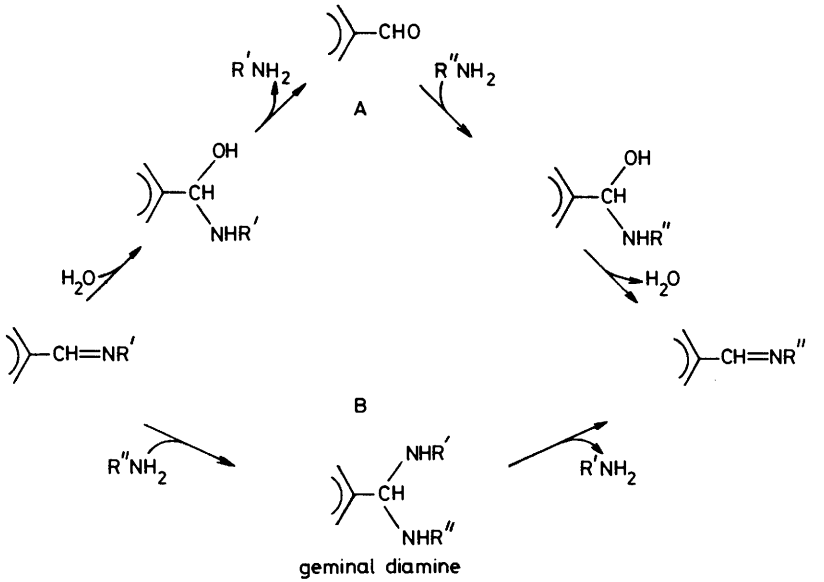
Chemical Models of Enzymic Transimination

By Mauri J. Mäkelä and Timo K. Korpela
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, UNIVERSITY OF TURKU, SF-20500 TURKU 50, FINLAND

1 Introduction

The reaction called transimination (in older literature transaldimination or trans-Schiffization) means the reaction of amines to imines so that the original imine is ruptured and a new one is formed. Chemically, transimination is a symmetric and reversible process which can follow two paths: either free carbonyl and two carbinolamines occur as the intermediates or the reaction involves only a geminal diamine, a structural analogue of the carbinolamines (routes A or B respectively in Scheme 1). The reaction can be classified as the transfer of an imino-group.



Scheme 1

In addition to aspects belonging to pure organic and physical chemistry, transimination is of biochemical interest since readily (and reversibly) formed Schiff bases play a vital role in cellular reactions. The biological processes have been often thought *a priori* to follow the shorter ('crosscut') pathway involving the geminal diamine whenever it seems possible, to the extent that investigators in the

field are increasingly attributing the word transimination to describe that particular mechanism.

In most reports on transimination the results have been considered in the light of pyridoxal 5'-phosphate (PLP*)-dependent enzymes. This large family of enzymes encompasses, for example, those catalysing various reactions of the amino-acids. The PLP coenzyme is linked to the protein moiety *via* a Schiff base or an imine bond. The catalytic act starts when this bond is converted into a new imine bond between PLP and the substrate amino-acid. Transimination is the first substrate-induced event to occur and apparently triggers the whole succeeding catalytic process. The study of the mechanism of transimination is therefore of value in exploring the events taking place during enzyme catalysis. This is not an easy task, however, because enzyme reactions are too fast and complex to be approached straightforwardly. The modern picture of enzymic transimination is therefore, to a great extent based on the work with chemical model ('biomimetic') reactions rather than on experiments with enzyme-catalysed reactions.

Fundamentals of the chemical aspects of the reactions of imines have been discussed by Jencks[1] and by Spencer.[2] This article will consider the subject more in view of the problems arising from the enzyme mechanisms. The last section sketches the present understanding of the transimination reactions catalysed by pyridoxal phosphate enzymes and shows how the elements of the puzzle can be fitted together.

2 Methods for Studying Transimination

Any chemical model system should be selected, in the first place, to be as congruent with its enzymic counterpart as possible. This is, in a way, a vicious circle since the course of an unknown enzyme reaction has to be approximated by chemical models which cannot be clearly formulated. However, studying various models probably scopes the mechanisms which the enzyme may utilize,[3] and in any case, the scarcity of other approaches to enzyme mechanisms necessitates the model studies.[4]

Because the aldehydes and the corresponding Schiff bases of the model systems generally have specific light absorptions at moderately high wavelengths, u.v.–v.i.s. spectroscopy has generally been used to monitor the reactions. The imines formed between alkylamines and *o*-hydroxypyridine aldehydes have the lowest energy maximum at 400—430 nm at pH values where the imine nitrogen is protonated (pK = 10—12). The free imine base absorbs at about 340 nm.[5,6] The change in the hybridization of the aldimine (C-4') carbon from sp^2 (Schiff base) to sp^3 (geminal diamine) induces a distinct change in the properties of the chromophore. The

* *Abbreviations:* AspAT, aspartate aminotransferase (EC 2.6.1.1); BHDA, benzhydrylidenedimethylammonium ion; EDA, ethylene diamine; GD, geminal diamine; MBP, *N*-*p*-methoxybenzylidenepyrrolidinium cation; PLP, pyridoxal 5'-phosphate; SB, Schiff base.

[1] W. P. Jencks, *Prog. Phys. Org. Chem.*, 1964, **2**, 63.

[2] T. A. Spencer, *Bioorg. Chem.*, 1977, **6**, 313.

[3] A. J. Kirby, *Adv. Phys. Org. Chem.*, 1980, **17**, 184.

[4] W. P. Jencks, 'Catalysis in Chemistry and Enzymology', McGraw-Hill, New York, 1969, p. 5.

[5] R. J. Johnson and D. E. Metzler, *Methods Enzymol.*, 1970, **18A**, 433.

[6] C. M. Metzler, A. Cahill, and D. E. Metzler, *J. Am. Chem. Soc.*, 1980, **102**, 6075.

latter structure absorbs at 325—330 nm in neutral or acidic aqueous solution and at 300—320 nm in alkali.[7-10] Corresponding changes occur in the spectra when aminothiols react with the aldehydes to form cyclic adducts.[11,12]

The bimolecular imine-transfer between chemically similar amines is not, however, well suited for u.v. spectroscopic monitoring due to the fact that there are only minute differences between the spectra of the imines and also that the geminal diamine intermediate does not appear in measurable extent. This problem can be avoided by using hydroxylamines or semicarbazides as the attacking amines because their reaction products absorb at different wavelengths from those of the parent amines.[13-16] Unfortunately, this measuring system may mean a compromise for the congruency of the enzyme model.[17]

Transimination reactions are generally fast and therefore rapid mixing techniques are often used in connection with the u.v. spectroscopy in kinetic work.[10,15,16] Relaxation techniques have been employed only occasionally with the model studies.[9] Since this technique is central to the enzyme research, studies on appropriate model systems with comparable time resolution are required.

Very large up-field shifts occur in the n.m.r. resonances of both ^{1}H and ^{13}C nuclei at the azomethine bond when Schiff bases are converted into geminal diamines or the respective sp^3 structures. This technique requires, however, relatively high concentrations of the reactants or long acquisition times, thus limiting the applicability of n.m.r. to the verification of molecular structures of stable intermediates.[10,18-21] Care should be taken when combining n.m.r. and u.v. results obtained under largely different conditions.[10]

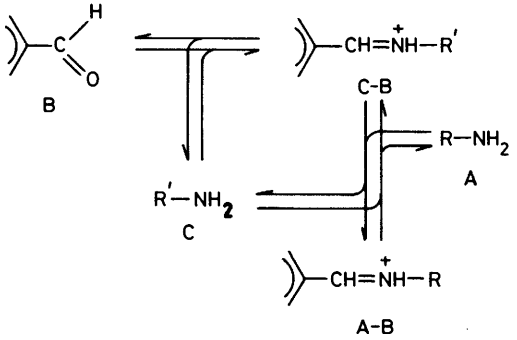
Geminal diamines have a low quantum-yield as compared to Schiff bases,[22] making it possible to monitor the transimination by fluorometer. Enzyme studies especially would benefit from the excellent sensitivity of this method, by way of reduced consumption of enzyme. Additional knowledge of the rather complex fluorescence properties of PLP enzymes and their model systems are awaited, however. In model systems including an optically active reactant (*e.g.* amine), circular dichroism may be the method of choice. Polarography may also have some use.[23]

