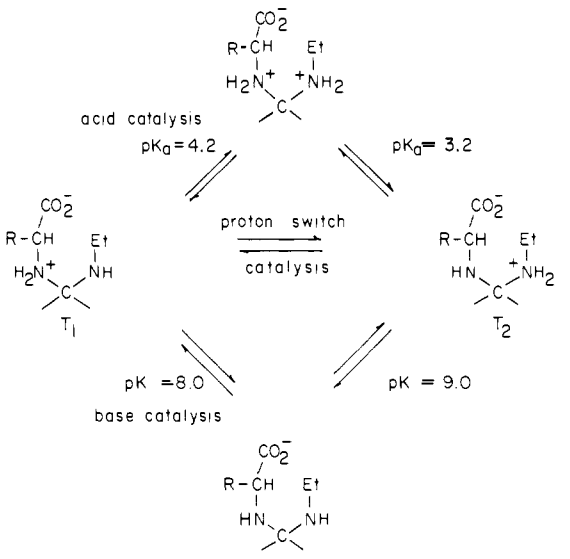
Results and Discussion

A reversible path parallel to one involving known hydrolysis/condensation reactions has been shown to exist in imine exchange reactions of PLP Schiff bases. This parallel reaction is first order in the parent ethylamine Schiff base and first order in exchanging aminoacidate ion. Furthermore, if the monoprotonated reactant, $HPLPEta^{2-}$, is taken as a reference point from

(27) (a) V. R. Williams and J. B. Neilands, *Arch. Biochem. Biophys.*, **53**, 56 (1954). (b) Wayne Felty and Daniel L. Leussing, *J. Inorg. Nucl. Chem.* **36**, 617 (1974).

(28) S. F. Mason, *J. Chem. Soc.*, 5010 (1957).

Scheme IV. Protonation of the Gem Diamine Nitrogen Atoms



which to assess the hydrogen ion dependence of the rates, the values of the forward pseudo-first-order rate constant, k_{III}, can be expressed as a simple polynomial in $[H^+]$

$$k_{III} = (k_1 + k_2[H^+] + k_3[H^+]^2 + \cdots)\alpha_{HPLPEta}[Aa^{i-}] \quad (22)$$

where the fraction of total Eta Schiff base present as $HPLPEta^{2-}$ is defined in eq 23.

$$\alpha_{HPLPEta} = \frac{[HPLPEta^{2-}]}{[PLPEta^{3-}] + [HPLPEta^{2-}] + [H_2PLPEta^{1-}] + \cdots} \quad (23)$$

The H^+ dependence of k_{III} is illustrated in Figures 2 and 3 where it is seen that $k_{III}/(\alpha_{HPLPEta}[Aa^{i-}])$ plotted vs. pH causes the data points to fall along single curves respectively for Ala^{1-} and Asp^{2-}.

The independence on Zn(II) concentration of the points shown in Figures 2 and 3 demonstrates that the metal ion is kinetically inert and essentially serves the function fo complexing the tridentate aminoacidate–PLP Schiff base after the rate-limiting step for imine exchange has occurred. Along the hydrolysis/condensation pathways Zn(II)-dependent terms have been found (Table II). Great significance should not be placed on this difference because the amine exchange experiments were designed to minimize the presence of Zn(II)–PLPEta complexes.

Equation 22 is the forward rate equation for a reaction system in which $HPLPEta^{2-}$ disappears along the parallel paths

$$HPLPEta^{2-} + Aa^{i-} \xrightarrow{k_1}$$

$$HPLPEta^{2-} + Aa^{i-} + H^+ \xrightarrow{k_2}$$

$$HPLPEta^{2-} + Aa^{i-} + 2H^+ \xrightarrow{k_3}$$

. . .

Kinetically indistinguishable are the reactions

$$HPLPEta^{2-} + Aa^{i-} \xrightarrow{k_1^T}$$

$$H_2PLEta^- + Aa^{i-} \xrightarrow{k_2^T}$$

$$H_3PLPEta + Aa^{i-} \xrightarrow{k_3^T}$$

. . .

