# A Stereochemical Aspect of Pyridoxal 5'-Phosphate Dependent Enzyme Reactions and Molecular Evolution

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**Abstract**   We have studied the stereospecificities of various pyridoxal 5'-phosphate (PLP) dependent enzymes for the hydrogen transfer between the C-4' of a bound coenzyme and the C-2 of a substrate in the transamination catalyzed by the enzymes. Stereospecificities reflect the structures of enzyme active-sites, in particular the geometrical relationship between the coenzyme-substrate Schiff base and the active site base participating in an α-hydrogen abstraction. The PLP enzymes studied so far catalyze only a *si*-face specific (*pro-S*) hydrogen transfer. This stereochemical finding suggests that the PLP enzymes have the same topological active-site structures, and that the PLP enzymes have evolved divergently from a common ancestral protein. However, we found that D-amino acid aminotransferase, branched chain L-amino acid aminotransferase, and 4-amino-4-deoxychorismate lyase, which have significant sequence homology with one another, catalyze a *re*-face specific (*pro-R*) hydrogen transfer. We also showed that PLP-dependent amino acid racemases, which have no sequence homology with any aminotransferases, catalyze a non-stereospecific hydrogen transfer: the hydrogen transfer occurs on both faces of the planar intermediate. Crystallographical studies have shown that the catalytic base is situated on the *re*-face of the C-4' of the bound coenzyme in D-amino acid aminotransferase and branched chain L-amino acid aminotransferase, whereas the catalytic base is situated on the *si*-face in other aminotransferases (such as L-aspartate aminotransferase) catalyzing the *si*-face hydrogen transfer. Thus, we have clarified the stereospecificities of PLP enzymes in relation with the primary structures and three-dimensional structures of the enzymes. The characteristic stereospecificities of these enzymes for the hydrogen transfer suggest the convergent evolution of PLP enzymes.

**Key words:** Pyridoxal phosphate, aminotransferase, amino acid racemase, amino deoxychorismate lyase, stereochemistry, molecular evolution, stereobiochemistry

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Pyridoxal 5'-phosphate (PLP) enzymes participate in various significant reactions of amino acid metabolism: PLP is the most versatile coenzyme, and catalyzes a variety of reactions including transamination, decarboxylation, racemization, aldol condensation, α,β-elimination and the β,γ-elimination of amino acids [5, 9]. The chemical mechanism for PLP enzyme reactions was first studied in the early 1950s by Metzler *et al.* [34] and Braunstein *et al.* [4]. Based on stereochemical studies from the standpoint of molecular orbital theory, Dunathan and Voet (1974) proposed that PLP enzymes have evolved from a common ancestral protein [10]. More than 300 PLP enzymes have been identified and characterized, and primary structures of many of them have been determined. Recently, the three-dimensional structures of about 20 different kinds of PLP enzymes have been shown. This structural information on PLP enzymes has accordingly enabled discussion on their molecular evolution. Christen and his coworkers divided aminotransferases into four subgroups on the basis of their primary structures, hydropathy patterns, and secondary structures [33]. They categorized PLP enzymes into three families (α, β, and γ) on the basis of their regio-specificities [1]. More recently, Grishin *et al.* (1995) classified PLP enzymes into seven fold types using a highly progressed protein alignment method based on their structural and hydrophobicity characteristics [16]. Comparison of amino acid sequences and protein folds has shown that PLP enzymes are of a multiple evolutionary origin (Table 1).

The stereospecificities of PLP enzymes which catalyze transamination for a hydrogen transfer between a coenzyme and a substrate coincide well with their primary structure characteristics. The stereochemistry of a reaction is one of criteria of molecular evolution. In this paper, we review the stereospecificities for the hydrogen transfer of PLP enzymes and the relationship between these stereospecificities, and the primary and tertiary structures.

**Table 1.** Families of PLP enzymes with reported three-dimensional structures.

| Fold type[a] | Enzyme | Family[b] | Stereospecificity | Reference |
|---|---|---|---|---|
| I | L-Aspartate aminotransferase | α | *si* | [28] |
| | Ornithine aminotransferase | α | *si* | [43] |
| | ω-Amino acid: pyruvate aminotransferase | α | | [57] |
| | Dialkylglycine decarboxylase | α | | [55] |
| | Prokaryotic ornithine decarboxylase | | | [37, 38] |
| | Tyrosine phenol-lyase | α | | [3] |
| | Tryptophanase | α | *si* | [23] |
| | Cystathionine β-lyase | γ | | [7] |
| | Glutamate-1-semialdehyde aminomutase | | | [17] |
| | Aromatic amino acid aminotransferase | α | | [40] |
| | Phosphoserine aminotransferase[c] | α | *si* | |
| | Serine hydroxymethyltransferase | α | *si* | [41] |
| | 8-Amino-7-oxononanoate synthase | α | | [2] |
| | Cystathionine-γ-synthase | | | [8] |
| II | Tryptophan synthase β subunit | β | *si* | [19] |
| | Threonine deaminase | | | [13] |
| | O-Acetylserine sulfhydrylase | β | | [6] |
| III | Alanine racemase | | both | [42] |
| | Eukaryotic ornithine decarboxylase[d] | | | |
| IV | D-Amino acid aminotransferase | | *re* | [48] |
| | Branched-chain L-amino acid aminotransferase | | *re* | [39] |

[a]Folds of PLP enzymes as defined by [16].
[b]Family of PLP enzymes as defined by [1].
[c]PDB coordinate was released as the code number 1BT4. The title is 'Phosphoserine Aminotransferase from *Bacillus circulans* subsp. *Alkalophilus*' and the authors are G. Hester, T. N. Luong, M. Moser, and J. N. Jansonius.
[d]Grishin, N. V. *et al.* 1998. *Abstr. Annual Meet. Protein Society*, San Diego, USA, p. 144.

## Mechanism of Aminotransferase Reactions and the Stereochemistry of Their Hydrogen Transfer

The general mechanism of aminotransferase reactions is shown in Fig. 1. PLP binds to the enzyme protein through a Schiff base (internal Schiff base) formation with the ε-amino group of an active-site lysyl residue (Fig. 1A). The pyridine nitrogen of the bound PLP is then protonated in usual physiological conditions. The addition of a substrate amino acid to the holoenzyme causes a transaldimination forming the external Schiff base with the substrate (Fig. 1B). The next step is crucial to determine the kind of reactions. One of the three bonds belonging to the α-carbon atom of the amino acid moiety is cleaved, and an anionic intermediate is formed (Fig. 1C). The scissile bond of the external aldimine intermediate must be oriented perpendicular to the coenzyme planar π-bonding system to maximize the σ-π orbital overlap in the transition state [9] as recognized by crystallographic studies of PLP enzymes [28, 37, 55]. The α-hydrogen is initially abstracted from the amino acid moiety of the external Schiff base complex (Figs. 1B, 1C, and 2) to form a planar intermediate (Fig. 1C). The abstracted hydrogen is then transferred to C-4' of the bound coenzyme to form a ketimine intermediate through an active-site base (Fig. 1D). The transfer of hydrogen between C-2 of the substrate moiety and C-4' of the coenzyme is characteristic of enzymatic transamination. The ketimine intermediate (Fig. 1D) is then hydrolyzed to

pyridoxamine 5'-phosphate (PMP) and a keto acid (Fig. 1E). A half reaction of transamination, i.e., the conversion of an amino acid to a keto acid, is thus completed. The other half reaction, the formation of a new amino acid from a keto acid including the regeneration of PLP, proceeds according to the reverse process.

