Stereospecificity of Thermostable Ornithine 5-Aminotransferase for the Hydrogen Transfer in the L- and D-Ornithine Transamination

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ABSTRACT: The thermostable ornithine 5-aminotransferase of a thermophile, *Bacillus* sp. YM-2, is unique in acting on both enantiomers of ornithine, although less effectively on the D-enantiomer. We studied the stereospecificity of the enzyme for the hydrogen abstraction from C-5 of the substrate moiety and the addition and removal of the hydrogen at C-4′ of the cofactor (pyridoxal phosphate and pyridoxamine phosphate) moiety of the external Schiff base intermediate in the transamination of L- and D-ornithine. L- and D-[5-^{3}H]ornithines were prepared by incubation of L- and D-ornithines with the enzyme in ^3H_2O, respectively. When the L-[5-^{3}H]ornithine was incubated with L-ornithine 5-aminotransferase of a mesophile, *Bacillus sphaericus*, which catalyzes the stereospecific abstraction of *pro-S* hydrogen from C-5 of L-ornithine, most of the tritium was released into the solvent. The D-[5-^{3}H]ornithine also reacted with the enzyme of *B. sphaericus* in the presence or absence of the amino acid racemase of *Pseudomonas putida*. Tritium was released only in the presence of the racemase, which catalyzes the racemization of ornithine but does not act on C-5 of ornithine. These results show that the *Bacillus* sp. YM-2 ornithine 5-aminotransferase stereospecifically abstracts the *pro-S* hydrogen from C-5 of L- and D-ornithine. When the apo form of the enzyme was incubated with pyridoxamine 5′-phosphate that was stereospecifically tritiated at C-4′ and 2-oxoglutarate in the presence of L-ornithine or D-ornithine, tritium was released exclusively from (4′*S*)-[4′-^{3}H]pyridoxamine. Therefore, addition and abstraction of hydrogen at C-4′ of the cofactor moiety stereospecifically occur on the *si* face of the external Schiff base intermediate in the overall transamination catalyzed by *Bacillus* sp. YM-2 ornithine 5-aminotransferase irrespective of the C-2 configuration of the amino donor.

The pyridoxal phosphate (PLP)-dependent aminotransferase reactions proceed through the abstraction of a hydrogen from the carbon bearing the amino group to be transferred, and the anionic intermediate is formed from the external Shiff base complex of a substrate and a cofactor [reviewed by Martinez-Carrion et al. (1985)]. The hydrogen that is abstracted is transferred to C-4′ of the cofactor, and consequently, the pyridoxamine 5′-phosphate (PMP)[1] form of the enzyme and keto acid are produced through the ketimine intermediate. Three stereochemical possibilities exist for the hydrogen transfer: the stereospecific transfer on the *si* or *re* face and, alternatively, the non-stereospecific transfer on both faces of the plane of the π-electron system of the intermediate. The stereospecificity reflects the active-site structure of the enzyme, especially the geometrical relationship between the catalytic base of the enzyme for the hydrogen transfer and the bound cofactor. The stereospecificities of various aminotransferases for the hydrogen transfer have been examined. A hydrogen is transferred on the *si* face of the intermediate in the transaminations catalyzed by L-aspartate aminotransferase (AspAT; Besmer & Arigoni, 1969), L-alanine aminotransferase (Austermuhle-Bertrola, 1973), dialkylamino acid aminotransferase (Bailey et al., 1970), pyridoxamine pyruvate aminotransferase (Ayling et al., 1968), L-glutamate decarboxylase (Sukhareva et al., 1971), L-tryptophan synthetase (Dunathan & Voet, 1974), and L-serine hydroxymethyltransferase (Voet et al., 1973). The last three enzymes are not aminotransferases but catalyze the transamination as a side reaction. Recently, we found that D-amino acid aminotransferase (DAT) and branched-chain L-amino acid aminotransferase (BCAT) catalyze the *re* face transfer of the hydrogen (Yoshimura et al., 1993). However, no information is available about the stereospecificity for the hydrogen transfer of ω-aminotransferases such as ornithine 5-aminotransferase (OAT, EC 2.6.1.13).

