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The first steps. The attack on the carbonyl carbon of pyridoxal cofactor in pyridoxal-dependent enzymes

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Abstract—A study of the reaction between gaseous aldehydes and amines has implicated proton transfer from wall-associated water. Carbinolamine formation and subsequent dehydration to imine with assistance by wall associated hydroxyl bearing species has not previously been specifically suggested to obtain in the multitude of enzyme processes using pyridoxal cofactor. However, the data now available make it clear that imine formation in the active site of those systems requires *at least one water or hydroxyl bearing amino acid* for proton transfer.

Introduction. Somewhat more than three decades ago, Dunathan and Voet[1a,3a] summarized the many roles played by pyridoxal after it formed an aldimine with an amino acid. They concluded that the transfer of a hydrogen to the formerly aldehydic carbon of pyridoxal from the substrate on which it had reacted always occurred in the same absolute sense, interpreting the regularity as a consequence of evolution, and argued that '*...This regularity in protonation stereochemistry suggests a remarkable regularity in the geometry of cofactor binding to the apoenzyme.*' They then went on to suggest (based on a personal communication from D. Arigoni) '*...that the pyridoxyl phosphate—lysine—Schiff base is not held in a rigid geometry...*' (emphasis added). Thus the observed regularity of geometric factors in the amino acid aldimine occurs concurrent with, or subsequent to, diamine formation and release of the active site lysine (Scheme 1). The evolutionary theme was subsequently expanded upon by Christen and coworkers.[1b,3b]

If it is argued that the approach of pyridoxal cofactor and the active site lysine to each other follows a Bürgi-Dunitz[2] (or nearly so) pathway and, further, that the charges which result are ameliorated by proton transfers,[3] then it follows that both carbinolamine formation and the subsequent dehydration of carbinolamine to imine must take advantage of whatever is available on the walls of the active site to mitigate proton transfer. A cartoon process utilizing two (2) waters to this end is presented in Scheme 2.

Having found that surface involvement was necessary for proton transfer between gaseous amine and aldehyde reactants on the way to the imine[4] we were prompted to examine the likelihood of similar proton transfer in carbinolamine formation and dehydration in enzymatic systems. We report that investigation here.

Discussion. The reversible formation of imines[5] is part of the multifaceted chemistry of pyridoxal-dependent enzymes.[6] Currently, it is understood that the *internal* aldimine formed reversibly from pyridoxal and the active site lysine subsequently produces a 1,1-diamine by reversible addition of an amino acid. Reversible elimination of the active site lysine then yields the *external* aldimine. The latter, depending upon the details of the enzyme cavity, then suffers the fate that awaits it, be it proton loss from carbon leading to any of transamination, elimination, racemization, and condensation or decarboxylation.[7] The structural bases for the differing outcomes are a subject of intense investigation, as is active site restructuring in general.[8] Nonetheless, despite the understanding of what must happen in the enzymatic process and what is known about the overall gen-