Second-order rate constants calculated for the disappearance of $HPLPEta^{2-}$, $H_2PLPEta^-$, and $H_3PLPEta$ along the paths defined in the latter description are given in Table IV.

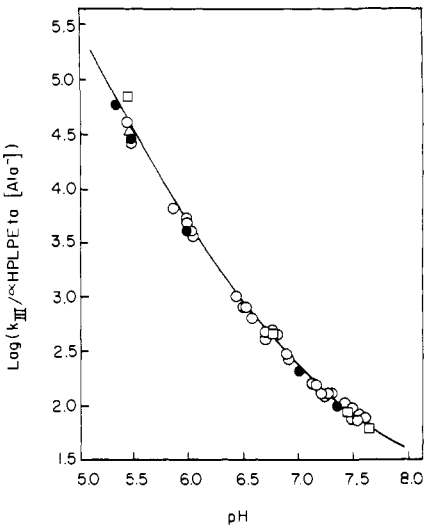


Figure 2. Reduced Ala^- exchange rates. Total Zn(II): (○) 5.0 mM; (●) 6.0 mM; (□) 7.2 mM; (△) 10.0 mM.

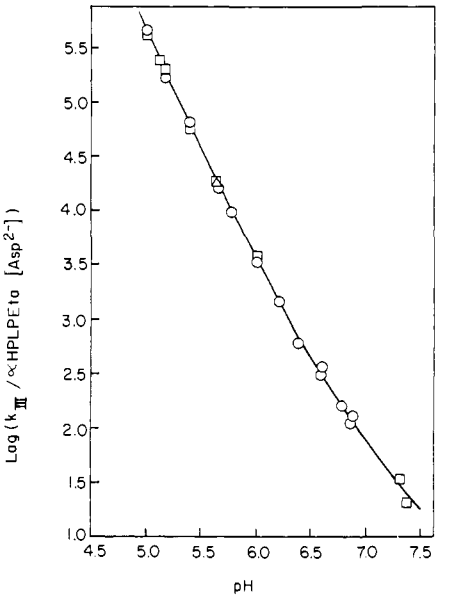


Figure 3. Reduced Asp^{2-} exchange rates. Total Zn(II): (○) 6.0 mM; (□) 8.0 mM; (△) 10.0 mM.

In the Asp^{2-} series of experiments, it was found that the inclusion of a reaction pathway involving a total of four protons was necessary to describe the dependence of k_{III} on pH at the lower range. Because no similar proton involvement was found in the Ala^- experiments, it is indicated that $H_4PLPEta^+$ is not the species undergoing attack. An alternate reaction is one in which $H_3PLPEta$ is attacked by the tautomer of $HAsp^{-1}$ in which the 4-CO_2^- group is protonated. Assuming a p$K_a \sim 4.7$ for HO_2C-$CH_2CH(NH_2)CO_2^-$ a value of 1.1×10^3 M^{-1} s^{-1} is calculated for the rate constant for this more likely reaction. The close agreement with 2.2×10^3 M^{-1} s^{-1} calculated for the reaction of Asp^{2-} with $H_3PLPEta$ indicates that the 4-carboxylic acid group of the amino acid is not effective catalytically.

For each transimination pathway involving a given Schiff base $H_iPLPEta^{i-3}$ a reaction series analogous to that shown in Scheme II exists. By applying the "steady-state" approximation to the concentrations of the gem diamines, it can be shown that the following relationship exists between the forward rate constants k_i^T given in Table III and the microscopic constants defined in Scheme II:

$$k_i^T = \frac{k_{a,i}k_{cat,i}k_{b,i}}{k_{-a,i}k_{-cat,i} + k_{-a,i}k_{b,i} + k_{cat,i}k_{b,i}} \quad (24)$$