In ω-aminotransferase reactions, one of two prochiral hydrogens of the distal carbon is abstracted and transferred to the C-4′ of the bound cofactor. We have shown that the *pro-S* hydrogen is stereospecifically abstracted from C-5 of L-ornithine in the transamination catalyzed by OAT from a mesophile, *Bacillus sphaericus* (Tanizawa et al., 1982), which specifically acts on L-ornithine. We recently found that the thermostable OAT from *Bacillus* sp. YM-2 is unique in acting on both enantiomers of ornithine, although D-ornithine is poorer as an amino donor than the L-enantiomer (Jhee et al., 1995). This is the first example of an aminotransferase acting on both enantiomers of amino acids with a significant efficiency. The bonds to be cleaved and formed in PLP-conjugated enzyme reactions are suggested to be situated perpendicular to the planar π-system of the cofactor−substrate complex because of the low activation energy of the reaction with this configuration (Dunathan, 1971). The configuration of the bound ornithine in the active site of the *Bacillus* sp. YM-2 OAT probably is regulated by

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[1] Abbreviations: AspAT, aspartate aminotransferase; BCAT, branched-chain L-amino acid aminotransferase; DAT, D-amino acid aminotransferase; OAT, ornithine 5-aminotransferase; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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binding of the C-2 of ornithine with the enzyme protein. If C-2 of L-ornithine is bound to the same site of the OAT as that of the D-enantiomer, there exists the possibility that the hydrogens transferred from C-5 of L- and D-ornithine are stereochemically different.

EXPERIMENTAL PROCEDURES

Materials. OATs of *Bacillus* sp. YM-2 (Jhee et al., 1995) and *B. sphaericus* IFO 3525 (Yasuda et al., 1979) and amino acid racemase with low substrate specificity of *Pseudomonas putida* ATCC 17642 (Lim et al., 1993) were purified to homogeneity as described previously. BCAT of *Escherichia coli* (Kuramitsu et al., 1985) was a gift from Professor H. Kagamiyama of Osaka Medical Collage. AspAT from pig heart was purchased from Boehringer, Germany. ^3H_2O (3.7 GBq/g) was obtained from Du Pont (Wilmington, DE). Dowex 50-X4 was from Dow Chemicals (Midland, TX). The other chemicals were of analytical grade.

Hydrogen Exchange at C-5 of L*-Ornithine Catalyzed by the Bacillus sp. YM-2 OAT. Bacillus* sp. YM-2 OAT (600 μg) was dissolved in 1 mL of ^2H_2O containing 10 μmol of potassium phosphate buffer (p^{2}H 8.0), and H_2O was removed by repeated concentrations with a Centricon 10 concentrator (Amicon, Inc., Massachusetts). The enzyme solution (100 μL) was mixed with 600 μL of ^2H_2O containing 6 μmol of potassium phosphate buffer (p^{2}H 8.0) and 29.7 μmol of L-ornithine. The reaction mixture was incubated in the NMR tube at 25 °C and subjected to the NMR measurement at the appropriate times.

Preparation of L*- and* D*-[5-^{3}H]Ornithines.* L-[5-^{3}H]-Ornithine and D-[5-^{3}H]ornithine were prepared from L-ornithine and D-ornithine, respectively, with *Bacillus* sp. YM-2 OAT in ^3H_2O as follows. Each 40 μmol of L- and D-ornithine was dissolved in 200 μL of ^3H_2O (10 mCi/g) containing 30 μmol of Tris-HCl buffer (pH 8.0) and 0.025% (w/v) sodium azide. The reaction was started by addition of *Bacillus* sp. YM-2 OAT (80 μg, 6.08 units) and carried out at 30 °C for 24 h. The reaction mixture was brought to about pH 2 with 2 M HCl, followed by centrifugation. The supernatant solution was applied to a Dowex 50-X4 column (50−100 mesh, H^+ form, 0.5 × 5 cm). The column was washed with distilled water until the radioactivity was decreased below 1000 cpm per 100 μL, and each L- and D-[5-^{3}H]ornithine was eluted with 10 mL of 2 M HCl. The yields of L-[5-^{3}H]ornithine and D-[5-^{3}H]ornithine were 85 and 83%, respectively. The specific activities of L-[5-^{3}H]- and D-[5-^{3}H]ornithine were 3.67×10^4 and 3.9×10^2 dpm/μmol, respectively.