[7] M. H. O'Leary, *Biochim. Biophys. Acta*, 1971, **242**, 484.
[8] E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, 1954, **76**, 169.
[9] P. S. Tobias and R. G. Kallen, *J. Am. Chem. Soc.*, 1975, **97**, 6530.
[10] T. Korpela, M. Mäkelä, and H. Lönnberg, *Arch. Biochem. Biophys.*, 1981, **212**, 581.
[11] D. Mackay, *Arch. Biochem. Biophys.*, 1962, **99**, 93.
[12] D. Mackay, *Biochim. Biophys. Acta*, 1963, **73**, 445.
[13] E. H. Cordes and W. P. Jencks, *Biochemistry*, 1962, **1**, 773.
[14] E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, 1962, **84**, 826.
[15] H. Fischer, F. X. DeCandis, S. D. Ogden, and W. P. Jencks, *J. Am. Chem. Soc.*, 1980, **102**, 1340.
[16] J. L. Hogg, D. A. Jencks, and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 4772.
[17] B. Chance, in 'Techniques of Chemistry', ed. A. Weissberger, Vol. 6, ed. G. G. Hammes, Wiley, New York, 1974, p. 171.
[18] E. H. Abbott and A. E. Martell, *J. Am. Chem. Soc.*, 1970, **92**, 1754.
[19] E. H. Abbott and A. E. Martell, *J. Am. Chem. Soc.*, 1971, **93**, 5852.
[20] M. H. O'Leary and J. R. Payne, *J. Biol. Chem.*, 1976, **251**, 2248.
[21] R. D. Lapper, H. H. Mansch, and I. C. P. Smith, *Can. J. Chem.*, 1975, **53**, 2406.
[22] T. Beeler and J. E. Churchich, *J. Biol. Chem.*, 1976, **251**, 5267.
[23] M. Cortijo, J. S. Jimenez, and J. Llor, *Biochem. J.*, 1978, **171**, 497.

In studies of enzyme-catalysed transimination, stopped-flow techniques have been attempted, but they have frequently proved to be too slow. On the other hand, the methods with more rapid responses show various relaxations, the sequence of which is difficult to synchronize with the actual chemical events within the supposed mechanism.[24] Only recently, indications of relevant observations have been attained by temperature-jump methods.[25] Polarized light spectrophotometry and circular dichroism have been used to monitor conformational movements during PLP enzyme reactions.[26,27] Proton transfers are central in both enzymic and non-enzymic transimination systems and hence studies concerning isotope effects (*e.g.* 'proton inventory technique'[28]) will share an important role in elucidating the detailed mechanisms.

3 Characteristics of Nucleophilic Amine Catalysis

Two mechanisms of catalysis thoroughly studied in biomimetic systems and for which there is evidence in certain enzymic reactions are general acid–base catalysis and covalent or electrophilic–nucleophilic catalysis in which the catalyst forms a covalent bond with the substrate.[1,29] The principle of nucleophilic amine catalysis, or in fact, the principle of transimination, is described in Scheme 2. The most noted



Scheme 2

example of such a reaction is anilinium catalysis in the oxime or semicarbazone formation from benzaldehyde discovered by Cordes and Jencks.[13,14] In slightly acidic conditions aromatic primary amines accelerate the reactions considerably more than would be expected on the basis of their pK-values. The aromatic amines catalyse the reaction by means of a very reactive intermediate (C–B, in Scheme 2), the formation of which is rate-determining in mild acid. The intermediate imine can be 'trapped' by semicarbazide or hydroxylamine (A, in Scheme 2). The reaction mechanism of the catalysis by aliphatic primary amines is the same but these more

[24] S.-H. Liu and J. L. Haslam, *Biochemistry*, 1974, **13**, 3079.
[25] L. Schirch, *J. Biol. Chem.*, 1975, **250**, 1939.
[26] V. L. Makarov, V. M. Kochkina, and Yu. M. Torchinsky, *FEBS Lett.*, 1980, **114**, 79.
[27] V. L. Makarov, V. M. Kochkina, and Yu. M. Torchinsky, *Biochim. Biophys. Acta*, 1981, **659**, 219.
[28] M. H. O'Leary, H. Yamada, and C. J. Yapp, *Biochemistry*, 1981, **20**, 1476.

basic amines differ from their aromatic counterparts with respect to reaction kinetics. In certain alkaline conditions the reaction is independent of the catalyst concentration because the attack of the final carbonyl acceptor (A) is rate-limiting.[1,13,14] The secondary amines (*e.g.* proline or morpholine) are particularly effective catalysts. In this case the reaction rate depends on the concentration of the final carbonyl acceptor, the attack of which is thus rate-determining. The reaction rates are increased with decreasing pH because the acid accelerates the addition of secondary amine to aldehyde.[1,29]

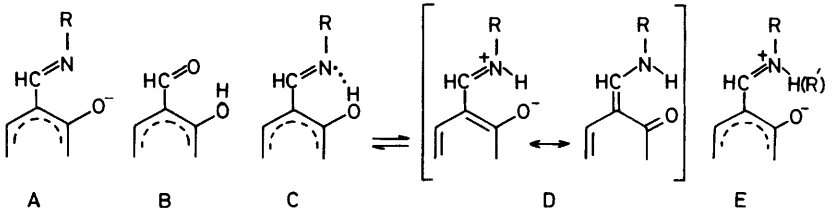
Efficient nucleophilic catalysis of the overall reaction between the carbonyl group and the acceptor amine generally requires that the following conditions are met[13,14,29–31] (see also Scheme 2): (i) The catalyst (C) must be more reactive towards substrate (B) than the final acceptor (A). (ii) The catalyst–substrate intermediate (C–B) must be more susceptible to the attack by the final acceptor than by the catalyst, or the equilibrium concentration of the possible non-reactive geminal diamine intermediate (another catalyst molecule attached to C–B[32]) must be low. (iii) The equilibrium constant for the catalyst substrate intermediate (C + B = C–B) must be smaller than that for the final product (C–B + A = A–B). In other words, catalysis of the reaction occurs if the formation of the intermediate (C–B) is kinetically rapid but is thermodynamically unfavourable so that the intermediate does not accumulate but, instead, reacts rapidly with the ultimate carbonyl acceptor to give products.[33]

The structures of all the reactants participating in transimination affect the observed rates and equilibria. Thermodynamic stability of the —C=N— linkage increases with the type of amine used, in the order NH_3 < aliphatic amines < aromatic amines < amines containing an adjacent electronegative atom with a free electron pair (H_2N—OH, for example). In contrast to the overall equilibrium constants, the rate and equilibrium constants for the formation of the tetraedric addition compound appear to be dependent on the basicity of the amine.[30] The structure and reactivity correlations of the compounds acting in the nucleophilic amine catalysis are comprehensively reviewed elsewhere.[1,30,34]

The reactivity of the intermediate imine (in the imino-group transfer reaction) is of central importance. The rate enhancement caused by the imine may at first seem surprising since the $\rangle C{=}N-$ group is less reactive than the $\rangle C{=}O$ group. The greater basicity of the $\rangle C{=}N-$, however, permits the easy formation of highly reactive cationic imine (also called protonated imine,[29] immonium ion,[35] or iminium ion[2]), $\rangle C{=}NH^+-$.[1,30] The reaction of secondary amines to aldehydes necessarily produces imines carrying the positive charge. As stated by Cordes and

[29] W. P. Jencks and E. H. Cordes, in 'Chemical and Biological Aspects of Pyridoxal Catalysis', ed. E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi-Fanelli, Pergamon Press, Oxford, 1963, p. 57.
[30] P. Y. Sollenberger and R. B. Martin, in 'The Chemistry of the Amino Group', ed. S. Patai, Wiley Interscience, London, 1968, p. 349.
[31] E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, 1962, **84**, 832.
[32] R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, 1966, **241**, 5851.
[33] W. P. Jencks, ref. 4, p. 73.
[34] W. P. Jencks, ref. 4, p. 42.
[35] D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, 1963, **85**, 188.

