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REVIEW

Stereochemistry and Evolution of Aminotransferases

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I. Stereochemistry of aminotransferase reactions.

Aminotransferases require pyridoxal 5'-phosphate as a coenzyme, and catalyze the transfer of amino group between amino acids and keto acids. The reaction mechanism of aminotransferase is as follows (Fig. 1). PLP is bound to an ε-amino group of the active-site lysyl residue of enzyme protein through a Schiff base (internal Schirff base) linkage (Fig. 1 A). With a substrate amino acid, PLP forms a substrate-PLP Schiff base complex (external Schiff base, B). An active site amino acid residue (-B:) of the enzyme serves as a catalytic base and abstracts an α-hydrogen from the Schiff base complex (C) to yield an anionic intermediate (D). The proton abstracted is then transferred to C4' of the cofactor to form a ketimine intermediate (E). Usually, the proton abstracted is exchanged with the solvent proton during the reaction. Therefore, when the amino acid labeled at α-position is used as an amino donor, the labeled proton (^{2}H or ^{3}H) is released into the solvent during transamination. The intermediate (E) is then hydrolyzed into pyridoxamine 5'-phosphate (PMP) and keto acid (F). The half reaction of transamination, i.e. the conversion of amino acid to keto acid is thus completed. The other half reaction, a new amino acid formation from keto acid (amino acceptor) including the regeneration of PLP proceeds through the reverse process.

In the process of proton transfer to or from C-4' of the cofactor (Fig. 1 D), proton can be transferred on both faces of the plane of the conjugated π-system of the cofactor-substrate imine (Fig. 2). In the aminotransferase reaction, the C-H bond to be broken and formed is considered to be situated perpendicular to the pyridine ring. The proton transfer is suggested to be a suprafacial intermolecular prototropic shift by a single base. Thus, The *pro-S* proton and *pro-R* proton are transferred on the *si-* and *re-* face of plane of the cofactor, respectively (Fig. 2). Though both manners of the transfer are possible, a hydrogen is added or removed only on the *si*-face in the transaminations so far studied catalyzed by L-aspartate aminotransferase[1a], L-alanine aminotransferase[1b], dialkylamino acid aminotransferase[1c], pyridoxamine pyruvate aminotransferase[1d], L-glutamate decarboxylase[1e], L-tryptophan synthase[1f], L-aspartate β-decarboxylase[1g], and L-serine hydroxymethyltransferase[1h].

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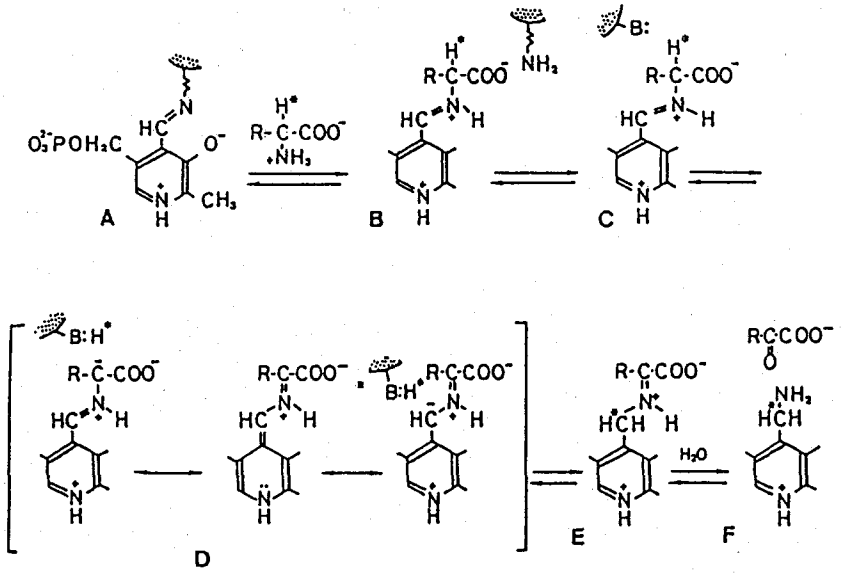

Figure 1. Reaction mechanism of aminotransferase.