Configuration of C-5 of L*- and* D*-[5-^{3}H]Ornithines.* The reaction mixture containing 150 μmol of Tris-HCl buffer (pH 8.5), 34 μmol of L-[5-^{3}H]ornithine, 50 μmol of 2-oxoglutarate, and 1 unit of OAT from *B. sphaericus* in a final volume of 0.5 mL was incubated at 37 °C for 14 h. D-[5-^{3}H]-Ornithine (33.2 μmol) was also reacted with OAT from *B. sphaericus* under the same conditions as that for L-[5-^{3}H]-ornithine except that the reaction mixture contained 0.67 unit of the amino acid racemase of *Ps. putida*. After incubation, each reaction mixture was dried with a Speed Vac Concentrator (Savant, New York) and dissolved in 200 μL of distilled water. The procedure was repeated two times. A 2 μL aliquot of the final solution of the reaction mixture with L-[5-^{3}H]ornithine and a 90 μL aliquot of that with D-[5-^{3}H]-ornithine were subjected to radioactivity assay with a Packard Tri-Carb scintillation spectrometer with Clear-sol I (Nacalai Tesque, Kyoto, Japan) as a scintillator. The tritium abstracted from each L- and D-[5-^{3}H]ornithine was expressed as a volatile radioactivity, which was obtained by subtraction of the radioactivity finally remaining from that initially added to the reaction mixture.

Preparation of Apoenzyme. Bacillus sp. YM-2 OAT was incubated with 40 mM L-ornithine (pH 7.4) at room temperature for 10 h and dialyzed against 2 M guanidine hydrochloride in 20 mM Tris-HCl buffer (pH 8.0) containing 0.1% 2-mercaptoethanol for 10 h, followed by dialysis against 20 mM Tris-HCl buffer (pH 8.0) containing 0.1% 2-mercaptoethanol at 4 °C for 12 h. The resultant apoenzyme had no activity in the absence of PLP and recovered 85% of the initial activity by addition of 0.1 mM PLP. The reconstituted enzyme showed the same spectrum as the native holoenzyme. Apo-AspAT and apo-BCAT were prepared according to the previous methods (Yoshimura et al., 1993).

Preparation of (4′S)- and (4′R)-[4′-^{3}H]Pyridoxamine 5′-Phosphates. PMPs stereospecifically tritiated at C-4′ were prepared by incubation of randomly labeled [4′-^{3}H]PMP with the apo form of AspAT and BCAT (Yoshimura et al., 1993). AspAT and BCAT catalyze the stereospecific exchange of *pro-S* and *pro-R* hydrogen of C-4′ of PMP with the solvent hydrogen, respectively. (4′*S*)-[4′-^{3}H]PMP (1.20×10^6 dpm/μmol) and (4′*R*)-[4′-^{3}H]PMP (1.08×10^6 dpm/μmol) were thus obtained.

Stereospecificity of the Bacillus sp. YM-2 OAT for the Abstraction and Addition of Hydrogen at C-4′ of the Cofactor in the Half- and Overall Reactions. The reaction mixture (200 μL) for the half-reaction contained 10 μmol of Tris-HCl buffer (pH 8.0), 0.5 μmol of 2-oxoglutarate, 1.98 nmol of (4′*S*)-[4′-^{3}H]PMP or 1.65 nmol of (4′*R*)-[4′-^{3}H]PMP, and 6 nmol of the apo form of the *Bacillus* sp. YM-2 OAT. The reaction was carried out at 30 °C for 15 min and terminated by addition of 90 μL of 2 M HCl. The mixture was immediately frozen in liquid nitrogen and dried with a Speed Vac concentrator. The residue was dissolved in 100 μL of distilled water and subjected to the radioactivity assay. The hydrogen abstracted from C-4′ of the cofactor is theoretically transferred to the amino acceptor, but actually, the abstracted hydrogen is exchanged with that of the solvent during the transfer. The tritium released from PMP was expressed as a volatile radioactivity in the solvent. The reaction conditions for the overall transamination were the same as those for the half-reaction except that the reaction mixture contained 2 μmol of L- or D-ornithine.