During the process of hydrogen transfer to or from the C-4' of the coenzyme (Fig. 1C), three stereochemical possibilities exist: the hydrogen is either stereospecifically transferred on the *re-* or *si-*face of the plane (*re-* and *si-*faces are defined in relation to the C-4' of the coenzyme as the center of the plane) of the conjugated π-system of the cofactor-substrate imine, or transferred non-stereospecifically onto both faces (Fig. 3). In the aminotransferase reactions, the Cα-H bond that is either broken or formed is situated perpendicular to the pyridine ring as described above (Fig 2). The hydrogen transfer is regarded as a suprafacial transfer through a single catalytic base [9]. Accordingly, the *pro-S* hydrogen or *pro-R* hydrogen is transferred onto the *si-* or *re-*face, respectively. The stereospecificities of various aminotransferases for hydrogen transfer have been previously examined [10]. Although three manners of hydrogen transfer are possible, hydrogen is merely added or removed to or from the *si-*face in transaminations catalyzed by L-aspartate aminotransferase, L-alanine aminotransferase, dialkylamino acid aminotransferase, pyridoxamine pyruvate aminotransferase, L-glutamate decarboxylase, L-tryptophan
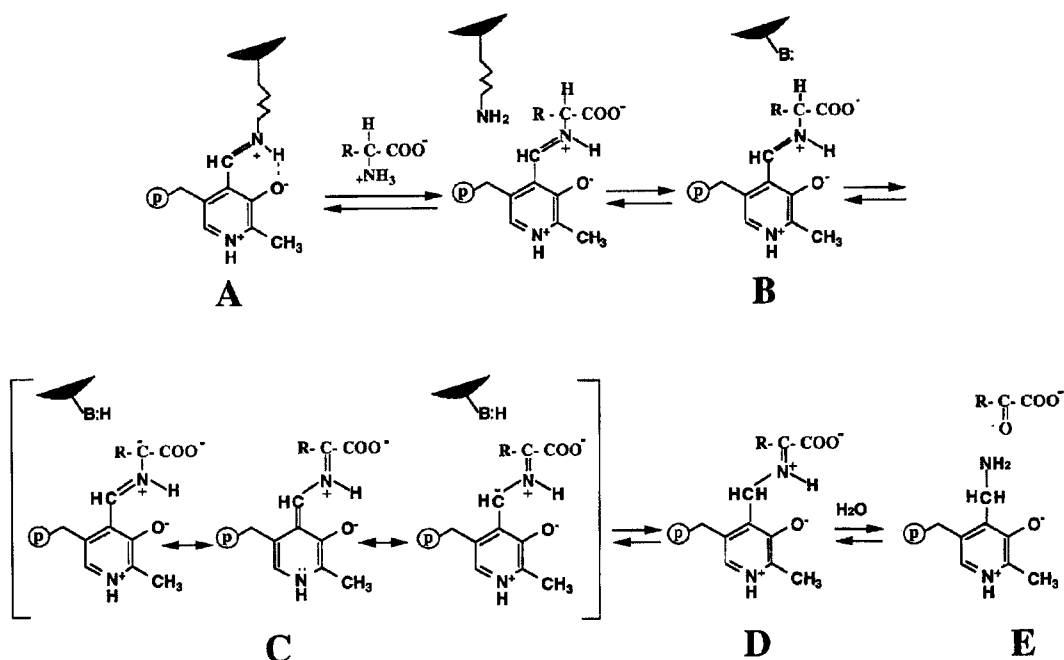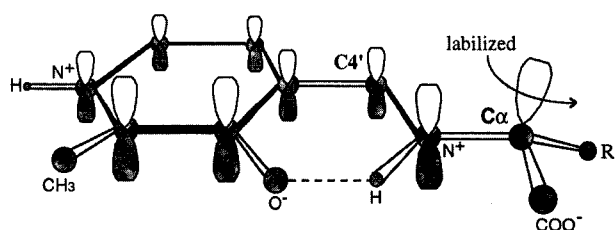
**Fig. 1.** Reaction mechanism of transamination.



**Fig. 2.** Specific orientation on the Cα-N bond in the external aldimine intermediate determines which bond is labilized.

In the reaction of transamination, the conformation in which hydrogen is held perpendicular to the plane of the pyridine ring maximizes orbital overlap between the nascent carbanion and the π system of the aldimine.
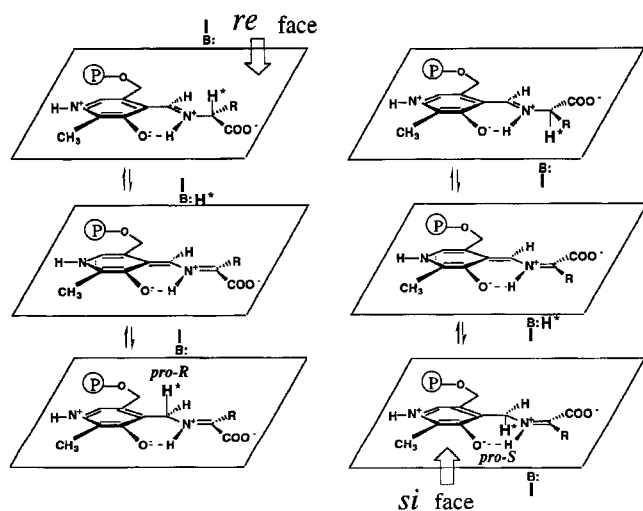


**Fig. 3.** Hydrogen transfer stereospecificity.

synthase, and L-serine hydroxymethyltransferase. The last three enzymes are not aminotransferases; however, they catalyze the transamination as a side reaction. These results suggest a similarity in the active site structure of these seven enzymes. Consequently, Dunathan proposed a hypothesis in which all PLP enzymes have most likely evolved divergently from a common ancestral protein [10].

