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CONFORMATION AND REACTION SPECIFICITY IN PYRIDOXAL PHOSPHATE ENZYMES

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The important biochemical role of molecules with extensive pi-electron systems has long been recognized. These molecules constitute the bulk of known enzyme cofactors and, as such, participate in every variety of biochemical reaction. The mechanism of action of these cofactors often reflects the influence of their pi systems on the chemical properties of contiguous sigma bonds.

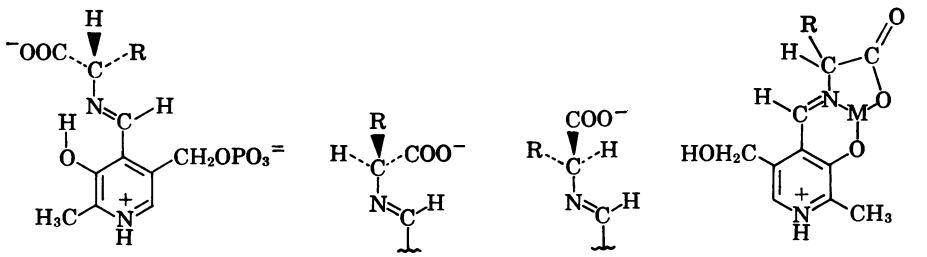
The pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP) forms of vitamin B_6 function in this way. The hypothesis of Snell[1] and of Braunstein[2] concerning the mechanism of action of the B_6 cofactors is well supported by numerous studies of the last 10 years.[3–5] This mechanistic picture emphasizes the function of the cofactor in weakening the sigma bonds around a carbon atom, usually the α-carbon of an amino acid, which is adjacent to the cofactor pi system. This labilization can be explained in two ways, one emphasizing the equilibrium position by considering the gain in delocalization energy in the product,[6] the other emphasizing the kinetic process by focusing on the transition state for bond breaking.

An important factor in the "activation" of sigma bonds by a pi system is the stereochemical relationship of the sigma bond to the adjacent pi orbitals. Early work by Corey[7] on the stereochemistry of enolization of a steroidal ketone demonstrated a clear preference for the geometry in which the α-carbon to hydrogen bond lies in a plane perpendicular to the plane defined by the carbonyl group and α-carbon. Recent work has reaffirmed the validity of this principle.[8, 9] This experimental result is supported by calculations which predict maximum ground state sigma-pi interaction when the geometry is that described.[10] This interaction will increase in the transition state where the geometry approaches that of the coplanar product.

In a common step of all PLP enzyme reactions the pi system of a Schiff base formed between PLP and an amino acid is extended by loss of a group from the amino acid α-carbon. This is accompanied by an important increase in the pi system's delocalization energy.[6] If this gain in delocalization energy is to aid the bond breaking process, the transition state must assume a geometry which places the bond to be broken in a plane perpendicular to that of the pyridoxal imine system. Thus the geometric requirements for effective sigma-pi interaction should be applicable to the whole group of B_6 enzymes.

The pyridoxal phosphate-amino acid imine (I) is shown in a conformation about the α-carbon to nitrogen bond which places the α-carbon to hydrogen bond in a

position to be activated by the pyridoxal phosphate pi system. This conformation, or one differing from that shown by a rotation of 180° about the α-carbon to nitrogen bond, is appropriate to transamination, α,β-elimination, β,γ-elimination, β-decarboxylation, and various synthetase reactions.[11] The conformation (II) is appropriate to loss of R, as in serine hydroxymethylase or threonine aldolase, and conformation (III) for α-decarboxylation.



I II III IV

Although nonenzymatic model reactions have been found which correspond to each of these reaction types, the model reactions in general proceed via imines of pyridoxal in which no control is exercised over the α-carbon to nitrogen bond conformation.[3] It is not surprising that in some cases the model system shows several reactivities. When metal ions are included in model systems, rates of transamination and of dehydroxymethylation are increased, while decarboxylation is very much inhibited. It has been suggested that complex formation between the pyridoxal imine and a metal ion enhances the first two reactions by an inductive effect of the metal ion, while decarboxylation is inhibited due to metal ion-carboxylate bonding.[12] The proposed complex (IV) has ideal geometry for transamination or dehydroxymethylation since the appropriate conformations are readily accessible to the ligand ring. However, the carboxylate group is held near the plane of the PLP ring, a geometry unfavorable for decarboxylation and undoubtedly an important factor in the observed inhibition. In a study of pyridoxal catalyzed decarboxylation and condensation reactions of aminomalonate, Matthew and Neuberger report that cupric ion increases the rate of decarboxylation.[13] This is to be expected since the pyridoxal imine chelate of aminomalonate has a carboxylate group which can assume the appropriate geometry for decarboxylation.