As shown in Fig. 2, the stereospecificity of hydrogen transfer reflects the topographical situation of the coenzyme-substrate Schiff base and the catalytic base of the enzyme, and is thus related to the structure of the active-site of enzyme. Based on the result that different PLP-enzymes show the same stereospecificity, Dunathan has proposed the hypothesis that all the PLP-enzymes may have evolved from a common ancestral protein.[2]

Recently, Christen and his coworkers classified the aminotransferases into 4 groups on the basis of comparison of their amino acid sequences (Table I).[3] Aminotransferases belonging to subgroups (I), (II), and (IV) are considerably homologous, but D-amino acid aminotransferase of *Bacillus* sp. YM-1[4] (D-AAT) and branched-chain L-amino acid aminotransferase of *Escherichia coli*[5] (BCAT), which show significant homology in their primary structures[6], are considered to be considerably different from other aminotransferases. Enzymes listed in Table I of which stereospecificities for the proton transfer were determined were asparatate aminotransferase (Asp AT) of subgroup I and alanine aminotransferase of subgroup IV. Thus, we were interested in stereochemistries of the reactions of the aminotransferases belonging to subgroups II and III. We have established the simple method of determination of the stereospecificity for the C4' proton transfer, and examined the stereo chemistry of the reactions catalyzed by D-AAT and BCAT of subgroup III,[7] and L-ornithine aminotransferase of *Bacillus* sp. YM-2 (OAT) of subgroup II.[8]

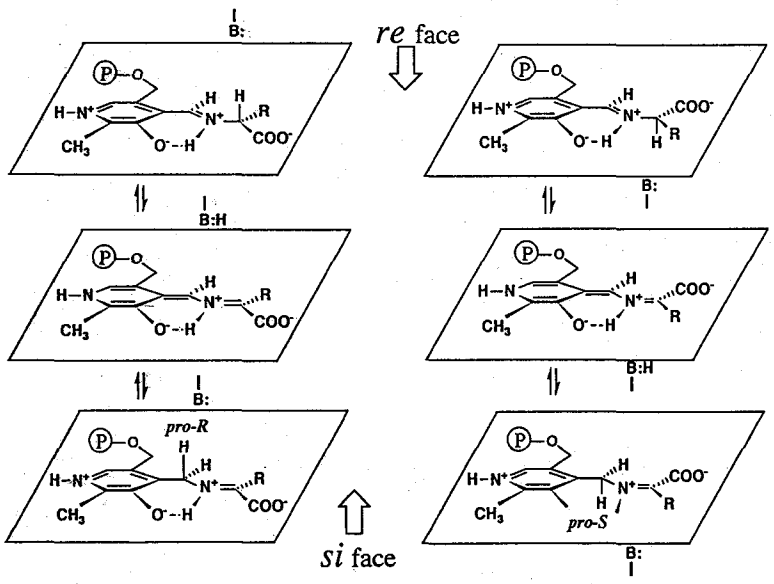

Figure 2. Stereochemistry of proton transfer catalyzed by aminotransferase.

Ⅱ. Method of the determination of stereospecificity for the C4'-proton transfer in aminotransferase reactions.

Stereochemistry of the C4'-proton transfer of aminotransferase reaction has been determined by the analysis of position of the label (^2H or ^3H) introduced into PMP formed by the *half reaction of PLP form of enzyme and labeled amino donor in* ^2H_2O or ^3H_2O. This conventional method is time-consuming, needs a large amount of enzyme, and sometimes produced a lot of radioactive waste. Thus, we developed a simpler method for the determination of the hydrogen transfer stereospecificity.[7] In this method, an apo-form of aminotransferase is prepared, and mixed with (4'-*S*)-[4'-^{3}H] PMP or (4'-*R*)-[4'-^{3}H] PMP. After the enzyme is reconstituted, an amino acceptor or an amino acceptor and amino donor are added to the enzyme solution. Half (with an amino accepter) and overall (with an amino acceptor and an amino donor) reactions proceed with the release of C4'-proton (s) of PMP into the solvent. Subtraction of the amount of radioactivity remained in the lyophilyzed reaction mixture from that initially present in PMP gives the amount of tritium released from C-4' of PMP. If the tritium is released only from (4'-*S*)-[4'-^{3}H] PMP, the proton transfer occurs on the *si*-face of the cofactor. In contrast, if the tritium is released from (4'-*R*)-[4'-^{3}H] PMP, the transfer occurs on the *re*-face. Two kinds of PMP stereospecifically ^{3}H-labeled at 4'-position are required for this method. Their preparation has become possible by finding of stereospecific exchange of C-4' proton of PMP catalyzed by AspAT and BCAT.

Table I. Subgroups of aminotransferases[3)]

Subgroup	Enzyme
I.	Aspartate AT[a)]
	Tyrosine AT
	Histidinol-phosphate AT
II.	Acetylornithine AT
	Ornithine AT
	Diaminopelargonate AT
	Glutamate semialdehyde AT
III.	D-Amino acid AT
	Branched-chain L-amino acid AT
IV.	Alanine AT
	Serine AT
	Phosphoserine AT

a) AT; Aminotransferase

Ⅲ. Stereospecific exchange of C-4' hydrogen with the solvent proton by BCAT.