Jencks, the order of reactivity of pyridoxal phosphate and its imines increases from A to E, shown in Scheme 3.[13,29] The reactivity order has been thereafter verified by



Scheme 3

pyridoxal analogues.[36] Pyridoxal compounds exist as two tautomers, *viz.* the enolimine and the ketoenamine (Scheme 3, C and D, respectively).[5,6,37] The resonance-stabilized form is predominant in polar solvents and obviously it is more reactive than the enolimine. The facile equilibrium between the tautomers may play a decisive role in the catalysis by pyridoxal enzymes.[37,38]

The chemical reactivity of pyridoxal imines originates from the electrophilic centre at the C-4′-atom; the greater its electrophilicity, the greater is its propensity to be attacked by a nucleophile.[39]

The efficiency of catalysis by primary amines in semicarbazone formation is considerably less with aromatic *o*-hydroxyaldehydes than with benzaldehydes. This is because internal hydrogen bonding with the former aldehydes effectively resists the generation of the reactive cationic imine.[14] It should be also noted that protonation of the pyridine nitrogen of pyridoxal imines may increase their reactivity up to 40—600-fold towards nucleophiles.[9,40]

4 Intermolecular Transimination Models

Intermolecular model systems having an adequate congruency with transimination by PLP enzymes should be restricted mainly to pyridine aldehydes or aromatic aldehydes containing an electronegative function at the *p*-position to the aldehydic group, and especially to their *o*-hydroxy-derivatives. Such models have been exclusively studied with pyridoxal[13,14,41,42] or PLP[11-14,32,43] as the aldehyde component. There remains a need to use simpler aldehydes in the later mechanistic framework.

In the early investigations one of the main problems was whether or not the final acceptor amine can form Schiff base by reacting directly with the catalyst–

[36] S. Shinkai, S. Shiraishi, and T. Kunitake, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 3656.
[37] M. Lehtokari, J. Puisto, R. Raunio, and T. Korpela, *Arch. Biochem. Biophys.*, 1980, **202**, 533.
[38] D. E. Metzler, *Adv. Enzymol.*, 1979, **50**, 1.
[39] W. Korytnyk, H. Ahrens, and N. Angelino, *Tetrahedron*, 1970, **26**, 5415.
[40] E. E. Snell and S. J. DiMari, 'The Enzymes', ed. P. D. Boyer, Vol. II, 3rd ed., Academic Press, New York, 1970, p. 335.
[41] Y. Matsushima, *Chem. Pharm. Bull.*, 1968, **16**, 2046.
[42] Y. Matsushima, *Chem. Pharm. Bull.*, 1968, **16**, 2151.
[43] N. D. Schonbeck, M. Skalski, and J. A. Shafer, *J. Biol. Chem.*, 1975, **250**, 5343.

substrate imine, and if so, what is the significance of the route. The reports unanimously agreed on the existence of the route, but its quantitation was not always based on exact results.

The rates of the reactions of amines with a free aldehyde and its Schiff base can be compared under certain fixed conditions, but this does not necessarily illustrate the role of nucleophilic catalysis. For example, the reaction of semicarbazide with PLP catalysed by primary amines is 30—50-times faster in the pH region (~11) where the acid–base catalysis of both reaction routes is insignificant[13] (see Figure 1). Comparison of the rates of any other pH region produces incommensurable values if reaction rate constants independent of acid–base catalysis are not calculated. The route of nucleophilic catalysis is shielded in that pH range from external acid-catalysis (whereas the reaction of PLP with semicarbazide is not, as seen in Figure 1) as a result of the internal hydrogen bonding within the Schiff bases.

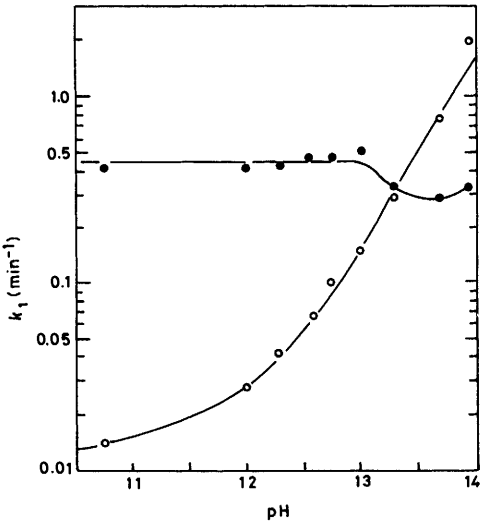


Figure 1 *First-order rate constants for pyridoxal phosphate semicarbazone formation from pyridoxal phosphate* (○) *and from the Schiff base of pyridoxal phosphate with methylamine* (●) *as a function of pH.* (Reproduced by permission from *Biochemistry* 1962, **1**, 776)

Cationic imines formed from secondary amines are good electrophiles and thus nucleophilic catalysis by them is effective (see Section 3). Morpholine catalysis is more effective in the case of PLP than of pyridoxal, with which the observed rate acceleration levels off when catalyst concentration is increased. This is due to the formation of the unreactive internal cyclic hemiacetal at high catalyst concentrations.[13,32,44–46]

[44] Y. Matsushima and A. E. Martell, *J. Am. Chem. Soc.*, 1967, **89**, 1322.
[45] F. Olivo, C. S. Rossi, and N. Siliprandi, in 'Chemical and Biological Aspects of Pyridoxal Catalysis', ed. E. E. Snell, D. M. Fasella, A. Braunstein and A. R. Fanelli, I.U.B. Symposium Series, Vol. 30, MacMillan, New York, 1963, p. 91.
[46] K. Nakamoto and A. E. Martell, *J. Am. Chem. Soc.*, 1959, **81**, 5857.

In contrast to the reactions involving semicarbazide, the reactions of amino-groups in cysteine or penicillamine were observed to take place 2—4-times faster with free PLP than with its 6-amino-caproic acid or glutamic acid Schiff bases.[12,43] It was thus concluded that any enhancement of PLP reactivity in enzymes is not caused *per se* by the existence of PLP as a lysine Schiff base adduct.[43] However, the comparison of the rate constants were made without specifying the catalytic mechanism and therefore a more precise formulaton of the results is needed.

Since the active sites of PLP enzymes are rather hydrophobic and since non-aqueous solvents favour Schiff base formation, it should also be advantageous to study the respective models in organic solvents such as methanol.[37] Conversion of pyridoxylidene glycinamide into pyridoxylidene glycine, with the aid of a large excess of glycine, can be followed by u.v. spectroscopy in methanol solution. The reaction with imine was much more rapid than the imine formation.[41,42] The spectral changes mentioned above can be understood in the light of the strong internal hydrogen bonding which is possible in the case of glycine under appropriately acidic conditions.[37] The significance of the geminal diamine route in methanol has been observed in another system, as well.[10]

5 Intramolecular Models of Transimination

Several alkylamines containing another nucleophilic function, such as NH_2, SH, or OH can form cyclic adducts (imidazolines, thiazolidines, or oxazolidines, respectively) with aldehydes and ketones. These adducts (especially those of diamines) resemble the intermediate geminal diamines within enzymic transimination. The intramolecular reactions are generally faster than the corresponding intermolecular ones, and are frequently so much faster that it is possible to observe those types of reaction that are involved in enzyme catalysis.[3] The fast rate of intramolecular transimination may be explained to result from a co-operation of nucleophilic catalysis and the entropic advantage caused by the proximity of the reacting groups.

An essential requirement for the formation of the cyclic adduct is that the group attacking the azomethine carbon can effectively function as nucleophile. Thus, cyclic products are usually observed only in relatively alkaline solutions.[6,7] The protonation state of the azomethine nitrogen also affects the reaction (see Section 3).