RESULTS

Stereospecific Exchange of Hydrogen at C-5 of Ornithine with Solvent Deuteron in the Half-Reactions of Bacillus sp. YM-2 OAT. Figure 1 shows the ^{1}H-NMR spectral change of L-ornithine during the incubation with the *Bacillus* sp. YM-2 OAT in ^2H_2O. After incubation of the reaction mixture at 37 °C for 40 h, the peak integral of the C-5 protons was reduced to about a half, whereas those of other protons did not change. This shows that the *Bacillus* sp. YM-2 OAT

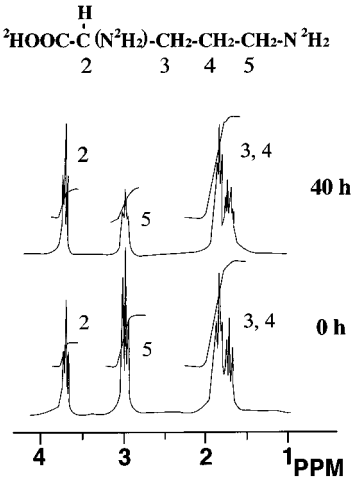

FIGURE 1: ^{1}H-NMR spectral change of L-ornithine during the incubation with thermostable *Bacillus* sp. YM-2 OAT in D_2O.

stereospecifically catalyzes the exchange of one of the two hydrogen atoms at C-5 with a solvent deuteron in the half-reaction of transamination. The *Bacillus* sp. YM-2 OAT acts on D-ornithine to produce the D-Δ^1-pyrroline-5-carboxylate, although slowly (Jhee et al., 1995). Accordingly, one of the hydrogens at C-5 of D-ornithine was expected to be abstracted during the transamination. However, no reduction of the peak integral of the C-5 protons of D-ornithine was observed in the half-transamination with the *Bacillus* sp. YM-2 OAT after 72 h under the same conditions as those for the half-transamination with L-ornithine. This is probably due to the very low reaction rate of the hydrogen exchange catalyzed by *Bacillus* sp. YM-2 OAT with D-ornithine as a substrate. The catalytic efficiency (V_{max}/K_m) for the transamination of D-ornithine by the *Bacillus* sp. YM-2 OAT is 0.17% of that of L-ornithine. We thus examined the C-5 hydrogen exchange of L- and D-ornithine with tritium.

Stereospecificity of the Bacillus sp. YM-2 OAT for the Hydrogen Abstraction from C-5 of L- *and* D-*Ornithines in ^3H_2O.* When L- and D-ornithines were incubated with the *Bacillus* sp. YM-2 OAT in ^3H_2O, they were tritiated. The specific radioactivities of D- and L-ornithines were 3.9×10^2 and 3.7×10^4 dpm/μmol, respectively. The rate of the tritium incorporation to D-ornithine was about 1% of that to L-ornithine. The exchange of a C-5 hydrogen of L-ornithine with a solvent deuteron in the half-transamination (Figure 1) suggests that the tritium was incorporated into C-5 of L-ornithine. Although no direct evidence for tritiation of D-ornithine at C-5 is available, the following result of the transamination of the tritiated D-ornithine catalyzed by the *B. sphaericus* OAT suggests that C-5 of D-ornithine was labeled.

The L-[5-^{3}H]ornithine prepared was incubated with the *B. sphaericus* OAT which specifically abstracts the *pro-S* hydrogen from C-5 of L-ornithine (Tanizawa et al., 1982). As shown in Table 1, 78.5% of the initial radioactivity is released into the solvent. Thus, the C-5 of the tritiated L-ornithine has the *S*-configuration. The tritiated D-ornithine also reacted with the *B. sphaericus* OAT in the presence or absence of the amino acid racemase with low substrate specificity of *Ps. putida*, which catalyzes the racemization of ornithine (Lim et al., 1993). In the presence of amino acid racemase, 85.6% of the initial radioactivity was released from the tritiated D-ornithine into the solvent. In contrast,

Table 1: Configuration of C-5 of L- and D-[5-^{3}H]Ornithine Prepared by Incubation of L- and D-Ornithine with the *Bacillus* sp. YM-2 OAT in ^3H_2O

	initial radioactivity (dpm)	released radioactivity[a] (dpm)	ratio[b] (%)
L-[5-^{3}H]ornithine	12200	9577	78.5
D-[5-^{3}H]ornithine			
−amino acid racemase[c]	2309	145	6.3
+amino acid racemase[d]	5994	4888	85.6

[a] Volatile radioactivity. [b] Ratio of the radioactivity released to that initially present in the reaction mixture. [c] The reaction mixture contained 10 μmol of Tris-HCl (pH 8.0), 6 μmol of D-[5-^{3}H]ornithine, 5 μmol of 2-oxoglutarate, and 0.15 unit of the *B. sphaericus* OAT in a final volume of 105 μL. Other conditions were the same as those for d. [d] The reaction conditions are given in the text.