It was reported that incubation of apo-AspAT with [4'-^{3}H]PMP in the absence of an amino acceptor results in a stereospecific exchange of C-4' *S*-^{3}H with the solvent hydrogen.[9)] We observed the similar stereospecific C-4' hydrogen exchange catalyzed by BCAT. When apo-BCAT was incubated with [4'-^{3}H]PMP, reconstitution of the enzyme (recovery of the activity) and release of ^{3}H into the solvent, which was shown by an increase in vaporable radioactivity of the solvent was observed (Fig. 3). The final ratio of the radioactivity released from [4'-^{3}H]PMP to the initial radioactivity was about 50%. This suggests the stereospecific release of ^{3}H. After the ^{3}H release ceased, PMP was recovered from the enzyme by acid and heat treatment. The PMP was purified by a reversed-phase column chromatography and incubated with apo-AspAT or apo-BCAT. As shown in Figure 4, both enzymes were reconstituted with PMP, but ^{3}H was released only in the reaction with apo-AspAT. This shows that the PMP recovered was tritirated specifically at 4'-*S* position. Therefore, BCAT catalyzes the C-4' pro-*R* hydrogen exchange with the solvent hydrogen. Based on these results, we can prepare (4'-*R*)-[4'-^{3}H]PMP and (4'-*S*)-[4'-^{3}H]PMP by the C-4' hydrogen exchange of [4'-^{3}H]PMP in ^1H_2O with apo-AspAT and apo-BCAT, respectively (Fig. 5).[7),8)]

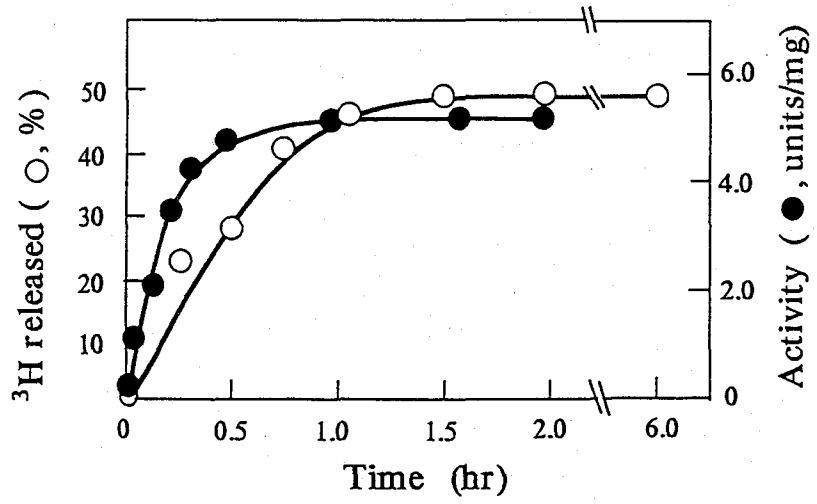

Figure 3. Release of tritium from [4'-^{3}H] PMP by apo-BCAT.

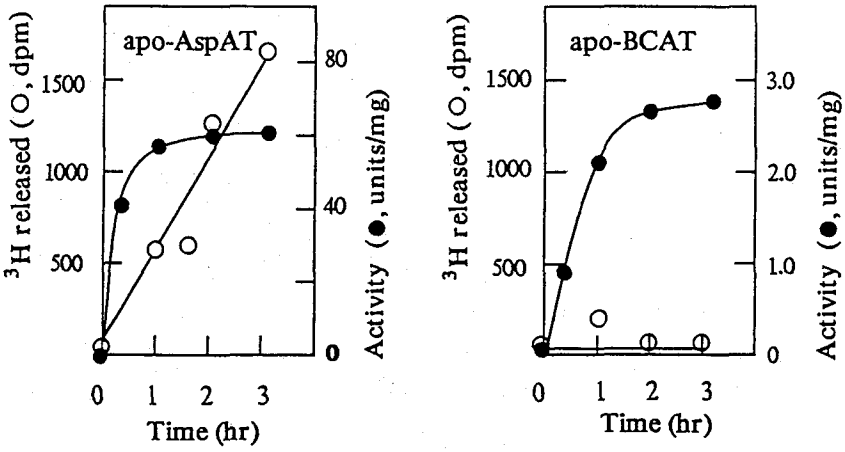

Figure 4. Release of tritium from [4'-^{3}H] PMP treated with apo-BCAT during the incubation with apo-AspAT and apo-BCAT.