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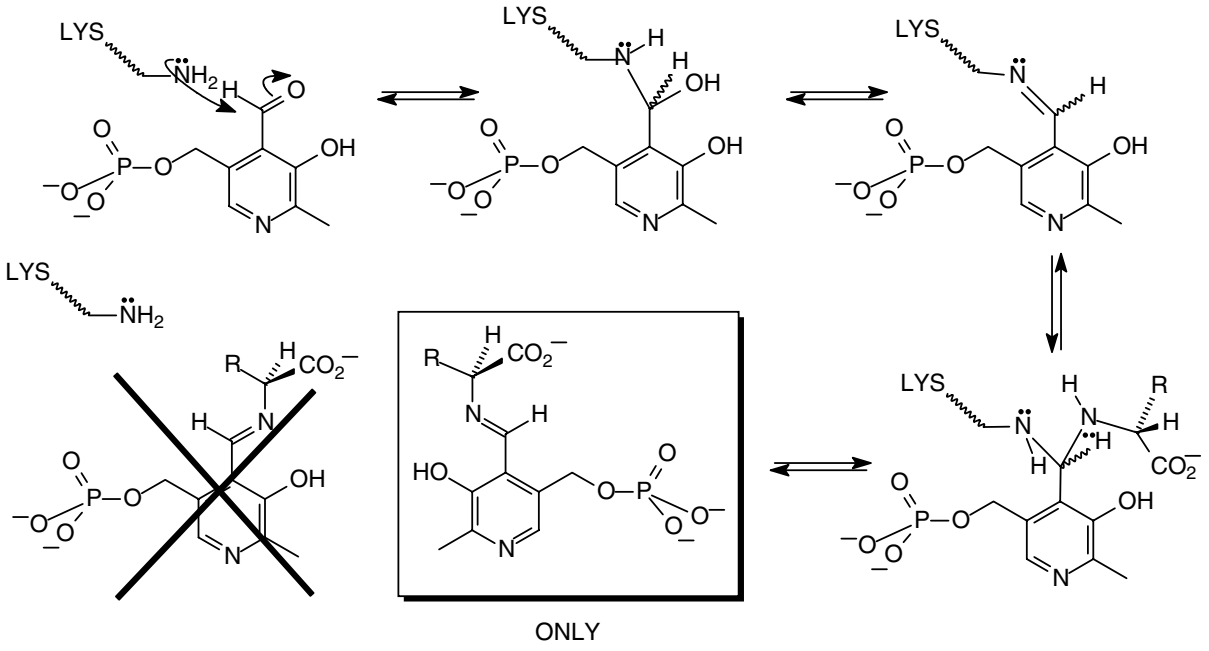
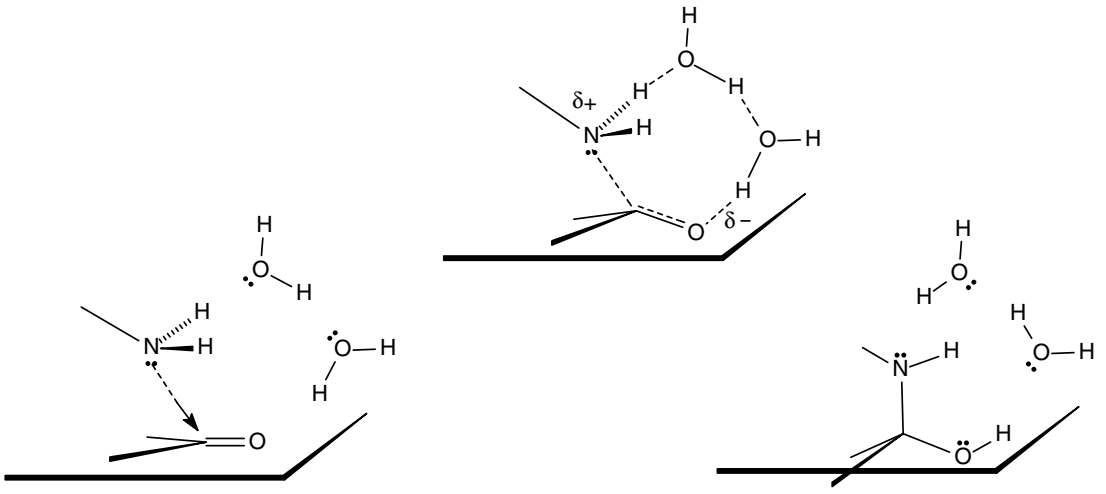


Scheme 1. A representation of the regularity in the geometry of cofactor binding to apoenzyme (after Ref. 1).



Scheme 2. A representation of carbinolamine formation following the Burgi-Dunitz pathway and using two (2) equivalents of water.