In the enzymatic reactions control of the conformation about the α-carbon to nitrogen bond must be an important function of the apoenzyme in providing reaction specificity. We assume that the position of the enzymatic site binding the carboxylate anion of the amino acid will be the most important factor in this control. In Figure 1 the PLP imine is shown in views along the carbon-nitrogen bond. In each of the three conformations (a), (b), and (c), the relationship of the carboxylate binding site (E^+) to the plane of the PLP ring is different. We will assume in the discussion that follows that the geometric relationships of Figure 1 are rigidly fixed in most PLP enzymes.

This view of PLP enzyme-substrate stereochemistry allows certain predictions to be made. If a change is made in the configuration at the α-carbon of the amino acid and if the carboxylate binding site continues to control the conformation about the α-carbon to nitrogen bond, a different group on the α-carbon may be subject to the

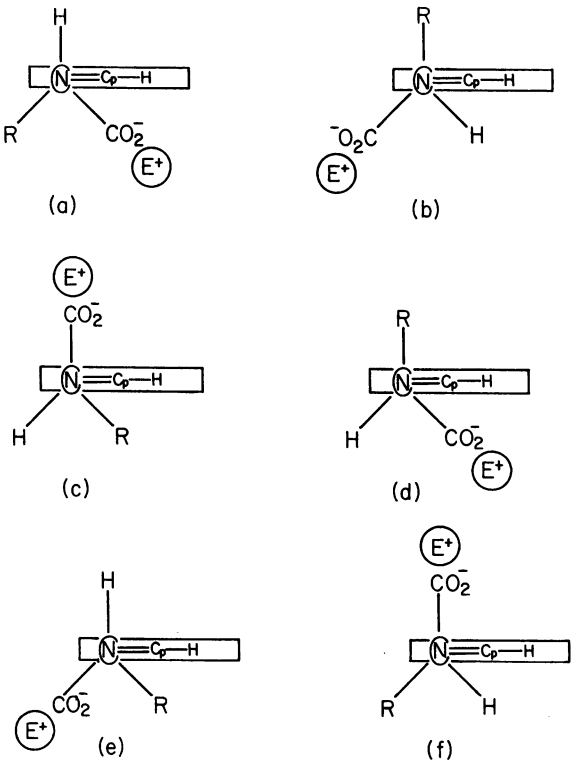


FIG. 1.—Various conformations of a pyridoxal amino acid imine as viewed along the α-carbon to nitrogen bond. The aldehydic carbon of PLP is shown (C_p) and the atoms of the pyridine ring are indicated by the rectangular box.

activating influence of the PLP ring. Thus, in Figure 1 a change in the amino acid configuration of (*a*) will lead to (*d*) in which the *R* group occupies the activated position, and in (*b*) a configuration change yields (*e*), the geometry appropriate for transamination. However, the carboxylate group remains in the activated position when the configuration is changed as in (*c*)–(*f*). It follows that a change in the configuration of a PLP enzyme's amino acid substrate may lead to catalysis of a different reaction by that enzyme. The enzymes with aldolase reactivity, L-serine hydroxymethylase and L-threonine aldolase, may show transaminase reactivity with appropriate D-amino acids; conversely, L-transaminases may show aldolase reactivity with appropriate D-amino acids. The latter cross-reactivity will be difficult to observe, since transaminases lack a binding site for the tetrahydrofolic acid cofactor.

A single cross-reactivity of this kind has been observed by Schirch and Jenkins in the transamination of D-alanine with L-serine hydroxymethylase.[14] The transamination itself is a very slow reaction; however, exchange of the alanine α-hydrogen is rapid in the presence of tetrahydrofolic acid. This indicates that the α-proton of D-alanine has the proper geometry for activation, even though other factors slow the over-all transamination.