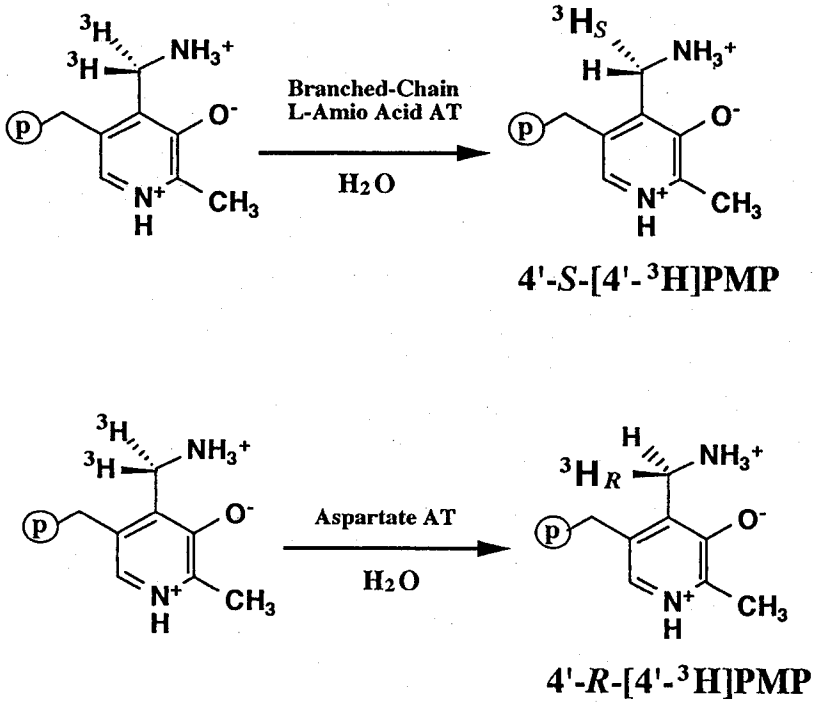

Figure 5. Preparation of (4'-*S*)-[4-^{3}H]PMP and (4'-*R*)-[4-^{3}H]PMP.

Ⅳ. Stereochemistry of the C-4' hydrogen withdrawal from PMP in the half and overall reactions by D-AAT, BCAT, and OAT.

When a PMP form of aminotransferase is converted to the PLP-form by incubation with an amino acceptor (a half reaction), one hydrogen at C-4' is withdrawn from C-4' PMP as described previously. We examined the stereospecificities of D-AAT, BCAT, and OAT for the hydrogen abstraction by measuring the ^{3}H release in the reaction of apo-form of enzyme with the stereospecifically tritirated PMP and α-ketoglutarate. The stereospecificities of these enzymes for the hydrogen abstraction during the overall reactions were also determined with the amino donors by the same procedure (Table II). In the half and overall reactions by D-AAT and BCAT, ^{3}H was released into the solvent specifically from (4'-*R*)-[4'-^{3}H]PMP, but was released from only (4'-*S*)-[4'-^{3}H]PMP by OAT and AspAT. Therefore, D-AAT and BCAT specifically abstract the C4'-pro-*R* hydrogen from PMP during both half and overall reactions, and OAT catalyzes C4'-pro-*S* hydrogen abstraction like AspAT.

Table II. Stereochemistry of the hydrogen withdrawing from PMP in the half and overall reactions by D-AAT, BCAT, and OTA.

	(4'-*S*)-[4'-^{3}H]PMP		(4'-*R*)-[4'-^{3}H]PMP	
	^{3}H-released[a,b]		^{3}H-released	
	dpm	%[c]	dpm	%
apo-D-AAT + KG[d]	36	—	1681	78.6
apo-D-AAT + KG + D-Ala	0	0	1657	75.9
apo-BCAT + KG	229	9.6	1233	57.6
apo-BCAT + KG + L-Val	0	0	1506	70.4
apo-OAT + KG	1472	72.2	0	0
apo-OAT + KG + L-Orn	1156	56.8	0	0
apo-AspAT + KG + L-Asp	1209	50.9	0	0

a. No tritium was released in the absence of enzyme.
b. Vaporable radioactivity of solvent.
c. Ratio of the radioactivity released to that initially added in the reaction mixture.
d. α-ketoglutarate