Condensation of a large number of aliphatic carbonyl compounds such as formaldehyde, glyoxalate, isobutyraldehyde, or acetone with diamines or amino-thiols has been studied (see *e.g.* references 47—50). Among aromatic aldehydes, benzaldehyde is known to form only Schiff bases with the bifunctional amines in aqueous solutions[9,51] and little evidence exists for the formation of cyclic adducts

[47] A. Hilton and D. L. Leussing, *J. Am. Chem. Soc.*, 1971, **93**, 6831.
[48] J. Hine and K. W. Narducy, *J. Am. Chem. Soc.*, 1973, **95**, 3362.
[49] R. G. Kallen, *J. Am. Chem. Soc.*, 1971, **93**, 6227.
[50] R. G. Kallen, *J. Am. Chem. Soc.*, 1971, **93**, 6236.
[51] D. J. Curran and S. Siggia, in 'The Chemistry of Carbon Nitrogen Double Bond', ed. S. Patai, Interscience, New York, 1970, p. 163.

with salicylaldehyde.[6,52] On the contrary, pyridine aldehydes as a rule undergo cyclization with diamines in aqueous alkali.[51] Five- and six-membered rings are produced most readily.[6,9,19] Equilibrium data for various cyclic transimination systems are presented in Table 1.

A. Thiazolidines and Oxazolidines.—In the earliest studies the reaction between cysteine and PLP was considered to proceed *via* the hemi-mercaptal pathway.[53,54] Later the key intermediate was kinetically shown to be Schiff base which was also the rate-limiting factor of the reaction.[11,12] Schonbeck *et al.*[43] determined pH-dependent kinetic constants for each step of the reaction of PLP with cysteine or penicillamine. The reaction between cysteine and formaldehyde obeys a similar reaction path as that of cysteine and PLP.[49,50]

Some oxazolidines are synthesized from PLP or pyridoxal with substituted β-amino-alcohols in non-polar solvents.[55] Although the pK values of the alcoholic hydroxy-groups are several orders of magnitude higher than those of thiols or amines it is proposed that their mechanisms are comparable.[50] It has also been suggested that serine could cyclize with PLP in aqueous solution.[54]

B. Imidazolidines and Comparable Heterocycles.—Pyridine-4-aldehyde is converted by treatment with 1,3-diaminopropane into a thermodynamically stable cyclic hexahydropyrimidine in aqueous alkali.[7,20] Under the same conditions PLP cyclizes rapidly but only partially with the diamine.[7] Metzler *et al.*[6] have disentangled, using log-normal analysis of u.v. spectra, the different structures and their ionic species formed when 5′-deoxypyridoxal (closely related to PLP) is allowed to react with 1,3-diaminopropane or ethylene diamine (EDA). Cyclization was predominant only in alkaline solution[6] (see Table 1). N.m.r. spectroscopic studies of this reaction where pyridoxal, instead of PLP, were used did not support the existence of the geminal diamines.[19] Possibly conditions were not sufficiently basic, because u.v. spectra indicate their presence in alkali.[10]

The intramolecular imine transfer between PLP and EDA has been the subject of the most thorough model-studies. The u.v. spectra of PLP–EDA solution in the pH range 7.5—14 show the existence of a rapid pH-dependent equilibrium between two reaction products, one of which is Schiff base and the other its C-4′ saturated derivative.[9] The magnitude of the association constant of the sp^3 hybridized component excludes the presence of a carbinolamine and therefore the adduct must be a geminal diamine.[56]

The predominant ionic and tautomeric equilibria[6,9] for the PLP–EDA system in aqueous solution at pH 7.5—14 are shown in Scheme 4. Strikingly, the ratio of geminal diamine to Schiff base was found to vary by a factor of less than 4 over the whole pH range. The geminal diamine prevails slightly over the open chain

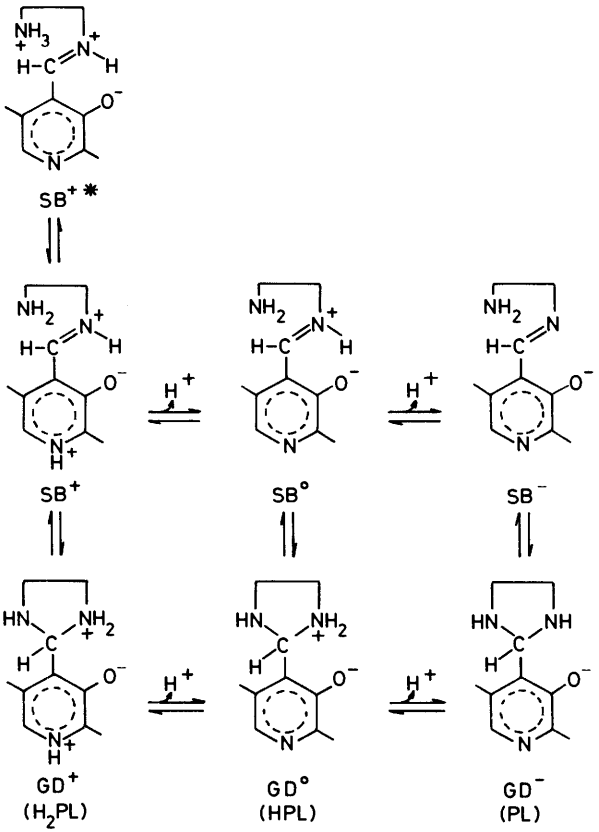
[52] R. S. McQuate and D. L. Leussing, *J. Am. Chem. Soc.*, 1975, **97**, 5117.
[53] F. Bergel and K. R. Harrap, *J. Chem. Soc.* (*C*), 1961, 4051.
[54] M. V. Buell and R. E. Hansen, *J. Am. Chem. Soc.*, 1960, **82**, 6042.
[55] J. M. Osbond, *Vitamins Hormones*, 1964, **22**, 389.
[56] E. G. Sander and W. P. Jencks, *J. Am. Chem. Soc.*, 1968, **90**, 6154.

Table 1 *Equilibrium data based on intramolecular transimination studies*

Reaction	*K*	*Remarks*	*Refs.*
Aminothiols			
HCHO + cys[a] = TC		Ionic strength 1.0 M, 25 °C	49
$K_1 = TC^+/cys^+ \times HCHO$	$2.24 \times 10^5\ M^{-1}$		
$K_2 = TC^\pm/cys^\pm \times HCHO$	$5.25 \times 10^5\ M^{-1}$		
$K_3 = TC^-/cys^- \times HCHO$	$1.14 \times 10^8\ M^{-1}$		
PLP + cys $\overset{K_1}{=}$ SB $\overset{K_2}{=}$ TC		Apparent values for pH 7.8 at 25 °C and anaerobic conditions	43
K_1	$2.60 \times 10^2\ M^{-1}$		
K_2	$6.00 \times 10^2\ M^{-1}$		
$K_{overall} = K_1 \times K_2$	$1.56 \times 10^5\ M^{-1}$		
Diamines:			
Isobutyraldehyde + EDA = GD		pH independent values, 35 °C	48
K_0[b]	$2.24 \times 10^3\ M^{-1}$		
K_1[c]	$6.58 \times 10^2\ M^{-1}$		
Isobutyraldehyde + *N*,*N*′-dimethyl-EDA = GD		pH independent values, 35 °C	48
K_0[d]	$7.99 \times 10^2\ M^{-1}$		
K_1[e]	$1.32 \times 10\ M^{-1}$		
HCHO + THF = cationic imine $\overset{K}{=}$ GD		pH independet constant, 25 °C, ionic strength 1.0 M, a model of serine transhydroxymethylase	9, 32
$K = (=NH^+-CH_2-N=)/(=N^+=CH_2)$	7.9×10^{-4}		
Salicylaldehyde + 1.3-DAP = SB $\overset{K}{=}$ GD		For the anionic form (pH moderately above 11.5)	52
K	1.5		
PLP + EDA $\overset{K_1}{=}$ SB $\overset{K_2}{=}$ GD		Apparent value for various mono-amines at pH 8.5	9
K_1	$1.2 \times 10^3\ M^{-1}$		
K_2 PL[f]	1.2	pH independent values, 25 °C, ionic strength 1.0 M	
HPL[f]	0.84		
H_2PL[f]	0.36[g]		
5′-deoxypyridoxal + EDA = SB $\overset{K_2}{=}$ GD		pH independent values, 25 °C, ionic strength 0.2 M	6
K_2 PL[f]	1.9		
HPL[f]	0.77		
5′-deoxypyridoxal + 1,3-DAP = SB $\overset{K_2}{=}$ GD		pH independent values, 25 °C, ionic strength 0.2 M	6
K_2 PL[f]	11		
HPL[f]	7		

[a] The upper indexes ($\pm$) refer to the ionic form of the compounds. [b] Values for $H_2N-(CH_2)_2-NH_2$. [c] Values for $^+H_3N-(CH_2)_2-NH_2$. [d] Values for $CH_3NH-(CH_2)_2-HNCH_3$. [e] Values for $CH_3NH_2^+-(CH_2)_2-HNCH_3$. [f] Refers to the ionic forms of Schiff bases (see Scheme 4). [g] Metzler *et al.*[6] assigned this value to be due to an open chain *trans*-structure shown in Scheme 5.