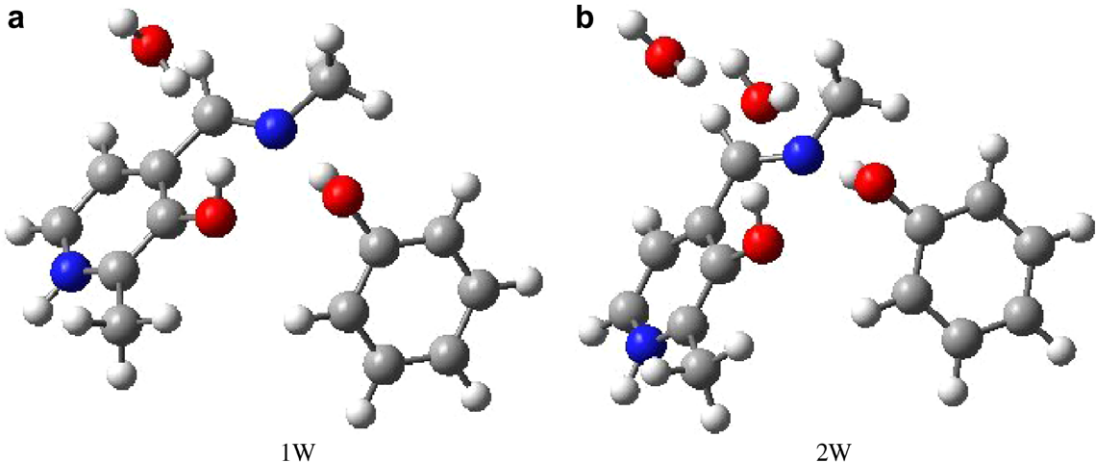


Figure 1. Internal aldimine and suggested positions of water eliminated/present critical to imine formation and assisted by TYP_{225}.

Table 1. Selected pyridoxal-dependent enzymes and residues that may be directly involved in imine formation

Enzyme (EC)	Source	PDB-ID[a]	Residue(s)	Atom distances (Å)
Fold type I				
Aminotransferase (AT) subclass I				
Aspartate AT (2.6.1.1)	Pig cytosolic	1AJS[b]	TYR_{225}	3.53 (O–N)
Aromatic AT (2.6.1.57)	*Escherichia coli*	3TAT[b]	TYR_{225}	3.65 (O–N)
L-Histidinol phosphate AT (2.6.1.9)	*Bacillus circulans*	1GEW[b]	TYR_{187}	3.40 (O–αC)
		1FG3[c]	TYR_{187}	3.20 (O–LYS:N)
			TYR_{110}	3.37 (O–Substrate:N)
		1GEY[d]	TYR_{187}	3.65 (O–LYS:N)
			TYR_{110}	3.42 (O–Substrate:N)
		1FG7[e]	TYR_{187}	3.42 (O–LYS:N)
			TYR_{110}	3.71 (O–αC)
AT subclass II				
Ornithine AT (2.6.1.13)	*Homo sapiens*	2OAT[d]	LYS_{292}	3.14 (N–N)
Dialkylglycine decarboxylase (4.1.1.64)	*Pseudomonas cepacia*	2DKB[b]	GLN_{246}	3.88 (N–N)
Glutamine-1-semialdehyde aminomutase (5.4.3.8)	*Synechococcus sp.*	4GSA[b]	THR_{305}	3.66 (O–αC)
Diaminopelargonic acid synthase (----)	*Escherichia coli*	1GJ3[e]	THR_{309}	3.67 (O–LYS:N)
Phosphoserine AT subclass				
Phosphoserine AT (2.6.1.52)	*Escherichia coli*	1BT4[b]	SER_{175}	4.59 (O–αc)
Tyrosine-phenol lyase subclass				
Tryptophan indole lyase (4.1.99.1)	Proteus vulgaris	1AX4[b]	SER_{263}	3.83 (O–N)
Cystathionine β-lyase subclass				
Cystathionine β-lyase	*Escherichia coli*	1CL1[b]	TYR_{111}	3.95 (O–αC)
Ornithine decarboxylase subclass				
Prokaryotic ornithine decarboxylase (4.1.1.17)	*Lactobacillus sp.*	1C4K[b]	SER_{352}	4.81 (N–αC)
Serine hydroxymethyl transferase subclass				
Serine hydroxymethyl transferase	*Homo sapiens*	1BJ4[b]	THR_{254}	4.13 (O–αC)
3-Amino-5-hydroxybenzoic acid synthase subclass				
3-Amino-5-hydroxybenzoic acid synthase	*Amycolatopsis Mediterranei*	1B9H[b]	SER_{183}	3.84 (O–αC)
Fold type III				
Alanine racemase (5.1.1.1)	*Bacillus stearothermophilus*	1SFT[b]	TYR_{43}	3.67 (O–αC)
Fold type IV				
D-Amino acid AT (2.6.1.21)	*Bacillus sp.*	2DAA[d]	TYR_{31}	3.86 (O-LYS:N)
Branched chain AT (2.6.1.42)	Mitochondrial	1EKF[b]	TYR_{207}	4.34 (O–N)
Fold type V				
Maltodextrin phosphorylase	*Escherichia coli*	1AHP[e]	THR_{641}	4.21 (O–αC)
Unclassified				
Cystalysin (2.6.1.5)	*Treponema denticola*	1C7N[e]	TYR_{64}	3.57 (O–αC)
Aldolase[f]				
D-2-deoxyribose-5-phosphate aldolase (4.1.2.4)	*Escherichia coli*	1JCL	LYS_{201}	3.41 (N–N)