**Evolution of Aminotransferases**

Aminotransferases, which form one of the largest groups of PLP enzymes, can be divided into four subgroups based on their primary structures [33]. Subgroup **i**: aspartate aminotransferase (AspAT), alanine aminotransferase, tyrosine aminotransferase, histidinol-phosphate aminotransferase, and phenylalanine aminotransferase; subgroup **ii**: ornithine δ-aminotransferase (OAT), acetylornithine aminotransferase, ω-amino acid:pyruvate aminotransferase (w-APT), γ-aminobutyrate aminotransferase, and 7,8-diaminopelargonate aminotransferase; subgroup **iii**: D-amino acid aminotransferase (D-AAT) and branched-chain L-amino acid aminotransferase (BCAT); and subgroup **iv**: serine aminotransferase and phosphoserine aminotransferase (PSAT). The (N-1) profile analysis suggests that aminotransferases belonging to subgroups (**i**), (**ii**), and (**iv**) are significantly similar to each other [33]. However, the primary structures of D-AAT [53] and BCAT [29] of subgroup **iii**, which also show a significant similarity to each other [52], are quite distinct from those of other aminotransferases. Furthermore, Goldsmith *et al.* recently classified D-AAT and BCAT as Fold type IV and other aminotransferases as Fold type I (Table 1) [16]. Dunathan studied the evolution of PLP

enzymes based on their stereospecificities for hydrogen transfer. However, AspAT and alanine aminotransferase of subgroup **i** are the only aminotransferases whose stereospecificities for hydrogen transfer and evolutionary pedigree have been clarified based on their primary structures. We were particularly interested in the stereospecificities for hydrogen transfer of aminotransferases belonging to subgroups **ii**, **iii**, and **iv**.

## Methods of Determination of the Stereospecificity for the C-4' Hydrogen Transfer in Transamination

The stereospecificity for the hydrogen transfer in enzymatic transamination has been studied by analyzing the position of the $^2H$ or $^3H$ introduced into the PMP formed by the half reaction between the PLP form of enzyme and the labeled amino donor in $^2H_2O$ or $^3H_2O$, respectively. However, when $^3H$ is used, this conventional method is time-consuming, needs a large amount of enzyme, and leads to the production of radioactive waste. Thus, a simpler method was developed for the determination of stereospecificity [59]. In addition to other aminotransferases, various PLP enzymes such as kynureninase [44] can intrinsically catalyze transamination, as either a main reaction or a side reaction between substrate amino acids and the bound PLP to form PMP. In a reverse transamination reaction of bound PMP with an amino acceptor, a PLP form is produced. [4'-$^3H$]PMP randomly labeled was prepared according to the reported method [54]. (4'-$S$)-[4'-$^3H$]PMP and (4'-$R$)-[4'-$^3H$]PMP were prepared based on the stereospecific exchange of the C-4' hydrogen of PMP catalyzed by AspAT and BCAT, respectively [59]. The apo-form of the enzyme was incubated with (4'-$S$)-[4'-$^3H$]PMP or (4'-$R$)-[4'-$^3H$]PMP to produce the PMP form enzyme, which was then incubated with an amino acceptor. In the course of the conversion of the PMP form to the PLP form, one of the two C-4' hydrogens of the coenzyme was abstracted and transferred to the C-2 of the acceptor moiety. However, most of the hydrogens abstracted were exchanged with the solvent hydrogen during the transfer [9]. Thus, we can determine the stereospecificity for the hydrogen transfer by measuring the amount of tritium released into the solvent from the (4'-$S$)-[4'-$^3H$]PMP or (4'-$R$)-[4'-$^3H$]PMP during the reaction. The subtraction of the remaining amount of radioactivity in the lyophilized reaction mixture from that initially found in the PMP indicates the amount of tritium released from the C-4' of the PMP. If tritium is released only from the (4'-$S$)-[4'-$^3H$]PMP, the hydrogen transfer occurs on the *si*-face of the intermediate plane. Conversely, if tritium is released from the (4'-$R$)-[4'-$^3H$]PMP, the hydrogen is transferred on the *re*-face (Fig 3). With stereospecifically tritiated PMPs, we showed the hydrogen transfer stereospecificities of OAT from *Bacillus* sp. YM-2 [24] as belonging to subgroup **ii**, D-AAT from *Bacillus* sp. YM-1 [53], BCAT from *E. coli* [29] as belonging to subgroup **iii**,

**Table 2.** Stereospecificities for hydrogen transfer of aminotransferases.

| Aminotransferases | (4'$S$)-[4'-$^3H$]PMP[a] $^3H$-released[c] | | (4'$R$)-[4'-$^3H$]PMP[b] $^3H$-released | |
|---|---|---|---|---|
| | dpm | %d | dpm | % |
| apo-Aspartate AT | 1,209 | 50.0 | 0 | 0 |
| apo-Ornithine AT | 1,746 | 72.2 | 0 | 0 |
| apo-D-Amino Acid AT | 36 | 1.5 | 1,681 | 78.6 |
| apo-BCAT[e] | 232 | 9.6 | 1,233 | 57.6 |
| apo-Phosphoserine AT | 1,985 | 82.1 | 79 | 3.7 |

[a]The intial radioactivity in the reaction mixture was 2,418 dpm.
[b]The intial radioactivity in the reaction mixture was 2,139 dpm.
[c]Vaporable radioactivity.
[d]Ratio of radioactivity released to that initially added in the reaction mixture.
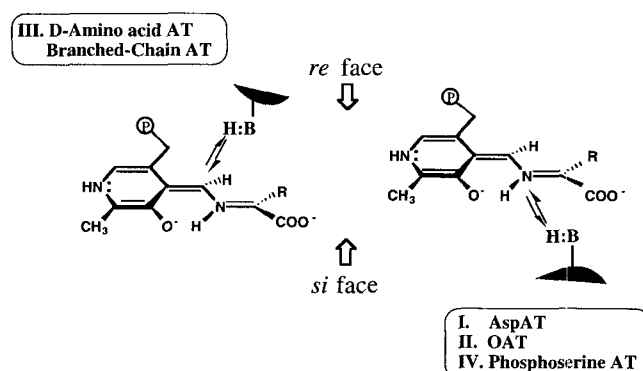[e]Branched-chain L-Amino Acid Aminotransferase.



**Fig. 4.** Orientation of the catalytic Base (-B:) with the coenzyme in the active sites of the aminotransferases.

and PSAT from *E. coli* [50] as belonging to subgroup **iv**. As shown in Table 2, $^3H$ was released specifically from (4'-$S$)-[4'-$^3H$]PMP in the reactions with OAT [24], PSAT (Jhee, K.-H., T. Yoshimura, N. Esaki, and K. Soda. in preparation), and AspAT [59]. In contrast, $^3H$ was released exclusively from (4'-$R$)-[4'-$^3H$]PMP in the reactions with D-AAT and BCAT [59]. This indicates that only the enzymes belonging to subgroup **iii** catalyze a *re*-face hydrogen transfer (Fig. 4).