Table IV. Rate Constants for Direct Transimination (25 °C, $I = 0.5$)

reactants	Ala^-, M^{-1} s^{-1}	Asp^{2-}, M^{-1} s^{-1}
$HPLPEta^{2-} + A^{i-}$	25 ± 1	
$H_2PLPEta^- + A^{i-}$	(4.4 ± 0.1) × 10^2	(1.2 ± 0.1) × 10^2
$H_3PLPEta + A^{i-}$	(2.6 ± 0.1) × 10^3	(2.6 ± 0.1) × 10^3
$H_3PLPEta + HA^-$ [a]		(1.1 ± 0.2) × 10^3

[a] $HA^- = HO_2CCH_2CH(NH_2)CO_2^-$.

At high values of $k_{cat,i}$ and $k_{-cat,i}$, the limiting relationships apply.

$$k_i^T(\text{lim}) = \frac{k_{a,i}k_{b,i}}{k_{-a,i}/K_{cat,i} + k_{b,i}} \quad (25)$$

In eq 25 $K_{cat,i}$ is the equilibrium constant for the tautomerization $T_{1,i} \rightleftharpoons T_{2,i}$, as defined in II.

From eq 25 it is seen that the limiting transimination rates depend on the rates of formation and dissociation of the gem diamines and on $K_{cat,i}$. It seems reasonable to assume that this last constant has a value approximately equal to that of the ratio of the protonation constants of free ethylamine and aminoacidate, i.e., ~10.

Hogg, Jencks, and Jencks[16] found that exchange between MCP^+ and NH_2OH is subject to catalysis, even by excess $^+NH_3OH$ in the reaction solution. By way of contrast, in the present investigation no concentration dependency by a solution component was observed other than that described by the parallel second-order reaction paths. Further experiments employing buffers that had pK_a values lying in the pH range of interest also did not show concentration dependencies consistent with the catalysis of the intermediate step. Mes buffers at pH 6.0 and 6.7 and Hepes buffers at pH 7.2 (both varied from 0.005 to 0.10 M) were found to produce small (5–10%) rate increases which showed no trends with the buffer concentration. At pH 7.6, 0.060 M Hepes produced a 15% rate increase but this decreased to 12% at 0.08 M. Imidazole showed somewhat larger effects. At both pH 6.8 and pH 7.3 concentration-independent rate enhancements scattered around 20% were obtained in the range 0.005–0.07 M buffer. However, as the buffer concentration was further increased in the pH 6.8 media, the rate enhancement was observed to steadily decrease until it became negligible at 0.12M buffer.

The reaction systems studied here are complicated by a large number of equilibria coupled to the kinetic processes. The diverse and small buffer effects that have been observed are consistent with the action of secondary salt effects on these equilibria. The somewhat higher influence of imidazole on the exchange rates may signify the ability of this substance to catalyze Schiff base hydrolysis and formation along a transimination pathway in a manner similar to that observed for secondary amine catalyzed Schiff base formation.[29,30]

The second-order rate constants for the overall reaction of $H_2PLPEta^-$ given in Table IV have values similar to those found by Hogg et al.[16] at considerably higher buffer concentrations than employed here, while the rates exhibited by $H_3PLPEta$ are even faster. Because the buffer-catalyzed proton transfers were earlier found to have rate constants already at the diffusion-controlled limit, the rates observed here must arise from faster formation and/or dissociation of the gem diamines. Furthermore, the lack of influence of acid and base catalysts in a manner consistent with eq 24 indicates that the limiting equation, eq 25, applies even at low buffer concentrations. The implication is that proton transfers between the T_1 and T_2 forms of the gem diamines are faster than expected for catalysis via diffusion-catalyzed processes, and are therefore probably internally catalyzed. Molecular models shows that the PLP phosphate group is able to swing close to the gem diamine nitrogen atoms and, if protonated as in analogues **12c**, **13a**, and **13b**, could serve as a bifunctional proton switch catalyst.

(29) E. H. Cordes and W. P. Jencks, *Biochemistry*, **1**, 773 (1962).
(30) Thomas C. Bruice and R. M. Topping, *J. Am. Chem. Soc.*, **85**, 1493 (1963).