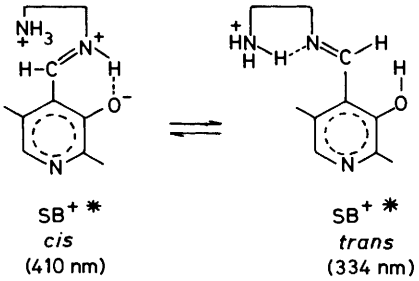
Abbreviations: cys = cysteine, TC = a thiazolidine-4-carboxylate, EDA = ethylene diamine, SB = Schiff base, GD = geminal diamine, THF = tetrahydrofolic acid, 1,3-DAP = 1.3-diaminopropane



Scheme 4

structures only in the anionic (PL) form. Near neutral SB^+ appears to be a minor tautomer while SB^+ is favoured by a factor of about 2 over GD^+.[9] Corresponding analysis of the system where 5′-deoxypyridoxal was used in place of PLP[6] agrees in outline with the above results (Table 1). Metzler *et al.*,[6] however, concluded that a hydrogen-bonded *trans* structure of the Schiff base (Scheme 5) rather than GD^+ was responsible for the absorption at 340 nm in neutral solutions as proposed by Tobias and Kallen[9] (Scheme 4). Since the equilibria of these systems are exceedingly complex, a more detailed study including temperature and solvent effects ought to be made.[6]

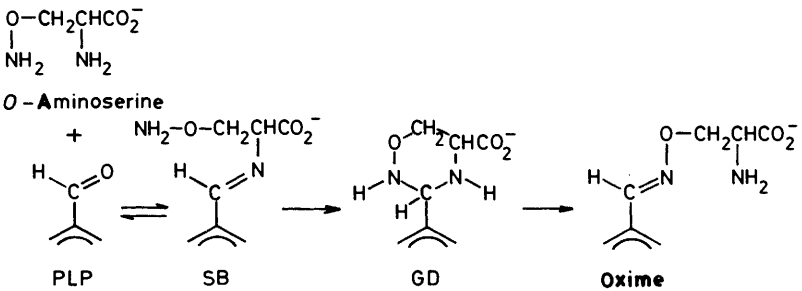
The rate of interconversion of ring-chain tautomers in the PLP–EDA system is about $4 \times 10^4 s^{-1}$ near pH 10 as measured by the temperature-jump relaxation method. Since the upper limit of the rate constant for the hydrolysis of the Schiff base is $2.7 \times 10^{-3} s^{-1}$ under the same conditions, the pathway *via* geminal diamine is favoured by a factor of at least 1.5×10^7 in this model reaction.[9] Comparison of the turnover numbers of PLP enzymes with those of the PLP–EDA model has led



Scheme 5

to the conclusion that there is no absolute need for apoenzyme to catalyse the transimination step.[9,57]

The reaction of *O*-amino-D-serine {2-amino-(3-amino-oxy)propionic acid} with PLP proceeds in alkali *via* the cyclic geminal diamine (GD) to oxime on the basis of kinetic, u.v., and n.m.r. data[10] (Scheme 6). The reaction rate of an (amino-oxy)-acetate model compound indicates that the oximation resulting from the direct attack of the (amino-oxy)function represents about 1 % of that proceeding through the GD pathway.[58] Because the decomposition of the geminal diamine is quite slow in alkali the overall oximation reaction of PLP by *O*-amino-D-serine can be divided into two parts and studied separately. Therefore, and because the nitrogen atoms of *O*-aminoserine are chemically unequal, the reaction is useful for studying the disintegration mechanism of geminal diamines. In particular, the model system parallels mechanisms by which amino-oxy compounds inhibit PLP enzymes.

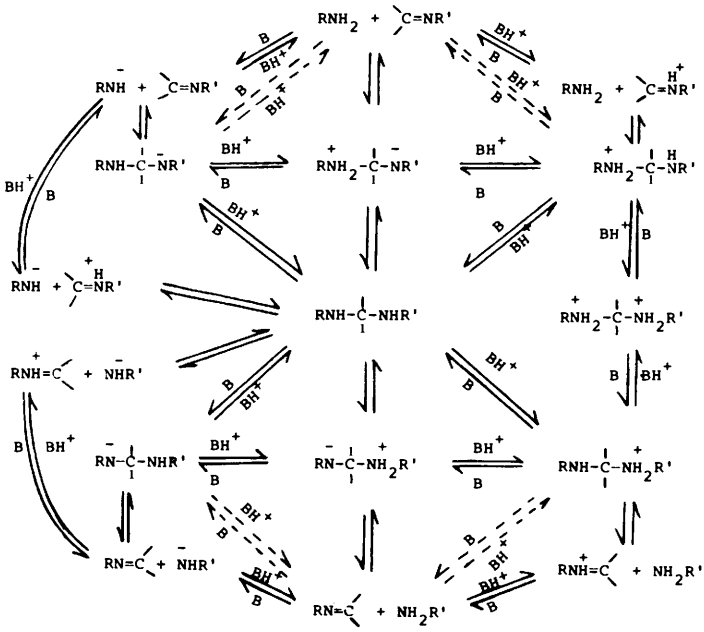


Scheme 6

6 Mechanisms of the Proton Transfers

Transimination is an apparently simple symmetric reaction, made complicated by the fact that two protons must be removed from the attacking amine and added to the leaving amine.[16,59] A summary of possible proton transfer mechanisms is shown in Scheme 7.[16]

[57] M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci., USA*, 1971, **68**, 1678.
[58] T. K. Korpela and M. J. Mäkelä, *Anal. Biochem.*, 1981, **110**, 251.
[59] K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, 1964, **86**, 2413.



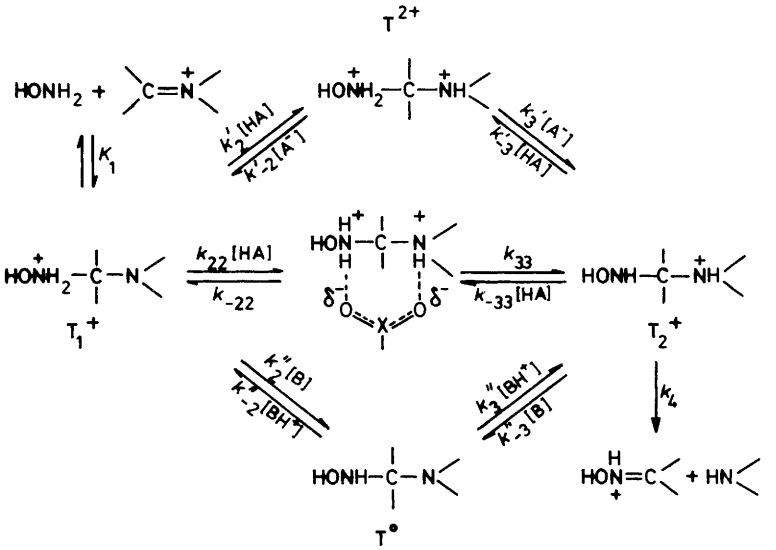
Scheme 7

(Reproduced by permission from *J. Am. Chem. Soc.*, 1977, **99**, 4772)