## Stereochemistry of Aminotransferase Reactions

D-AAT and BCAT are the first examples of PLP enzymes that catalyze a *re*-face hydrogen transfer. This suggests that the catalytic bases for the hydrogen transfer of both enzymes are situated on the *re*-face of the plane of the conjugated π-system of the external Schiff base intermediate (Fig. 4). The stereostructures of D-AAT and BCAT, in particular the topographic arrangement of the catalytic base to the C-4' of the intermediate, are opposite to those of AspAT, OAT, and PSAT (Fig. 5). The active-sites Lys258 of AspAT, Lys292 of OAT, and Lys197 of PSAT participating in the α-hydrogen abstraction are located on the *si*-face of the coenzyme-substrate Schiff base plane.
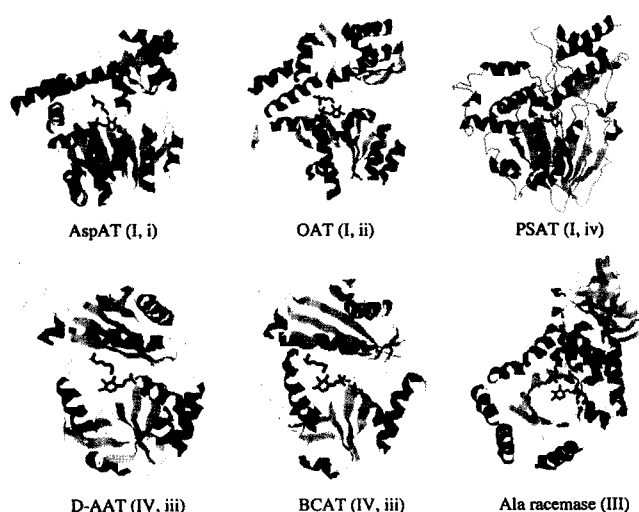
Fig. 5. Schematic representation of the overall fold of the aminotransferases and alanine racemase (Ala racemase).

Only one subunit is shown. AspAT, OAT, PSAT, D-AAT, and Ala racemase consist of 2 identical subunits, and BCAT consists of 6 identical subunits. The capital and small numbers in parentheses indicate the fold type and subgroup of each aminotransferase (see Table 1 and Fig. 4). The overall structures of subgroups i, ii, and iv (AspAT, OAT, and PSAT) are quite similar to one another, and those of D-AAT and BCAT exhibit almost the same characteristics. Ala racemase (fold type III) has a totally different fold type from others. PLP and active site lysyl residues (K258; AspAT, K292; OAT, K197; PSAT, K145; D-AAT, K159; BCAT, K39; Ala racemase) are shown in black and blue. Lysyl residues of AspAT, OAT, and PSAT are located on the si-face of the planar pyridine ring of PLP and those of D-AAT, BCAT, and Ala racemase are on the re-face (see text). Each 3-D structure was taken from the Brookhaven Protein Data Bank (PDB) under the code names of 1ASN for the AspAT, 1OAT for the OAT, 1BT4 for the PSAT, 4DAA for the D-AAT, 1A3G for the BCAT, and 1SFT for the Ala racemase. This figure was produced by RasMol software.

The Lys145 of D-AAT and Lys159 of BCAT, which are bound with PLP, are both regarded as catalytic bases, and are situated on the reverse face, namely, the re-face of the Schiff base plane (Fig. 5). The fold types of AspAT (subgroup i), OAT (subgroup ii), ω-APT (subgroup ii), and PSAT (subgroup iv) resemble one another [47], and are classified into fold type family I. However, the fold types of D-AAT and BCAT are completely different from those of any other PLP enzymes, and are classified into family IV (Table 1, Fig. 5). Therefore, the classifications of aminotransferases based on primary structure, three dimensional structure, and the stereochemistry of hydrogen transfer coincide with one another. As suggested by Dunathan, the stereospecificity of PLP enzymes for a hydrogen transfer probably reflects their molecular evolution. The present findings suggest the convergent evolution of aminotransferases.

## Stereospecificity for a Hydrogen Transfer in Transamination Catalyzed by Amino Acid Racemases

We studied the stereochemistry of a C-4' hydrogen transfer in a transamination catalyzed by amino acid racemases

Table 3. Stereospecificities for hydrogen transfer of amino acid racemases.

| Racemases | (4'S)-[4'-³H]PMP[a] ³H-released[c] | | (4'R)-[4'-³H]PMP[b] 3H-released | |
|---|---|---|---|---|
| | dpm | % | dpm | % |
| apo-AAR[d] | 666 | 43 | 655 | 49 |
| apo-AAR[e] | 668 | 43 | 715 | 53 |
| apo-AAR[f] | 675 | 44 | 627 | 47 |
| apo-AR[g] | 513 | 31 | 1,193 | 80 |
| apo-FAR[h] | 526 | 34 | 1,421 | 84 |

[a]The intial radioactivity in the reaction mixture was 1,540 dpm.
[b]The intial radioactivity in the reaction mixture was 1,340 dpm.
[c]Vaporable radioactivity.
[d]Amino acid racemase from Pseudomonas putida [30].
[e]Amino acid racemase from Pseudomonas striata [45].
[f]Amino acid racemase from Aeromonas punctata [21].
[g]Alanine racemase from Bacillus stearothermophilus [20].
[h]Fragmentary alanine racemase from Bacillus stearothermophilus [56].