V. Stereochemistry and evolution of aminotransferases.

The results obtained demonstrate for the first time that the pro-*R* hydrogen is added or withdrawn at C-4' of the cofactor in the aminotransferase reactions. Therefore, aminotransferases of subgroup III catalyze the *re*-face transfer of the C-4' proton (Table III). As described previously, the stereochemistry of this hydrogen transfer reflects the geometrical relations between the cofactor-substrate complex and the catalytic base of enzyme. The identity of D-AAT and BCAT in the stereospecificities indicates that their active site stereo-structures are homologous with each other, but different from those of other aminotransferases. This is compatible with the classification of aminotransferases according to their primary structures: both the enzymes belong to the same group which differs from other three groups of aminotransferases.[3]

Recently, three-dimensional structures of D-AAT[10] and ω-amino acid: pyruvate aminotransferases[11] (ω-APT) were demonstrated. The latter enzyme shows a high sequence homology with L-ornithine aminotransferase[11], and is thus considered to be classified into the subgroup II. Though the amino acid sequence of ω-APT differs from that of AspAT, the three-dimensional structure (folding) of enzyme resembles that of AspAT. The three-dimensional structure of D-AAT is completely different from those of AspAT and ω-APT[10]. These results suggest that AspAT of subgroup I and ω-APT of subgroup II evolved divergently from the same protein, but the ancestral protein of D-AAT (subgroup III) should be different from that of AspAT and ω-APT. Classification of aminotransferases based on

Table III. Stereochemistry and subgroups of aminotransferases.

Subgroup	Enzyme	Stereochemistry
I.	Aspartate AT	*si*
II.	Ornithine AT	*si*
III.	D-Amino acid AT	*re*
	Branched-chain L-amino acid AT	*re*
IV.	Alanine AT	*si*

a) AT; Aminotransferase

the stereochemistry of C4'-hydrogen transfer is compatible with that based on the three-dimensional structure as well as the primary structure. Therefore, we conclude that PLP-dependent aminotransferases have evolved from at least two ancestral proteins.

References

(1) (a) Besmer, P., and Arigoni, D., *Chimia*, **23**, 190 (1969). (b) Austermuhle-Bertrola, E., *Ph. D. Dissertation.* **No. 5009**, *ETH*, Zurich (1973). (c) Bailey, G. B., Kusamrarn, T., and Vuttivej, K., *Fed. Proc.*, **29**, 857 (1970). (d) Ayling, J. E., Dunathan, H. C., and Snell, E. E., *Biochemisty*, **7**, 4532-4542 (1968). (e) Sukhareva, B. S., Dunathan, H. C., and Braunstein, A. E., *FEBS Lett.*, **15**, 241-244 (1971). (f) Dunathan, H. C., and Voet, J. G., *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3888-3891 (1974). (g) Chang, C. C, Laghai, A., O'Leary, M. H., and Floss, H. G., *J. Biol. Chem.*, **257**, 3564-3569 (1982). (h) Voet, J. G., Hindenlang, D. M., Blanck, T. J. J., Ulevitch, R. J., Kaljen, R. G., and Dunathan, H. C., *J. Biol. Chem.*, **248**, 841-842 (1973).

(2) Dunathan, H. C., *Adv. Enzymol. Relat. Areas Mol. Biol.*, **35**, 79-134 (1971).

(3) Metha, P. K., Hale, T. I., and Christen P., Enzymes Dependent on Pyridoxal Phosphate and Other Carbonyl Compound as Cofactors, Pergamon Press, Oxford, (1991), pp 35-42.

(4) Tanizawa, K., Masu, Y., Asano, S., Tanaka, H., and Soda, K., *J. Biol. Chem.*, **264**, 2445-2449 (1989).

(5) Kuramitsu, S., Ogawa, T., Ogawa, H., and Kagamiyama, H., *J. Biochem.* (*Tokyo*), **97**, 993-999 (1985).

(6) Tanizawa, K., Asano, S., Masu, Y., Kuramitsu, S., Kagamiyama, H., Tanaka, H., and Soda, K., *J. Biol. Chem.*, **264**, 2450-2454 (1989).

(7) Yoshimura, T., Nishimura, K., Ito, J., Esaki, N., Kagamiyama, H., Manning, J. M., and Soda, K., *J. Am. Chem. Soc.*, **115**, 3897-3900 (1993).

(8) Jhee, K., Yoshimura, T., Esaki, N. and Soda, K., *Nippon Nougeikagaku Kaishi*, **67**, 341 (1993).

(9) Tobler, H. P., Christen P., and Gehring, H., *J. Biol. Chem.*, **261**, 7105-7108 (1986).

(10) Sugio, N., personal communication.

(11) Watanabe, N., Yonaha, K., Sakabe, K., Sakabe, N., Aibara, S., and Morita, Y., Enzymes Dependent on Pyridoxal Phosphate and Other Carbonyl Compound as Cofactors, Pergamon Press Oxford, (1991), pp 121-124.