Table V. Estimated Relative Susceptibilities to Nucleophilic Attack and Efficacies for Internally Catalyzed Proton Transfer

Schiff base	isomer	susceptibility	PMP analogue for gem diamine	catalytic effect	internal catalyst
$HPLPEta^{2-}$	3a	+	7	++	phenoxide
$H_2PLPEta^-$	4a	+	9c	++	phenoxide (weak), $-PO_3H^-$
	4b	++	9a	+	phenoxide (weak), $-PO_3^{2-}$ (weak)
	4c	++	9b	++	-OH
$H_3PLPEta$	5a	++	10a	++	$-PO_3H^-$
	5c	++	10b	+++	-OH, $-PO_3H^-$
$H_4PLPEta^+$	6	+++	11	+++	-OH, $-PO_3H^-$

The phenoxide oxygen atom of **11** lies close to the nitrogen atoms of the gem diamine group and during the course of nucleophilic addition to PLP this atom increases in basicity as the ring becomes more like that of PMP. The pK_a of the PMP phenol would appear to lie in the vicinity of 8.0, which matches that of the proton to be removed (T_1 of Scheme IV). A facile proton shift to the phenoxide followed by a slight rotation of the resulting phenol group would bring this proton under the influence of the slightly more basic nitrogen atom of the ethylamine residue. Another rapid proton transfer would then ensue. In addition to serving as a base catalyst the 3′-substituent, as the phenol in **9b** and **10b**, could also serve as a proton switch catalyst.

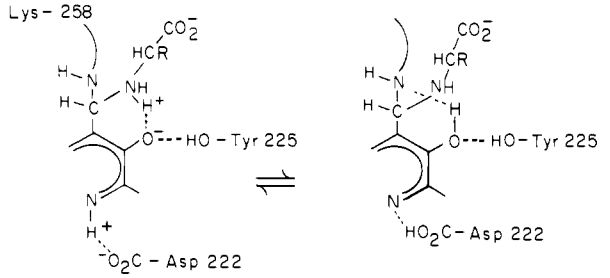
The variously protonated $H_iPLPEta$ Schiff bases are expected to show different susceptibilities to nucleophilic attack depending on how many and where protons are attached. Structures in which the aldimine nitrogen atom is unprotonated, **3c**, **3d**, **4c**, etc., are expected to show little tendency to add a second amine, whereas those in which this group is protonated will be reactive. Countering the effect of this proton will be mesomeric structures, such as **3b**, through which negative charge is transferred to the aldimine linkage. On the other hand, protonation at basic sites located on the pyridine ring will tend to activate the Schiff base by withdrawing negative charge.

On the basis of the discussion presented in the above two paragraphs, qualitative predictions of relative nucleophilic reactivity and ease of internal proton shift have been made and are summarized in Table V. A comparison with Table IV shows that the order of observed rates is the same as that predicted for increasing susceptibility of Schiff base to nucleophilic attack: $HPLPEta^{2-} < H_2PLPEta^- < H_3PLPEta$. The rate of exchange along the pathway starting with **4b** is expected to be relatively slow owing to the small fraction present as this form of $H_2PLPEta^{1-}$ and to the low internal catalytic efficiency for proton transfer within the gem diamine for which **9b** is an analogue.

Direct transimination in the PLP Schiff bases investigated here has been found to be faster than the indirect hydrolytic route for imine exchange. The rate advantage provided by the former pathway over the latter is, however, rather modest and amounts roughly to a factor of 2. The question then arises as to which pathway is favored in the enzymic environment. According to the X-ray structure of the enzyme–Asp^{2-} complex published by Ford, Eichle, and Jansonius,[9] the ω-carboxylate of Asp^{2-} forms an ion pair with Arg-292 while the α-carboxylate lies with the positively charged field generated by Arg-386 and Asn-194. These protein side chains are so positioned that the Asp^{2-} amine group is directed toward the aldimine linkage of the internal PLP–lysine-238 Shiff base. This orientation will facilitate *both* the rates of direct transimination and of the second step in the hydrolytic/condensation pathway.