In the mechanistic studies of transimination the possibility that the catalysis might involve addition of a proton to the neutral imine (upper right corner in Scheme 7) has been eliminated by utilizing cationic imines obtained from secondary amines so that the fixed alkyl group serves as a model of mobile protons attached to the imine nitrogen. Correspondingly, the carbonyl reactant has been kept simple to avoid complicated equilibria. Hence pyridine aldehydes have not been considered in detailed mechanistic studies thusfar. Benzhydrylidenedimethylammonium (BHDA)[15,59,60] and *N*-*p*-methoxybenzylidenepyrrolidinium (MBP)[16] have acted as substrates for attack by hydroxylamine or *N*-methylhydroxylamine. The aim in using both hydroxylamine and *N*-methylhydroxylamine has been to discover if the removal of the second proton from hydroxylamine is included in the mechanism.[16]

The proton transfers of transimination may be accomplished through three different routes of catalysis, *viz.* by base, acid, or bifunctional mechanisms of catalysis[16] (Scheme 8). Observations favouring each of them in the reactions of the model cations are dealt with here. Aspects of the disintegration of geminal diamines as well as effects of solvents on the catalytic mechanism are also discussed.

[60] A. J. Kresge. *Pure Appl. Chem.*, 1981, **53**, 189.



Scheme 8

A. Base Catalytic Proton Transfer.—General base-catalytic proton transfers required for the hydroxylaminolysis of MBP or BHDA are shown in Scheme 8 (lower reaction path). The initial cationic addition intermediate (T_1^+) is generated through a rapid equilibrium stage, K_1. The rate-limiting step is the trapping of the adduct T_1^+ by proton removal (k_2'') in order to prevent reversion to starting materials. By contrast, the protonation and expulsion of the leaving amine (k_3'' and k_4, respectively) are fast.[15,16,60]

Support for the base catalysis in the oximation of BHDA by hydroxylamine comes from the Brønsted plot which is non-linear[15,60] and reflects the simple proton transfer between electronegative atoms in water.[61] In addition, the positive deviation of water from the Brønsted plot, a large solvent isotope effect, and a maximum in the solvent deuterium isotope effects for the catalysis by oxy-anions at the break point of the Eigen curve give additional evidence for the trapping mechanism.[15] The reactions of MBP with both hydroxylamine and *N*-methylhydroxylamine show the same type of base catalysis as the trapping mechanism. Only a ten-fold difference in the rates of the attacking amines indicates steric effects from the methyl group of *N*-methylhydroxylamine. It was not possible to obtain satisfactory Brønsted plots for these reactions because of a change in the rate-determining step with changing buffer concentrations.[16]

The deprotonation of T_1^+ will be largely or entirely rate-determining because T^0 will generally add a proton to its more basic nitrogen atom to give T_2^+ faster than it will revert to T_1^+ [15,16] (see Scheme 8). The alternative mechanism in which the

[61] M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 1.

rate-limiting step is the protonation of T^0 by a buffer acid (k_3'' in Scheme 8) is kinetically equivalent to the above mentioned mechanism.[15] The Eigen curve for simple proton transfer reactions shows a break when the pK values of the catalyst and substrate are equal.[61] The break point in the Brønsted curve at pK = 2.85 and the estimated pK values of 3.3 and 6.6 for T_1^+ and T_2^+, respectively, support the rate-limiting proton removal from T_1^+.[15]

B. Acid Catalytic Proton Transfer.—Acid catalysis of the transimination reaction of MBP with hydroxylamine of *N*-methylhydroxylamine can occur through intermediate T^{2+} as shown in the upper part of Scheme 8. General acid or concerted catalysis of the first step (K_1) of the reaction is improbable because of the absence of a basic site for such catalysis in a cationic imine. The initial protonation of T_1^+ will be largely or entirely rate-determining because T^{2+} will generally lose its most acidic proton to give T_2^+ faster than it will revert to T_1^+.[16] The change in rate-determining step with increasing buffer or hydrogen ion concentration from a step which is strongly catalysed to one which shows no evidence for catalysis is consistent with the above mechanism.[16]

In contrast to the hydroxylaminolysis of MBP, the transimination of BHDA shows no detectable proton catalysis.[15,60] The absence of the acid catalysis may be attributed to the difficulty of protonating the weakly basic hindered dimethylamino-group of the T_1^+ intermediate. This may be due to an interfering influence by the phenyl groups of BHDA on the approach of an acid to the dimethylamino-nitrogen. This likely lowers the pK_a of this group by providing an unfavourable non-polar environment to the protonated species with T^{2+}.[15]

As stated before, detailed proton transfer mechanisms for the transimination reactions involving pyridine aldehydes have not been reported. Generally, the addition of basic nucleophiles such as an alkylamine occurs to the cationic iminium species and elimination of basic amines from carbinolamines requires prior *N*-protonation.[62,63] For these reasons Tobias and Kallen[9] suggested specific acid-catalysis for both the ring opening and closing reactions involving the PLP–EDA system, at least in alkaline conditions. The amino-oxy group in *O*-aminoserine may cyclize to PLP–aldimine even without prior imine protonation, since this function is an exceptionally powerful nucleophile.[10]

C. Bifunctional Proton Transfer.—The unexpectedly high catalytic activity of cacodylic and carboxylic acids within the transimination of MBP with hydroxylamine suggests that the catalysts can trap the intermediate T_1^+ through a bifunctional mechanism in a single encounter with T_1^+ (centre path in Scheme 8). Also, catalysis by water presumably occurs through such a proton-switch mechanism. The proton transfer itself can occur either through the stepwise mechanism shown in Scheme 8 or by a concerted mechanism. In the stepwise

[62] W. P. Jencks, ref. 4, p. 463.
[63] E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, 1963, **85**, 2843.

mechanism the first of these steps (k_{22} in Scheme 8) is expected to be slower, as is the case in the acid catalytic pathway.[16]

Hydroxylaminolysis of BHDA shows no detectable bifunctional acid–base catalysis by carboxylic acids.[15] This results from the fact that the intermediate T_1^+ cannot rapidly isomerize to a structure in which both the proton donor and acceptor are on the same side of the molecule as a result of interactions between the two phenyl and two methyl groups in T_1^+.[15,64,65]

Consideration of all three mechanisms of catalysis has led to the generalization that for catalysis in a stepwise mechanism of transimination, the proton transfer steps that are immediately adjacent (in time) to the attack or expulsion of the less basic amine molecule will be rate-limiting.[16]

D. Decomposition of Geminal Diamines.—The decomposition of geminal diamines has been established to proceed with preferential elimination of the less basic amine and the formation of the more stable iminium cation under conditions of thermodynamic control, but there are ambiguities in the course of the reaction under conditions of kinetic control.[66]

In hydroxylaminolysis of MBP the concentration of T_2^+ is several orders higher than that of the intermediate T_1^+ when proton transfer is at equilibrium. The reactivity of T_2^+ is, however, about four orders of magnitude smaller (the difference is approximately the same as in the case of concentrations) compared to its counterpart. In spite of this it is probable that the k_1 rather than the k_4 step in Scheme 8 becomes rate-determining at high catalyst concentrations.[16] In the transimination of benzylidene-anilines attack and expulsion of the less basic aniline is rate-determining.[67] In many reactions protonation and expulsion of the more basic of two possible amine leaving groups is preferred.[68–70] However, the reduction of a model compound for methylenetetrahydrofolic acid gives a product with the methyl group on the more basic nitrogen atom[71] and the ring of methylenetetrahydrofolic acid opens on the side of less basic nitrogen.[72]

Moad and Benkovic[66] studied the kinetically preferred mode of breakdown of variously substituted geminal diamines. Each of them was shown to disintegrate with preferential expulsion of the less basic amine leaving-group. This was independent of pH in the range 2—11 and of whether or not the reaction involved the formation of an aromatic or aliphatic iminium cation. However, on the basis of these results it was not possible to predict the kinetic course of the decomposition of the intermediates participating in the transimination reactions involving aromatic Schiff bases.