from various sources. The hydrogen transfer in side-reaction transaminations catalyzed by amino acid racemases was expected to be non-stereospecific, if the reaction proceeded through the proposed reaction mechanism [12]. Racemization requires both deprotonation and reprotonation from both the re- and si-faces of the same carbon atom of the substrate. We studied the stereospecificity of amino acid racemases with low substrate specificity of Pseudomonas putida [2], Ps. striata [8], and Aeromonas punctata [30], and alanine racemase of Bacillus stearothermophilus [45] and its fragmentary form [21] for the hydrogen transfer by the same method as that used for aminotransferases. As shown in Table 3, tritium was released from both (4'-S)- and (4'-R)-[4'-³H]PMP into the solvent, even in transamination catalyzed by these racemases [31]. This shows that these amino acid racemases catalyze the non-stereospecific hydrogen transfer between the coenzyme and the substrates. Alanine racemase of B. stearothermophilus and eukaryotic ornithine decarboxylase were classified into fold type III (Table 1). Accordingly, it is interesting to study the hydrogen transfer stereospecificity of eukaryotic ornithine decarboxylase. A crystallographic study of alanine racemase revealed that the active-site base Lys39 is situated on the opposite side of the PLP ring on which the active-site Lys258 side chain is located in AspAT [24]. PLP-binding lysyl residues are found in similar locations in both alanine racemase and D-AAT when the three-dimensional structures of both enzymes are superimposed. The Lys145 in D-AAT is situated on the re-face of the intermediate plane, and participates in the pro-R hydrogen transfer at C-4' (Figs. 4, 5). The active site Lys39 is also located on the re-face in the alanine racemase (Fig. 5), and its non-stereospecific hydrogen transfer most probably supports the participation of two bases in the α-hydrogen abstraction which occur on both faces of the intermediate plane. Ringe and coworkers suggested that the OH group of Tyr265, which is from
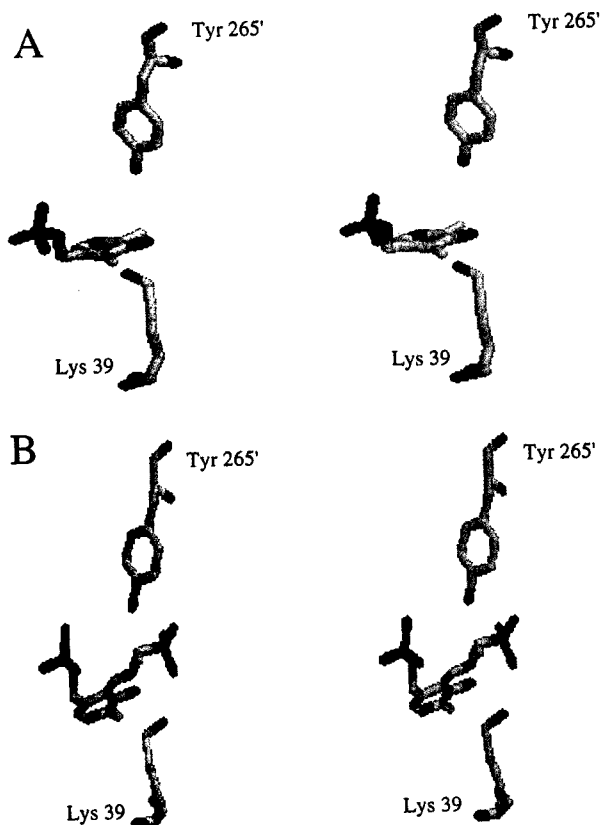
**Fig. 6.** Stereodiagram of the Ala racemase active site.
A. Ala racemase without substrate (PDB code, 1SFT) [42]. B. 1-Aminoethylphosphonic acid (substrate analogue)-PLP aldimine structure (PDB code, 1BD0) [46]. The putative catalytic residues Tyr265' (' indicates another subunit) and Lys39 are shown. The important difference between structures A and B is that the Lys39 and Tyr265' residues have shifted position due to the presence of a cofactor derivative (see text).

another subunit, probably functions as the second base in the active site [42]. A crystallographic study of the alanine racemase in the presence of a substrate analogue (1-aminoethylphosphonic acid: L-Ala-P) showed that the putative second base Tyr265' is appropriately positioned for an α-hydrogen abstraction from an L-enantiomer in the active site. Tyr265' is located at a distance of 3.2 Å from the α-carbon of L-Ala-P, in a position to act as a general base (Fig. 6) [46]. However, there is no direct evidence to show that the alanine racemase reaction follows a double base mechanism.

### Stereospecificity for the Hydrogen Transfer in a Transamination Half Reaction Catalyzed by Lyases

Besides aminotransferases and amino acid racemases, various other PLP enzymes such as aspartate β-decarboxylase and kynureninase catalyze transamination as a side reaction [35]. The hydrogen transfer stereospecificities of L-glutamate decarboxylase, L-tryptophan synthase, and L-serine hydroxymethyltransferase in transaminations have been determined as *si*-face specific [10]. We studied the

**Table 4.** Stereospecificities of SACL, ADCL, tryptophanase, and tryptophan synthase for the C-4' hydrogen withdrawal from PMP in the half reaction.

| Reaction system | $(4'S)$-[4'-$^3$H]PMP[a] $^3$H-releasedc | | $(4'R)$-[4'-$^3$H]PMP[b] $^3$H-released | |
|---|---|---|---|---|
| | dpm | % | dpm | % |
| apo-SACL[d] +pyruvate | 1,551 | 43 | 0 | 0 |
| apo-ADCL[e] +pyruvate | 0 | 0 | 2,381 | 66 |
| apo-tryptophanase+ pyruvate | 2,850 | 79 | 0 | 0 |
| apo-tryptophan synthase +pyruvate | 2,705 | 75 | 0 | 0 |

[a,b]The intial radioactivity in the reaction mixture was 3,608 dpm.
[c]Vaporable radioactivity.
[d]*S*-Alkylcysteine α,β-lyase [26].
[e]4-Amino-4-deoxychorismate lyase [14, 32].

stereospecificities for the hydrogen transfer of 4-amino-4-deoxychorismate lyase (ADCL) of *E. coli* (Jhee, K.-H., T. Yoshimura, N. Esaki, and K. Soda, in preparation), tryptophanase of *E. coli* [51], tryptophan synthase of *Salmonella typhimurium* [36], and S-alkylcysteine α,β-lyase (SACL) of *Bacillus* sp. [26], which catalyze the side-reaction transamination between bound PMP and PLP, by the same method as that used for aminotransferases. Tryptophan synthase (which was shown to be *si*-face specific [36]) was used as a control for the stereospecificity assay. This enzyme exhibits a high sequence homology with tryptophanase. Among these PLP enzymes, ADCL in particular was interesting because of its significant sequence homology with D-AAT (23% with the enzyme from *B.* sp YM-1) and BCAT (21% with the enzyme from the *E. coli*) [48]. Bacterial ADCL (*pabC*) is a key enzyme along with *p*-aminobenzoate synthase (*pabA* and *pabB* proteins) in the biosynthesis of *p*-aminobenzoate from chorismate and glutamine. It catalyzes the conversion of 4-amino-4-deoxychorismate (ADC) to *p*-aminobenzoate and pyruvate [14]. A reciprocal profile analysis gave Z scores greater than 13.5 between ADCL and the aminotransferases of subgroup **iii** [32]. A Z score value higher than 6 indicates a definitive structural relationship between the target and the probe sequences [15]. Accordingly, this high Z score indicates that ADCL is closely related in structure to the aminotransferases of subgroup **iii**, which contains D-AAT and BCAT, but is different from other aminotransferases. ADCL is also categorized as fold type IV which contains D-AAT and BCAT [16]. We studied the stereospecificities for the hydrogen transfer of PLP-dependent lyases. When (4'S)-[4'-$^3$H] PMP or (4'R)-[4'-$^3$H] PMP was incubated with the apo-forms of SACL, tryptophanase, and tryptophan synthase in the presence of pyruvate, tritium was exclusively released from (4'S)-[4'-$^3$H] PMP into the solvents except for ADCL (Table 4). Accordingly, in the reactions with
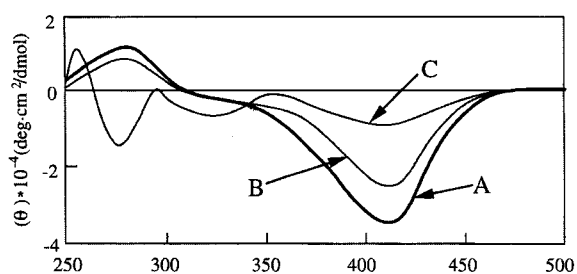
**Fig. 7.** Circular dichroism spectra of (A) ADCL, (B) D-AAT [53], and (C) BCAT [47].
CD spectra were taken in (A) 20 mM potassium phosphate buffer (pH 8.0) with an enzyme concentration of 5.1 mg/ml, (B) 50 mM potassium phosphate buffer (pH 7.4) with an enzyme concentration of 5.7 mg/ml, (C) 20 mM HEPES buffer (pH 8.0) containing 0.1 M KCl with an enzyme concentration of 0.46 mg/ml. All CD spectra were taken at 25°C.