The phosphate group of enzyme-bound PLP is anchored in a highly polar pocket directed away from the aldimine linkage.[8a,9] It appears unlikely that this group is available to catalyze proton transfer. Furthermore, the phenoxide oxygen atom also does not

appear to be able to function as an effective catalyst, because PLP is bound to the enzyme in the pyridinium form corresponding to **5a**. In **5a** this oxygen atom has low basicity. However, the pyridinium proton is hydrogen bonded to the terminal carboxylate of Asp-222 and the phenoxide atom is hydrogen bonded to the –OH of Tyr-225.[9] These interactions could make possible a mechanism through which the phenoxide is activated: transfer of the pyridinium proton to Asp-222 would simultaneously cause the phenoxide to become more basic and, therefore, to be more capable of receiving a proton from a gem diamine nitrogen atom.



Alternatively, the proton shift to Asp-222 could be accompanied by a proton shift from Tyr-225 to the PLP phenoxide. The PLP phenol could then serve as a proton switch catalyst.

It has been calculated that if a second-order reaction has been reduced to a first order one by way of the preequilibrium formation of a complex in which a favorable reaction geometry is preserved, the resulting first-order rate constant will be numerically larger than the original rate constant by a factor of 10^4, or more.[31] Thus, an enzymic direct transimination rate constant $\sim 10^6$ s^{-1} may be expected. To compete with this, a very high velocity for hydrolysis of the Lys-258 aldimine is required. From temperature-jump studies Fasella[32] found the primary bimolecular rate constant between Asp^{2-} and AAT has a value that is at least 10^7 M^{-1} s^{-1}. If the true value lies in the vicinity of this lower limit, than at millimolar Asp^{2-} concentrations, the limiting step in the displacement of Lys-258 is the rate of formation of the preexchange complex and it is less important whether imine exchange is direct or indirect. On the other hand, direct transimination provides a definite advantage in efficiency over the indirect route,[15] and this difference could be important if enzyme–substrate complex formation were faster.

Tobias and Kallen[15] report rate constants of ca. 4×10^4 s^{-1} for ring closing and ring opening with the PLP–En Schiff base at pH 10. These values correspond to k_a and k_{-a} of eq 25 and, owing to the reaction symmetry, also correspond to k_b and k_{-b}. K_{cat} for the reaction is unity, also owing to symmetry. Inserting these values into eq 25 yields a transimination rate constant in one direction of 2×10^4 s^{-1}. As a reasonable approximation, it can be assumed that the chemical properties of the amine groups in Eta and Ala^- do not differ significantly from those of En. Thus, the forward rate constant for the direct replacement of Eta in $HPLPEta^{2-}$ by Ala^- should differ from the exchange constant of $HPLPEn^{2-}$ essentially by only an entropy factor. The ratio of these constants, 8×10^2, is lower than expected, possibly because the terminal amine group of an En Schiff base becomes protonated around pH 10[33] and is withdrawn from the reaction pool. Nevertheless, the importance of the entropy term on these reaction rates is clearly evident. Tobias and Kallen[15] concluded that the enzyme need not activate the transimination pathway except by bringing the reactants together in a favorable geometry. The X-ray structure[9] confirms that this requirement is accomplished, but our results suggest that the enzyme may need, and has the potential, to intervene favorably during the intermediate proton transfer steps.

Acknowledgment. This investigation was supported by a grant from the National Science Foundation.

Appendix

Solution of the Equilibrium Equations. The method outlined below deals with a specific set of reactions; however, it is easily modified to encompass a fewer, or greater, number of species and interactions.

Consider an equilibrium system comprised of the unit species M, A, B, and H which interact in various ways to form the adducts MA, MA_2, MB, MB_2, HA, H_2a, HB, H_2B, AB, HAB, MAB, and MHAB. Given that the following are known, the total concentrations of M, A, and B, designated as TM, TA, and TB; an experimentally determined value of H at equilibrium; the overall formation constants for the adducts, for which β_i is the formation constant for the ith adduc, the following calculations may be made.