Table 2: Stereospecificity of the *Bacillus* sp. YM-2 OAT for the Hydrogen Transfer between the Substrate and the Cofactor

	(4′*S*)-[4-^{3}H]PMP ^{3}H-released[a] (dpm)	(4′*R*)-[4-^{3}H]PMP ^{3}H-released (dpm)
+ 2-OG[b]	1715	0
+ 2-OG + L-ornithine	1349	0
+ 2-OG + D-ornithine	1000	0

[a] Volatile radioactivity. [b] 2-Oxoglutarate.

tritium was only slightly released into the solvent in the absence of the amino acid racemase (Table 1). The amino acid racemase does not act on C-5 of ornithine. When L-ornithine was incubated with the amino acid racemase in D_2O, the peak integral of the C-2 protons disappeared, whereas those of C-5 did not change (data not shown). Moreover, we confirmed that no tritium was released from L-[5-^{3}H]ornithine into the solvent on incubation with the amino acid racemase. Thus, tritium was abstracted from C-5 of the tritiated D-ornithine, after D-ornithine was converted to the L-enantiomer. These results suggest that the *Bacillus* sp. YM-2 OAT stereospecifically abstracts a *pro-S* hydrogen from C-5 of both D- and L-ornithines.

Stereospecificity of the Bacillus sp. YM-2 OAT for the Abstraction and Addition of Hydrogen at C-4′ of the Cofactor. We studied the stereospecificity of the *Bacillus* sp. YM-2 OAT for the abstraction and addition of hydrogen at C-4′ of the cofactor in the half and overall reactions. When (4′*S*)-[4′-^{3}H]PMP or (4′*R*)-[4′-^{3}H]PMP was incubated with the apo form of the *Bacillus* sp. YM-2 OAT in the presence of 2-oxoglutarate, tritium was exclusively released from (4′*S*)-[4′-^{3}H]PMP into the solvent (Table 2). Accordingly, the *pro-S* hydrogen at C-4′ of PMP is abstracted in the half-reaction. Stereospecificities for the abstraction and addition of hydrogen at C-4′ of the cofactor in the overall transaminations were also determined with L- and D-ornithines as a substrate. When each enantiomer of the stereospecifically tritiated PMP was incubated with the apoenzyme and 2-oxoglutarate in the presence of L- or D-ornithine, tritium was released from (4′*S*)-[4′-^{3}H]PMP into the solvent specifically (Table 2). This suggests that the abstraction and addition of hydrogen at C-4′ of the cofactor occur on the *si* face of the plane of the conjugated π-system of the intermediate in the overall transamination of the *Bacillus* sp. YM-2 OAT irrespective of the C-2 configuration of the amino donor.