[a] Leading references for the pdb's cited are given in Ref. 11.
[b] Internal aldimine.
[c] 1,1-Diamine.
[d] External aldimine.
[e] Pyridoxylamine.
[f] This enzyme is a Class I aldolase and the pdb is that of the carbinolamine formed from D-2-deoxyribose-5-phosphate and LYS 167.

eral pathway for imine formation and hydrolysis in solution,[5] the details of these reactions in biological systems remain unclear.

In this vein, it is generally assumed that proton transfer from the acidic pyridoxal aromatic ring phenolic hydroxyl, lying, as it does, *ortho* to the aldehydic function, is part of the driving force for the initial step of the condensation with the active site lysine amino group. Since the carbinolamine is the initial product, if the proton is completely transferred phenoxide ion forms and any resulting negative charge must be ameliorated by adjacent species. Meanwhile, the same must be true of the positive charge that has developed on the lysine amino group. And the two opposite charges remain widely separated within the cavity. Alternatively, if the proton is incompletely transferred from the phenolic hydroxyl to the aldehydic oxygen, the strongly basic alkoxide ion (for that is the fate of the carbonyl oxygen) must form. On the one hand, proximity argues for proton transfer from phenol to aldehyde while, on the other, charge separation argues against. Similarly, basicity argues for proton transfer and charge separation argues against.

We suggest as an alternative that the phenolic proton activates the carbon of the carbonyl by helping polarize it while proton transfer from wall associated water and/or hydroxyl groups intrudes.

Consider the case of the transaminase enzyme aspartate aminotransferase (AAT, EC 2.6.1.1, which we choose because X-ray data for the internal aldimine with 2-methylaspartate are widely available).[9] The truncated structure shown in Figure 1 as **1W** represents the active site lysine (LYS_{258}) and a nearby tyrosine (TYR_{225}) present in the crystal along with a water molecule (that one eliminated in the imine forming reaction) inserted so as to give a dihedral angle, H–N–C–OH, of about 49°, the value computations suggest would be expected in such an elimination were there assistance by TYR_{225}. Alternatively, were a second water involved, the depiction in Figure 1 as **2W** could obtain. The latter is preferred (of lower energy) by computations[4] and is shown with a dihedral angle (H-N-C-OH) of 75°.[10]

Clearly, making use of this idea requires the understanding that exact positioning of residues and water is not assured by crystal structures nor is it assured even by the affirmation that water is present in the active site. Nonetheless, we have extended this idea by an examination of the data available in the Protein Data Bank (PDB)[9] where we searched for those structures that utilize pyridoxal as its internal aldimine and an external aldimine/ketimine.[11] Again, although we recognize the uncertainties present in interpreting enzyme crystal structures with active sites that have been altered (inadvertently or otherwise) by remote site substitution,[12] and we are aware of the further uncertainties of water location in operating enzymes, we found it possible to locate an amino acid active site residue that could potentially assist in the addition–elimination reactions involving lysine and pyridoxal in a host of systems. These are collected in the Table 1 (using the organization scheme of Schneider, Käck, and Lindqvist).[13]

We thus conclude that the enzymes processing pyridoxal to the internal aldimine do so with the use of a cyclic pathway involving an appropriately situated amino acid residue such as TYR_{225} in AAT and use an additional water molecule for both the initial addition to carbinolamine and the subsequent elimination of water. Further, we suspect that the large number of additions and eliminations involving biological imines employs this simple cyclic motif and that, once again, the regularity of this process has historical implications.

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10. The structure (**1ajs**) was downloaded from the PD[11] and viewed using software from Accelrys, Inc., San Diego, CA (DSViewerPro). After expansion of the view to the internal imine and tyrosine (TYR_{225}), the result was transferred to GaussView (GaussView, Version 2.0, Roy Dennington II, Todd Keith, John Millam, Ken Eppinnett, W. Lee Hovell, and Ray Gilliland, Semichem, Inc., Shawnee Mission, KS, 2003). Once in GaussView, the water (or waters) shown was (were) added and the position(s) adjusted to produce the stated

dihedral angles with typical hydrogen bonding distances of 270–280 pm.

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