SACL, tryptophanase, and tryptophan synthase, *pro-S* C-4' hydrogen of PMP is transferred to C-2 of pyruvate in a half reaction. Conversely, *pro-R* hydrogen is transferred in the ADCL reaction. ADCL is the first example of PLP-dependent lyase which catalyzes a *re*-face hydrogen transfer in a side-reaction transamination. Control experiments were simultaneously performed to exclude the possibility that the observed exchanges were due to a non-enzymatic transamination between PMP and pyruvate. Although the transaminations by SACL, tryptophanase, and ADCL are not their main physiological reactions, but rather side-reactions as described above, they probably play an important role in the regulatory mechanism of activities [35].

**Circular Dichroism Spectrum of ADCL**

The circular dichroism (CD) spectrum of ADCL showed a negative band in the wavelength region around 410 nm where the absorption maxima of D-AAT and BCAT were found (Fig. 7). Accordingly, the structural environment of the coenzyme in ADCL is probably similar to those of D-AAT and BCAT.

## CONCLUSION

We found the occurrence of three types of PLP enzymes from the viewpoint of the stereospecificity for the hydrogen transfer between the bound coenzyme C-4' and the substrate amino acid C-2: The enzymes catalyze either stereospecifically the hydrogen transfer on the *si*- or *re*-face of the intermediate plane, or non-stereospecifically on both faces. The primary and three-dimensional structures of the PLP enzymes studied so far are consistent with their hydrogen transfer stereospecificities. The PLP enzymes that show the same hydrogen transfer stereospecificity probably evolved from a common ancestral protein.

**Abbreviations:** AspAT, aspartate aminotransferase; BCAT, branched-chain L-amino acid aminotransferase; D-AAT, D-amino acid aminotransferase; OAT, ornithine δ-aminotransferase; ω-APT, ω-amino acid:pyruvate aminotransferase; PSAT, phosphoserine aminotransferase; ADCL, 4-amino-4-deoxychorismate lyase; SACL, S-alkylcysteine α, β-lyase; PLP, pyridoxamine 5'-phosphate; PMP, pyridoxamine 5'-phosphate

**Note Added in Proof**

After completion of this review, further relevant papers have been published [11, 18, 27, 49, 58]. Double base mechanism of alanine racemase have been reported by the two groups [49, 58].

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## REFERENCES

1. Alexander, F. W., E. Sandmeier, P. K. Mehta, and P. Christen. 1994. Evolutionary relationships among pyridoxal-5'-phosphate-dependent enzymes. Regio-specific α, β and γ families. *Eur. J. Biochem.* **219:** 953–960.
2. Alexeev, D., M. Alexeeva, R. L. Baxter, D. J. Campopiano, S. P. Webster, and L. J. Sawyer. 1998. The crystal structure of 8-amino-7-oxononanoate synthase: A bacterial PLP-dependent, acyl-CoA-condensing enzyme. *J. Mol. Biol.* **284:** 401–419.
3. Antson, A. A., T. V. Demidkina, P. Gollnick, Z. Dauter, R. L. von Tersch, J. Long, S. N. Berezhnoy, R. S. Phillips, E. H. Harutyunyan, and K. S. Wilson. 1993. Three-dimensional structure of tyrosine phenol-lyase. *Biochemistry* **32:** 4195–4206.
4. Braunstein, A. E. 1957. Les voies principales de l'assimilation et dissimilation de l'azote chez les animaux. *Advan. Enzymol.* **19:** 335–389.
5. Braunstein, A. E. 1973. *The Enzymes*, vol. 9. pp. 379–481. 3rd. ed.
6. Burkhard, P., G. S. J. Rao, E. Hohenester, K. D. Schnackerz, P. F. Cook, and J. N. Jansonius. 1998. Three-dimensional structure of O-acetylserine sulfhydrylase from *Salmonella typhimurium. J. Mol. Biol.* **283:** 121–133.
7. Clausen, T., R. Huber, B. Laber, H. D. Pohlenz, and A. Messerschmidt. 1996. Crystal structure of the pyridoxal-5'-

phosphate dependent cystathionine β-lyase from *Escherichia coli* at 1.83 angstrom. *J. Mol.Biol.* **262:** 202– 224.

8. Clausen, T., R. Huber, L. Prade, M. C. Wahl, and A. Messerschmidt. 1998. Crystal structure of *Escherichia coli* cystathionine γ-synthase at 1.5 Å resolution. *EMBO J.* **23:** 6827– 6838.

9. Dunathan, H. C. 1971. Stereochemical aspects of pyridoxal phosphate catalysis. *Adv. Enzymol. Relat. Areas Mol. Biol.* **35:** 79– 134.

10. Dunathan, H. C. and J. G. Voet. 1974. Stereochemical evidence for the evolution of pyridoxal-phosphate enzymes of various function from a common ancestor. *Proc. Natl. Acad. Sci. USA* **71:** 3888– 3891.

11. Eads, J. C., M. Beeby, G. Scapin, T. W. Yu, and H. G. Floss. 1999. Crystal structure of 3-amino-5-hydroxybenzoic acid (AHBA) synthase. *Biochemistry* **38:** 9840– 9849.