[64] P. Deslongchamps, *Tetrahedron*, 1975, **31**, 2463.
[65] P. Deslongchamps, *Heterocycles*, 1977, **7**, 1271.
[66] G. Moad and S. J. Benkovic, *J. Am. Chem. Soc.*, 1978, **100**, 5495.
[67] Ref. 21, in J. L. Hogg, D. A. Jencks, and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 4772.
[68] W. P. Jencks, ref. 4, p. 542.
[69] S. J. Benkovic, T. H. Barrows, and P. R. Farina, *J. Am. Chem. Soc.*, 1973, **95**, 8414.
[70] W. P. Bullard, L. J. Farina, P. R. Farina, and S. J. Benkovic, *J. Am. Chem. Soc.*, 1974, **96**, 7295.
[71] T. H. Barrows, P. R. Farina, R. L. Chrzanowski, P. A. Benkovic, and S. J. Benkovic, *J. Am. Chem. Soc.*, 1976, **98**, 3678.
[72] H. F. Fife and J. E. C. Hutchins, *J. Am. Chem. Soc.*, 1976, **98**, 2536.

E. Solvent Effects on Mechanism.—The effects of solvents on model reactions may be important for understanding enzymic reactions because it is known, for instance, that the active site of aspartate aminotransferase resembles methanol solution rather than water in its polar properties.[23,37] A comparison of the solvent effects of glycerol, ethylene glycol, and methanol on the catalytic rate constants of the reactions of BHDA[15] or MPB[16] with hydroxylamine is consistent with the trapping mechanism but it does not provide strong support for that mechanism because of the uncertainties introduced by large solvent effects on the reaction rate. Glycerol causes a small increase in the observed rate constants but a much larger increase is observed with the less viscous compounds ethylene glycol and methanol. The smallness of the changes and the observation that these solvents have somewhat similar effects on the uncatalysed step at high buffer concentrations make it difficult to draw clear-cut conclusions.[15]

The differential stabilization of the addition intermediates T_1^+ and the transition states for their reactions relative to hydroxylamine and the cationic imines BHDA and MBP causes the rate increase in methanol and other organic solvents.[15] Some of the stabilization may reflect the stabilization of protonated 'alpha-effect' compounds, since the intermediate T_1^+ is a protonated hydroxylamine derivative.[73] In part, the stabilization may be due to the protons which are more favourably solvated on amine cations in alcohol than in water.[74] In addition, protonated imines are at least 10^3-times more labile than uncharged imines in the presence of organic solvents whereas the solvent effect on the pK_a of the parent amine[75] is small.

7 Transimination as a Part of Enzymic Reactions

Transimination is the common initial step in the mechanism of PLP-dependent enzyme reactions. The imine transfer *via* a geminal diamine is generally included in these reactions by 'logical necessity'[76] although experimental evidence for such is still scanty. Progress in studies of transimination is hampered by the lack of exact knowledge of the u.v. properties of geminal diamines and of other reaction intermediates in conditions (*e.g.* 'pH') at the active site. It has been supposed that the geminal diamines absorb at about 340 nm.[77] In fact, some PLP enzymes exist in non-physiological alkaline pH as substituted aldimines having a u.v. absorption at 340 nm.[78] β-Cyanoalanine causes a reversible change in absorption of aspartate β-decarboxylase from 360 to 310—320 nm (pH 5.5).[79] If the latter absorption was due to a geminal diamine structure (and not a ketimine as suggested[79]) it resembles the u.v. absorption of the adducts between PLP and diamines in aqueous alkali.[9,10]

[73] C. D. Ritchie, R. J. Minasz, A. A. Kamego, and M. J. Sawada, *J. Am. Chem. Soc.*, 1977, **99**, 3747.
[74] A. F. Trotman-Dickenson, *J. Chem. Soc.*, 1949, 1293.
[75] M. Brault, R. H. Kayser, and R. M. Pollack, *J. Org. Chem.*, 1978, **43**, 4709.
[76] E. E. Snell, *Adv. Enzymol.*, 1975, **42**, 325.
[77] C. M. Metzler, D. E. Metzler, D. S. Martin, R. Newman, A. Arnone, and P. Rogers, *J. Biol. Chem.*, 1978, **253**, 5251.
[78] M. H. O'Leary and W. Brummund, Jr., *J. Biol. Chem.*, 1974, **249**, 3737.
[79] S. S. Tate and A. Meister, *Biochemistry*, 1969, **8**, 1660.

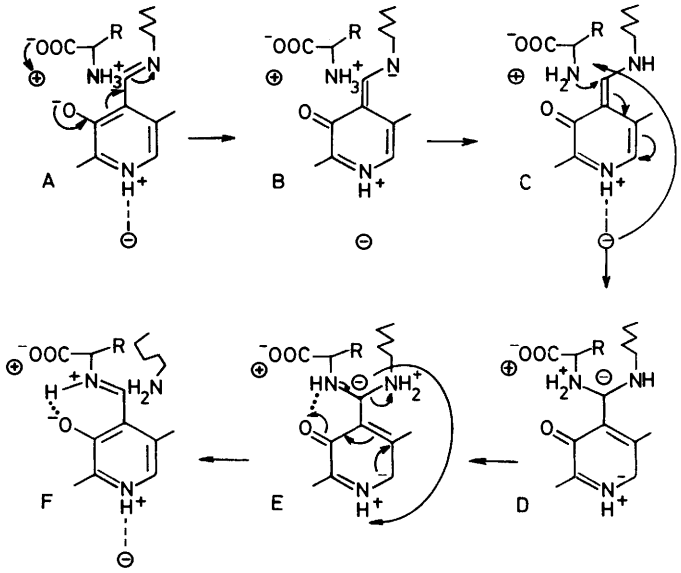
The first positive kinetic evidence for a geminal diamine intermediate was obtained with serine transhydroxymethylase on the basis of a transient absorption at 343 nm which was observed during the bimolecular phase of the reaction.[25,80-82] In addition, important experimental data on the transimination step exist for aspartate aminotransferase[83-85] and glutamate decarboxylase.[28,78] Two successive transimination steps can be considered to take place with δ-aminolevulinate dehydratase[86] and serine transhydroxymethylase.[32]

We describe now the hypothesis of enzymic transimination suggested by Ivanov and Karpeisky[87] and later supplemented by Metzler.[38] This model deals with aspartate aminotransferase but the main elements of the reaction step are presumably similar in almost all PLP enzymes.

Aspartate aminotransferase (AspAT) is the best studied PLP enzyme. It effects the reversible transfer of an amino-group from aspartate or glutamate to 2-oxoglutarate or to oxalacetate. Fully active AspAT ('α-form') is a dimeric protein containing two PLP molecules which lie symmetrically near the subunit interface.[88] Cytosolic and mitochondrial isoenzymes of AspAT are known. The coenzyme PLP is bound not only by the azomethine linkage but by ionic and hydrogen bonds *via* the phosphate, pyridine nitrogen, and phenolic hydroxy-group to appropriate groups in the protein.[87] The active site is somewhat shielded from the solvent and the chemical milieu therein resembles that in a methanolic solution.[23,37] In Scheme 9 (A) the azomethine bond is drawn to be in *trans*-conformation with respect to the phenolic hydroxy-group of PLP.[38] This conclusion was based on the u.v. properties of the coenzyme.[5] Recent *X*-ray studies, however, show that the conformation is *cis* in the low pH form of AspAT (D. E. Metzler, personal communication) and probably this is also true for the high-pH enzyme forms,[88] at least in the crystalline state. The azomethine bond of the 'idle' AspAT is buried in the protein[88] and is hence apparently protected in some degree from attacks of unspecific nucleophiles. A positively charged group on the apoenzyme lies near the 3-hydroxy-group of PLP imine which lowers the basicity of the imine nitrogen permitting it to occur in an unprotonated state above a p*K*-value of about 6.4.[38,87] A comprehensive survey of the structures of transaminase enzymes will appear in the near future.[89]

When a substrate molecule approaches the active centre (Scheme 9, A) and forms a Michaelis complex, the positively charged amino-group of the substrate (pK = 9.6) is attracted by the coenzyme ring which carries a negatively charged