If values of [A], [B], and [M] are assumed, then the concentrations of all of the adducts may be calculated, e.g.

$$[MA_2] = \beta_{MA_2}[M][A]^2$$

$$[HAB] = \beta_{HAB}[A][B][H]$$

etc.

These concentrations are then used to evaluate the mass-balance functions pertaining to the unknowns.

$$FM = TM - [M] - [MA] - [MA_2] - [MB] - [MB_2] - [MAB] - [MHAB]$$

$$FA = TA - [A] - [HA] - [H_2A] - [MA] - 2[MA_2] - [MAB] - [MHAB]$$

$$FB = TB - [B] - [HB] - [H_2B] - [MB] - 2[MB_2] - [MAB] - [MHAB]$$

The solution to the problem are those values of [M], [A], and [B] which cause FM = FA = FB = 0. This solution is unique if the concentrations are limited to positive values, which is the chemically meaningful solution.

It is highly improbable that the first estimates of [M], [A], and [B] will be chosen so that the problem is solved within a satisfactory degree of accuracy. These estimates then may be improved by using the Newton–Raphson method.

Pertaining to the present example the procedure is as follows. Setup simultaneous linear equations involving the partial derivatives

$$H_2PLEta^- + Aa^{i-} \xrightarrow{k_2^T}$$

$$\frac{\delta TA}{\delta M}\Delta M + \frac{\delta TA}{\delta A}\Delta A + \frac{\delta TA}{\delta B}\Delta B = -FA$$

$$\frac{\delta TB}{\delta M}\Delta M + \frac{\delta TB}{\delta A}\Delta A + \frac{\delta TB}{\delta B}\Delta B = -FB$$

For a given set of values for [M], [A], and [B], the partial derivatives may also be evaluated, just as FM, FA, and FB. Solving for ΔM, ΔA, and ΔB leads to improved values for the subunit concentrations.

$$[M]_{j+1} = [M]_j + \Delta M_j$$

$$[A]_{j+1} = [A]_j + \Delta A_j$$

$$[B]_{j+1} = [B]_j + \Delta B_j$$

The procedure is iterated until the corrections become smaller than the acceptable error in [M], [A], and [B].

Because the concentrations of the adducts have been previously

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calculated, computational time may be saved in evaluating the partial derivatives by using the relationships

$$\delta FM/\delta M = -\{[M] + [MA] + [MA_2] + [MB] + [MB_2] + [MAB] + [MHAB]\}/[M]$$

$$\frac{\delta FM}{\delta A} = -\frac{[MA] + 2[MA_2] + [MAB] + [MHAB]}{[A]}$$

$$\frac{\delta FM}{\delta B} = -\frac{[MB] + 2[MB_2] + [MAB] + [MHAB]}{[B]}$$

$$\frac{\delta TA}{\delta M} = -\frac{[MA] + 2[MA_2] + [MAB] + [MHAB]}{[M]} = \frac{\delta FM}{\delta A}\frac{[A]}{[M]}$$

The calculations given in Table III were made by using the constants given in Table I and total concentrations appropriate to the different stages in the reaction.

Before the solution was mixed, the concentrations of free PLP and PLPEta Schiff bases in the preequilibrated Schiff base reactant solution were calculated from the known total concentrations of PLP and Eta and the pH at which the solution was equilibrated. For the time a few milliseconds after mixing this Schiff base solution with an equal volume of Zn(II) and aminoacidate solution, the unit species were taken as Zn(II), PLP^{3-}, $PLPEta^{3-}$, Aa^{i-}, and H^+. The total concentrations of PLP and PLPEta were taken as half the concentrations of uncombined PLP and Schiff base in the initial reactant solution containing these species. The total concentrations of Zn(II) and Aa^{i-} were taken as half the total concentrations in the other reactant solution. $[H^+]$ was determined from the measured pH of the mixed solution because the pH remains essentially constant during imine exchange.