12. Faraci, W. S. and C. T. Walsh. 1988. Racemization of alanine by the alanine racemases from *Salmonella typhimurium* and *Bacillus stearothermophilus*: Energetic reaction profiles. *Biochemistry* **27:** 3267– 3276.

13. Gallagher, D. T., G. L. Gilliland, G. Xiao, J. Zondlo, K. E. Fisher, D. Chinchilla, and E. Eisenstein. 1998. Structure and control of pyridoxal phosphate dependent allosteric threonine deaminase. *Structure* **15:** 465– 475.

14. Green, J. M, W. K. Merkel, and B. P. Nichols. 1992. Characterization and sequence of *Escherichia coli pabC*, the gene encoding aminodeoxychorismate lyase, a pyridoxal phosphate-containing enzyme. *J. Bacteriol.* **174:** 5317– 5323.

15. Gribskov, M. 1992. Translational initiation factors IF-1 and eIF-2 alpha share an RNA-binding motif with prokaryotic ribosomal protein S1 and polynucleotide phosphorylase. *Gene* **119:** 107– 111.

16. Grishin, N. V., M. A. Phillips, and E. J. Goldsmith. 1995. Modeling of the spatial structure of eukaryotic ornithine decarboxylase. *Protein Sci.* **4:** 1291– 1304.

17. Hennig, M., B. Grimm, R. Contestabile, R. A. John, and J. N. Jansonius. 1997. Crystal structure of glutamate-1-semialdehyde aminomutase: An alpha-dimeric vitamin B-6-dependent enzyme with asymmetry in structure and active site reactivity. *Proc. Natl. Acad. Sci. USA* **94:** 4866– 4871.

18. Hester, G., W. Stark, M. Moser, J. Kallen, Z. Markovic-Housley, and J. N. Jansonius. 1999. Crystal structure of phosphoserine aminotransferase from *Escherichia coli* at 2.3 Å resolution: Comparison of the unligated enzyme and a complex with α-methyl-l-glutamate. *J. Mol. Biol.* **286:** 829– 850.

19. Hyde, C. C., S. A. Ahmed, E. A. Padlan, E. W. Miles, and D. R. Davies. 1988. Three-dimensional structure of the tryptophan synthase α2β2 multienzyme complex from *Salmonella typhimurium. J. Biol. Chem.* **263:** 17857– 17871.

20. Inagaki, K., K. Tanizawa, B. Badet, C. T. Walsh, H. Tanaka, and K. Soda. 1986. Thermostable alanine racemase from *Bacillus stearothermophilus*: Molecular cloning of the gene, enzyme purification, and characterization. *Biochemistry* **25:** 3268– 3274.

21. Inagaki, K., K. Tanizawa, H. Tanaka, and K. Soda. 1987. Purification and characterization of amino acid racemase with broad substrate specificity from *Aeromonas caviae. Agric. Biol. Chem.* **51:** 173– 180.

22. Inoue, K., S. Kuramitsu, K. Aki, Y. Watanabe, T. Takagi, M. Nishigai, A. Ikai, and H. Kagamiyama. 1988. Branched-chain amino acid aminotransferase of *Escherichia coli*: Overproduction and properties. *J. Biochem.* **104:** 777– 784.

23. Isupov, M. N., A. A. Antson, E. J. Dodson, G. G. Dodson, I. S. Dementieva, L. N. Zakomirdina, K. S. Wilson, Z. Dauter, A. A. Lebedev, and E. H. Harutyunyan. 1998. Crystal structure of tryptophanase. *J. Mol. Biol.* **276:** 603– 623.

24. Jhee, K.-H., T. Yoshimura, N. Esaki, K. Yonaha, and K. Soda. 1995. Thermostable ornithine aminotransferase from *Bacillus* sp. YM-2: Purification and characterization. *J. Biochem.* **118:** 101– 108.

25. Jhee, K.-H., T. Yoshimura, N. Esaki, and K. Soda. 1996. Stereospecificity of thermostable ornithine 5-aminotransferase for the hydrogen transfer in the L- and D-ornithine transamination. *Biochemistry* **35:** 9792– 9796.

26. Kamitani, H., N. Esaki, H. Tanaka, and K. Soda. 1990. Thermostable S-alkylcysteine α, β-lyase from a thermophile: Purification and properties. *Agric. Biol. Chem.* **54:** 2069– 2076.

27. Kern, A. D., M. A. Oliveira, P. Coffino, and M. L. Hackert. 1999. Structure of mammalian ornithine decarboxylase at 1.6 Å resolution: Stereochemical implications of PLP-dependent amino acid decarboxylases. *Structure Fold. Des.* **7:** 567– 581.

28. Kirsch, J. F., G. Eichele, G. C. Ford, M. G. Vincent, J. N. Jansonius, H. Gehring, and P. Christen. 1984. Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. *J. Mol. Biol.* **174:** 497– 525.

29. Kuramitsu, S., T. Ogawa, H. Ogawa, and H. Kagamiyama. 1985. Branched-chain amino acid aminotransferase of *Escherichia coli*: Nucleotide sequence of the *ilvE* gene and the deduced amino acid sequence. *J. Biochem.* **97:** 993– 999.

30. Lim, Y.-H., K. Yokoigawa, N. Esaki, and K. Soda. 1993. A new amino acid racemase with threonine α-epimerase activity from *Pseudomonas putida*: Purification and characterization. *J. Bacteriol.* **175:** 4213– 4217.

31. Lim, Y.-H., T. Yoshimura, Y. Kurokawa, N. Esaki, and K. Soda. 1998. Nonstereospecific transamination catalyzed by pyridoxal phosphate-dependent amino acid racemases of broad substrate specificity. *J. Biol. Chem.* **273:** 4001– 4005.

32. Mehta, P. K. and P. Christen. 1993. Homology of pyridoxal-5'-phosphate-dependent aminotransferase with the *cobC* (cobalamin synthesis), *nifs* (nitrogen fixation), *pabC* (*p*-aminobenzoate synthesis) and *malY* (abolishing endogenous induction of the maltose system) gene products. *Eur. J. Biochem.* **211:** 373– 376.

33. Mehta, P. K., T. I. Hale, and P. Christen. 1993. Aminotransferases: Demonstration of homology and division into evolutionary subgroups. *Eur. J. Biochem.* **214:** 549– 561.

34. Metzler, D. E., M. Ikawa, and E. E. Snell. 1954. A general mechanism for vitamin B6-catalyzed reactions. *J. Am. Chem. Soc.* **76:** 648– 652.

35. Miles, E. W. 1985. *In* Christen, P. and D. E. Metzler (eds.), *Transaminases*, pp. 470–481. John Wiley & Sons, New York.