Dual reactivities have been observed in other pyridoxal phosphate enzymes,

but in these cases both reactivities are the result of labilization of the same group. For example, aspartic β-decarboxylase is slowly inactivated in the presence of substrate by the conversion, through transamination, of its pyridoxal phosphate cofactor to the inactive pyridoxamine phosphate form.[15] Both the normal β-decarboxylation and the spurious transamination depend on labilization of the amino acid α-proton. The reaction paths differ in the stage at which hydrolysis of the cofactor-amino acid complex occurs, the spurious transamination resulting from a premature hydrolysis of the ketimine form of the Schiff base.

In contrast to the β-decarboxylase just described, an α-decarboxylase should never show reactions involving labilization of groups other than the carboxyl group. Although no such reactions have been reported, leucine decarboxylase has been observed to suffer inactivation in the presence of substrate.[16] A possible explanation would involve a spurious transamination yielding 2-keto-4-methylpentanoic acid and pyridoxamine phosphate or, if transamination accompanies decarboxylation, isovaleraldehyde and pyridoxamine phosphate. The former case, but not the latter, would represent a violation of the predicted reaction specifity of α-decarboxylases. However, recent work seems to indicate that the inactivation is due to cofactor dissociation rather than transamination.[17]

The stereochemical relationship between PLP and the carboxylate binding site in α-decarboxylases is "symmetric" in that the carboxylate site lies directly above the plane of the PLP ring. This leads to the prediction that α-decarboxylases should never show other reactivities since only the COO^- group can occupy the "activated" position. It also suggests that, among PLP enzymes, α-decarboxylases should be best able to accept as substrates amino acids of unnatural configuration. In Figure 1*c* and *f*, R and H exchange positions at the active site while the carboxylate anion remains in the activated position. In the case where R is nonpolar and/or small, this exchange may not prevent the binding and reaction of the amino acid of unnatural configuration. This lack of configurational specificity seems to be a property of the decarboxylase activity in a Pseudomonad studied by Dempsey.[18]

Several amino acid racemases appear to utilize PLP as a cofactor.[19, 20] These enzymes provide a path for interconversion of D and L amino acids which involves labilization of the α-proton.[20] The mechanism for PLP enzymatic racemization suggested by Snell[1] requires labilization by PLP of the α-proton of both the D and L amino acids and thus represents a violation of the view of PLP enzyme stereochemistry presented here.[21] However, it is not necessary that these enzymes catalyze the removal of the α-proton from the D and L isomers at the same rate. This is equivalent to the statement that reprotonation at the α-carbon need not yield L and D configurations with the same probability.

It is our belief that these racemases represent a class of B_6 enzymes which exhibit a high but not absolute stereospecificity in the labilization of the α-hydrogen. In terms of active site geometry, this implies a somewhat flexible conformational relationship between cofactor and substrate which provides a relatively high energy path for proton addition to and abstraction from one enantiomer and a low energy path for the other more "natural" enantiomer. Thus, with one enantiomer the rate of loss of α-proton should far exceed the rate of racemization. A comparison of the turnover numbers of an efficient transaminase and of glutamic acid racemase in-

dicates that this is possible if one assumes that the racemase can approach the efficiency of a transaminase in removing the α-proton from the most reactive substrate.[22] This hypothesis can be easily tested by observing the rate of loss of α-deuterium or tritium compared to the rate of racemization for the D- and L-amino acids. In the most extreme case, the rate of loss of label would equal the rate of racemization for one isomer while the rate of loss of label would be vastly greater than the rate of racemization for the other. One might predict that the D isomer would fit the first pattern and the L isomer the second pattern if racemases are to be considered "primitive" enzymes which have in part followed an evolutionary path toward complete L specificity.

This view of the active site stereochemistry of PLP cofactor enzymes should be of use as a working hypothesis relating reactivity and stereochemistry in the large family of B_6 enzymes. In addition, it allows formulation of more specific questions concerning the detailed mechanism of action of these enzymes.

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[22] A crude calculation can be made, based on data reported by Glaser.[20] If one assumes that his best preparation of racemase was 50% pure, and has a molecular weight of 100,000, the calculated turnover number is almost two orders of magnitude less than that of glutamic-aspartic transaminase (Banks, B. C. E., *et al.*, *J. Chem. Soc.*, 4235 (1961).