The concentrations for the final equilibrium state of the mixed solutions were calculated by using half the total concentrations of the PLP, Eta, Zn, and aminoacidate in the initial solutions before mixing. The measured pH was used to obtain $[H^+]$.

As a point of additional interest, if the pH of the reactant solution is not known independently, $[H^+]$ may be computed by adding to the above a mass balance equation on total titratable H^+, e.g., $TH = [H^+] - [OH^-] + [HA] + 2[H_2A] + [HB] + 2[H_2B] + [HAB] + [MHAB]$.

Registry No. Ethylamine, 75-04-7; alanine, 56-41-7; aspartic acid, 56-84-8; $HPLPEta^{2-}$, 85535-42-8; $H_2PLPEta^-$, 85535-40-6; $H_3PLPEta$, 64818-26-4; PLP, 54-47-7; Zn^{2+}, 23713-49-7; PLP^{3-}, 85535-41-7; Ala^-, 17807-53-3; Zn(PLPEtaH), 85552-60-9; $HPLPAla^{3-}$, 85535-43-9; $H_2PLPAla^{2-}$, 85535-44-0; $Zn(PLPAla)^{2-}$, 85115-54-4; $Zn(HPLPAla)^-$, 85552-61-0; $Zn(H_2PLPAla)$, 85552-62-1; $Zn(Ala)^+$, 12542-92-6; $Zn(Ala)_2$, 14647-06-4.

Communications to the Editor

Ortho-Metallation at a Multiply Bonded Dirhenium Center: The First Such Example Occurring at a Multiple Bond of the M_2L_8 Type

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Bidentate bridging ligands such as bis(diphenylphosphino)methane (dppm) that contain a single bridgehead atom have proven to be very effective in stabilizing molecules containing pairs of multiply bonded metal atoms.[1] In exploring the behavior of other bridging ligands of this general type, including those in which the ligand is unsymmetrical, we turned our attention to 2-(diphenylphosphino)pyridine (Ph_2Ppy). Prior work by Balch and co-workers[2-5] has demonstrated the effectiveness of Ph_2Ppy in stabilizing well defined homo- and heterobinuclear transition-metal complexes, particularly those of the group 8 metals. The extension of these studies to include multiply bonded pairs of atoms of the early transition series is especially noteworthy as judged by the recent results we have obtained on dirhenium complexes of this ligand. In the course of these investigations, we have isolated a derivative of the triply bonded Re_2^{4+} core,[1,6-9] possessing the stoichiometry $Re_2Cl_3(Ph_2Ppy)_2[(C_6H_5)(C_6H_4)Ppy]$ (**1**). This complex contains a novel tridentate bridging mode for this ligand in which ortho metalation has occurred at one of the phenyl rings. This is, to our knowledge, the first example of an ortho-metalation reaction involving a multiply bonded dimetal unit contained within an M_2L_8 skeleton. This result has an important bearing in the

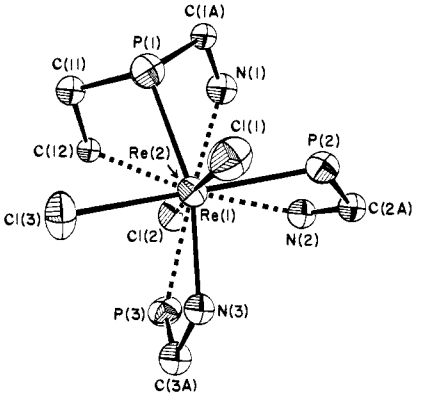

Figure 1. ORTEP drawing of the central part of the $Re_2Cl_3(Ph_2Ppy)_2[(C_6H_5)(C_6H_4)Ppy]$ molecule (**1**). The view is straight down the Re–Re axis so that Re(2) is hidden by Re(1). Re(2)–ligand bonds are shown with broken lines for clarity.

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