36. Miles, E. W., D. R. Houck, and H. G. Floss. 1982. Stereochemistry of sodium borohydride reduction of tryptophan synthase of *Escherichia coli* and its amino acid Schiff's bases. *J. Biol. Chem.* **257:** 14203–14210.

37. Momany, C., S. Ernst, R. Ghosh, N. L. Chang, and M. L. Hackert. 1995. Crystallographic structure of a PLP-dependent ornithine decarboxylase from *Lactobacillus* 30a to 3.0 Å resolution. *J. Mol. Biol.* **252:** 643–655.

38. Momany, C., R. Ghosh, and M. L. Hackert. 1995. Structural motifs for pyridoxal-5'-phosphate binding in decarboxylases: An analysis based on the crystal structure of the *Lactobacillus* 30a ornithine decarboxylase. *Protein Sci.* **4:** 84–854.

39. Okada, K., K. Hirotsu, M. Sato, H. Hayashi, and H. Kagamiyama. 1997. Three-dimensional structure of *Escherichia coli* branched-chain amino acid aminotransferase at 2.5 Å resolution. *J. Biochem.* **121:** 637–641.

40. Okamoto, A., Y. Nakai, H. Hayashi, K. Hirotsu, and H. Kagamiyama. 1998. Crystal structures of *Paracoccus denitrificans* aromatic amino acid aminotransferase: A substrate recognition site constructed by rearrangement of hydrogen bond network. *J. Mol. Biol.* **280:** 443–461.

41. Renwick, S. B., K. Snell, and U. Baumann. 1998. The crystal structure of human cytosolic serine hydroxymethyltransferase: A target for cancer chemotherapy. *Structure* **15:** 1105–1116.

42. Shaw, J. P., G. A. Petsko, and D. Ringe. 1997. Determination of the structure of alanine racemase from *Bacillus stearothermophilus* at 1.9-resolution. *Biochemistry* **36:** 1329–1342.

43. Shen, B. W., M. Hennig, E. Hohenester, J. N. Jansonius, and T. Schirmer. 1998. Crystal structure of human recombinant ornithine aminotransferase. *J. Mol. Biol.* **277:** 81–102.

44. Soda, K., M. Moriguchi, and K. Tanizawa. 1975. Regulation of the activity of microbial kynureninase by transamination of the enzyme-bound coenzyme. *Acta Vitaminol. Enzymol.* **29:** 335–338.

45. Soda, K. and T. Osumi. 1971. Amino acid racemase (*Pseudomonas striata*). *Methods Enzymol.* **17B:** 629–636.

46. Stamper, C. G., A. A. Morollo, and D. Ringe. 1998. Reaction of alanine racemase with 1-aminoethylphosphonic acid forms a stable external aldimine. *Biochemistry* **37:** 10438–10445.

47. Stark, W., J. Kallen, Z. Markovic-Housley, B. Fol, M. Kania, and J. N. Jansonius. 1991. *In* Fukui, T., H. Kagamiyama, K. Soda, and H. Wada (eds.), *Enzymes Dependent on Pyridoxal Phosphate and other Carbonyl Compounds as Cofactors*, pp. 111–115. Pergamon Press, Oxford.

48. Sugio, S., G. A. Petsko, J. M. Manning, K. Soda, and D. Ringe. 1995. Crystal structure of a D-amino acid aminotransferase: How the protein controls stereoselectivity. *Biochemistry* **34:** 9661–9669.

49. Sun, S. and M. D. Toney. 1999. Evidence for a two-base mechanism involving tyrosine-265 from arginine-219 mutants of alanine racemase. *Biochemistry* **38:** 4058–4065.

50. Tanaka, T., S. Yamamoto, T. Moriya, M. Taniguchi, H. Hayashi, H. Kagamiyama, and S. Oi. 1994. Aspartate aminotransferase from a thermophilic formate-utilizing methanogen, *Methanobacterium thermoformicicum* strain SF-4: Relation to serine and phosphoserine aminotransferases, but not to the aspartate aminotransferase family. *J. Biochem.* **115:** 309–317.

51. Tani, S., N. Tsujimoto, Y. Kawata, and M. Tokushige. 1990. Overproduction and crystallization of tryptophanase from recombinant cells of *Escherichia coli*. *Biotechnol. Appl. Biochem.* **12:** 28–33.

52. Tanizawa, K., S. Asano, Y. Masu, S. Kuramitsu, H. Kagamiyama, H. Tanaka, and K. Soda. 1989. The primary structure of thermostable D-amino acid aminotransferase from a thermophilic *Bacillus* species and its correlation with L-amino acid aminotransferases. *J. Biol. Chem.* **264:** 2450–2454.

53. Tanizawa, K., Y. Masu, S. Asano, H. Tanaka, and K. Soda. 1989. Thermostable D-amino acid aminotransferase from a thermophilic *Bacillus* species. Purification, characterization, and active site sequence determination. *J. Biol. Chem.* **264:** 2445–2449.

54. Tobler, H. P., P. Christen, and H. Gehring. 1986. Stereospecific labilization of the C-4' *pro-S* hydrogen of pyridoxamine 5'-phosphate in aspartate aminotransferase. *J. Biol. Chem.* **261:** 7105–7108.

55. Toney, M. D., E. Hohenester, S. W. Cowan, and J. N. Jansonius. 1993. Dialkylglycine decarboxylase structure: Bifunctional active site and alkali metal sites. *Science* **261:** 756–759.

56. Toyama, H., K. Tanizawa, T. Yoshimura, S. Asano, Y.-H. Lim, N. Esaki, and K. Soda. 1991. Thermostable alanine racemase of *Bacillus stearothermophilus*: Construction and expression of active fragmentary enzyme. *J. Biol. Chem.* **266:** 13634–13639.

57. Watanabe, N., K. Sakabe, N. Sakabe, T. Higashi, K. Sasaki, S. Aibara, Y. Morita, K. Yonaha, S. Toyama, and H. Fukutani. 1989. Crystal structure analysis of omega-amino: Pyruvate aminotransferase with a newly developed Weissenberg camera and an imaging plate using synchrotron radiation. *J. Biochem.* **105:** 1–3.

58. Watanabe, A., T. Yoshimura, B. Mikami, and N. Esaki. 1999. Tyrosine 265 of alanine racemase serves as a base abstracting α-hydrogen from L-alanine: The counterpart residue to lysine 39 specific to D-alanine. *J. Biochem. (Tokyo)* **126:** 781–786.

59. Yoshimura, T., K. Nishimura, J. Ito, N. Esaki, H. Kagamiyama, J. M. Manning, and K. Soda. 1993. Unique stereospecificity of D-amino acid aminotransferase and branched-chain L-amino acid aminotransferase for C-4' hydrogen transfer of the coenzyme. *J. Am. Chem. Soc.* **115:** 3987–3900.