[80] C.-F. Cheng and J. L. Haslam, *Biochemistry*, 1972, **11**, 3512.
[81] M. S. Chen and L. Schirch, *J. Biol. Chem.*, 1973, **248**, 7979.
[82] R. J. Ulevitch and R. G. Kallen, *Biochemistry*, 1977, **16**, 5355.
[83] P. Fasella and G. G. Hammes, *Biochemistry*, 1967, **6**, 1798.
[84] G. G. Hammes and J. L. Haslam, *Biochemistry*, 1969, **8**, 1591.
[85] G. G. Hammes and P. R. Schimmel, 'The Enzymes', ed. P. D. Boyer, Vol. II, 3rd edn., Academic Press, New York, 1970, p. 67.
[86] D. Shemin, 'The Enzymes', ed. P. D. Boyer, Vol. VII, 3rd edn., Academic Press, New York, 1972, p. 323.
[87] V. I. Ivanov and M. Ya. Karpeisky, *Adv. Enzymol.*, 1969, **32**, 21.
[88] G. C. Ford, G. Eichele, and J. N. Jansonius, *Proc. Natl. Acad. Sci., USA*, 1980, **77**, 2559.
[89] In 'Transaminases', ed. P. Christen and D. E. Metzler, Wiley & Sons, London, in preparation.



Scheme 9

(Redrawn by permission from *Advances in Enzymology*. 1979, **50**, 1)

phenolate group. Simultaneously the negative carboxylate pairs with a positive charge on the apoenzyme. Binding of the carboxylate markedly affects the phenolate ion whose negative charge is then distributed by resonance to the imino nitrogen, making the latter more basic. It has been proposed that its pK value increases from 6.2 to 8.0. On the other hand, neutralization of the negative charge on the carboxylate presumably lowers the pK value of the substrate amino-function from 9.6 to 7.7.[38,87] The reduced polarity inside the active site, as compared to the aqueous phase, destabilizes the zwitterion and further assists the deprotonation of the amino-function.

Non-enzymic model studies suggest that the addition of the substrate amino-function to AspAT takes place when the azomethine nitrogen of PLP imine is protonated (see Section 3). There is some uncertainty as to the conformation of the azomethine bond changes during the protonation.[27,88] In spite of the X-ray results mentioned above, Scheme 9 (B–C) still assumes the *trans*-form which does not change on protonation. The assumption can be excused because it is the most straightforward way for the substrate and lysine amino-groups to attack from opposing sides of the imine while keeping the rotation around the covalent bonds to a minimum. In addition, the partially protonated Schiff base (a *cis* form, see also Scheme 3, C or D) would be only slightly more reactive than the unprotonated Schiff base (Scheme 3, A). Another premise for maximal efficiency of the coenzyme at this reaction step is that its pyridine nitrogen be protonated (see Section 3).

With enzymes this requirement is usually fulfilled in the physiological pH region.[40,90]

What is the origin of the proton that is acquired by the imino-nitrogen? It is generally supposed from active-site mapping studies and by a consideration of possible changes in the pK values (see above) that the substrate amino-group donates a proton to the imine. This does not, however, exclude the possibility that this amino-proton is conserved while another function in a charge-transfer chain donates the proton.[28]

The transfer of the proton to the imine nitrogen (Scheme 9, B–C) generates an electrophilic centre on the imine carbon and favours rapid formation of the tetraedric geminal diamine (Scheme 9, C–D). The next step in the sequence is the elimination of the lysine residue from the adduct. As with the model reactions, this demands that one proton be transferred from the substrate nitrogen to lysine nitrogen (Scheme 9, D–E), probably with the aid of a mediator group. Metzler[38] proposed that the negative charge originally hydrogen bonded to the protonated pyridine nitrogen is transferred by a shuttle mechanism to a position near the azomethine and could hence serve as the mediator. Whenever the proton is transferred to the lysine nitrogen it separates from the adduct, probably by the action of the 3-hydroxyl of PLP as an intramolecular catalyst[91] (Scheme 9, E–F).

Changes in the hybridization at the aldimine carbon of PLP between sp^2 and sp^3 stages during the transimination are likely to require appreciable movements of the coenzyme with respect to the apoprotein part.[87] This is supported by considerable experimental evidence obtained with circular dichroism and polarized light spectroscopy.[26,27,77,87] A large deuterium solvent isotope effect also supports the occurrence of conformational changes during the interchange of the Schiff bases.[28] Views are widely scattered as to the detailed course of the rotations. The original hypothesis of Ivanov and Karpeisky[87] allows a 40° rotation around the C-2′—C-5′ axis of PLP to occur during the addition step. The same step for the rotation was suggested by Metzler.[77] According to Makarov *et al.*[26,27] the rotation proceeds predominantly in the protonation step (Scheme 9, B–C), but minor twists may take place in later transimination steps. A total angle change of 25° was suggested. Stereochemical aspects of PLP catalysed reactions have been treated recently by Floss and Vederas.[92]

It is apparent that the specificity elements of the substrate are important in 'opening' the structure of the enzyme toward substrate, even in the transimination step of the PLP reactions. With D-serine hydratase, for instance, the reaction is several million times slower if a carboxyl group is lacking within the substrate.[93] The X-ray studies being currently made will throw new light onto the mechanisms of PLP enzymes in the near future.[88]

[90] L. Davis and D. E. Metzler, 'The Enzymes', ed. P. D. Boyer, Vol. VII, 3rd edn., Academic Press, New York, 1972, p. 33.
[91] T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, 1965, **4**, 1463.
[92] H. G. Floss and J. C. Vederas, 'New Comprehensive Biochemistry', Vol. 3, ed. A. Neuberger and L. L. M. van Deenen, Stereochemistry, ed. Ch. Tamm, Elsevier Biomedical Press, Amsterdam, 1982, p. 161.
[93] C. S. Federiuk and J. A. Shafer, *J. Biol. Chem.*, 1981, **256**, 7416.

The challenging view of proper successive transfers of protons and tautomeric forms of PLP Schiff bases in driving and directing the catalysis in a plausible way has been clearly recognized.[38,87,94] The ability of a single coenzyme to catalyse such a large variety of reactions as PLP does, necessitates that it must be able to interact with catalytic 'hooks' provided by the protein part in many different combinations. The different rates of electron-transfer within conjugated systems, rotations of PLP imine bonds, proton transfers (extremely high rates of protons through 'tautomeric catalysis',[38] for example) and slower movements of the apoprotein are parameters that must all be compatible. An effective combination of them, which occurs in enzymes, could be more readily understood if the enzyme can use some kind of 'delay circuits' to achieve a correct timing of the successive catalytic events. It may be speculated that a wave movement in the subtle conformation of the protein network, the wave being reflected backwards after a suitable period of time, could be a method to achieve the time delay. A similar system could also be a means of conserving the binding energy between substrate and enzyme[95] until the energy is needed in the catalysis. Some correlation could appear between the size of an enzyme and the time from the formation of the Michaelis complex to the phase requiring the energy for the catalysis. An example of the possible operation of the proposed 'delay circuit' in the case of transimination reactions might be found in the time delay required for relocation of the 'mediator' charge[38] (see above).

The enzymic mechanism may be described as a 'series of rules'[96] or as a 'computerized program'. From an enzymological point of view it would be interesting to know whether the transimination step contains a 'run'-command to the enzyme to accomplish the whole catalysis, or if it is rather an autonomous 'subprogram'.

Acknowledgements. This article was made possible by a grant from *Magnus Ehrnrooth Foundation* to one of us (M. Mäkelä) which is gratefully acknowledged. We would like to express our deep appreciation to Professor David E. Metzler (Department of Biochemistry and Biophysics, Iowa State University, Iowa) for valuable advice and criticism of this work.

[94] T. C. Bruice, *Ann. Rev. Biochem.*, 1976, **45**, 359.
[95] W. P. Jencks, *Adv. Enzymol.*, 1975, **43**, 219.
[96] W. P. Jencks, *Adv. Enzymol.*, 1980, **51**, 78.