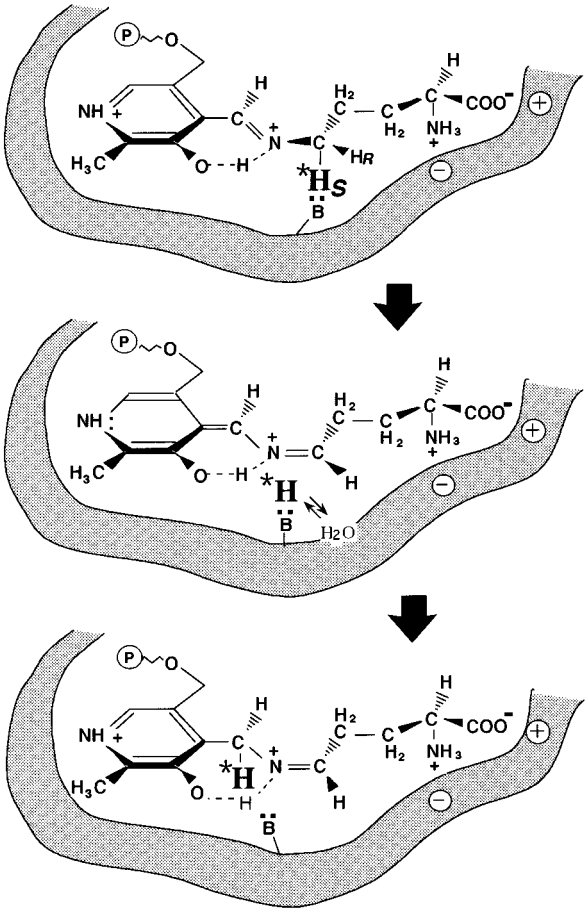

FIGURE 2: Hydrogen transfer by the *B*acillus sp. YM-2 OAT between C-5 of L-ornithine and C-4′ of cofactor in the transamination.

DISCUSSION

We here showed that the *pro-S* hydrogen of C-5 of D- and L-ornithine was specifically abstracted, and the addition and abstraction of hydrogen at C-4′ of the cofactor exclusively occur at the *pro-S* position in transamination by the *Bacillus* sp. YM-2. The intramolecular hydrogen transfer between C-2 of the substrate and C-4′ of the cofactor was observed in the pyridoxamine pyruvate aminotransferase (Dunathan, 1971) and AspAT (Gehring, 1984) reactions. The *pro-S* hydrogen abstracted from C-5 of D- or L-ornithine is probably transferred to the C-4′ of PLP on the *si* face of the planar π-system of the substrate−cofactor complex in transamination by the *Bacillus* sp. YM-2 OAT (Figure 2). The stereospecificity for the hydrogen transfer is not dependent on the configuration of ornithine. The K_m value for D-ornithine (17 mM) is 9 times higher than that for L-ornithine. Although the affinity of D-ornithine for the enzyme is lower than that for L-ornithine, the C-2 moiety of D-ornithine is bound to the same binding site as that for L-ornithine. The geometrical relationships between the C-5 of L- and D-ornithines and the plane of the π-electron system of the external Schiff base intermediates are probably the same.

Christen and his co-workers classified the aminotransferases into four subgroups on the basis of their primary structures (Mehta et al., 1993): subgroup I, aspartate aminotransferase, alanine aminotransferase, and others; subgroup II, acetylornithine aminotransferase, OAT, and others; subgroup III, D-amino acid aminotransferase (DAT) and branched-chain L-amino acid aminotransferase (BCAT); and subgroup IV, serine aminotransferase and phosphoserine aminotransferase. They suggest that aminotransferases belonging to subgroups I, II, and IV are considerably homologous in the structure. However, the primary structures of DAT (Tanizawa et al., 1989a) and BCAT (Kuramitsu et al., 1985) of subgroup III, which are significantly homologous with each other (Tanizawa et al., 1989b), are different from those of other aminotransferases. The recent crystallographical studies of DAT demonstrated that the fold of DAT is quite different from those of any other pyridoxal enzymes studied so far (Sugio et al., 1995). DAT and BCAT are probably evolved from an ancestral protein which differed from those of other aminotransferases belonging to subgroups I, II, and IV. We recently showed that the stereospecificities for the hydrogen transfer reflect the evolutionary relationship of the aminotransferases. We found that DAT and BCAT catalyze the transfer of *pro-R* C-4′ hydrogen of PMP (Yoshimura et al., 1993). In contrast, AspAT (Besmer & Arigoni, 1969) and alanine aminotransferase (Austermuhle-Bertrola, 1973) belonging to subgroup I and dialkylamino acid aminotransferase (Bailey et al., 1970) which was not cited to the above evolutionary pedigree trees but is homologous to human ornithine aminotransferase were reported to catalyze the *pro-S* hydrogen transfer. The stereospecificity reflects the active-site structure of the enzyme, especially the geometrical relationship between the catalytic base of the enzyme for the hydrogen transfer and the bound cofactor. The relative topological arrangement of the catalytic base of DAT to the C-4′ of the cofactor is opposite to that of AspAT (Sugio et al., 1995). We here showed that the *Bacillus* sp. YM-2 OAT catalyzes the transfer of *pro-S* C-4′ hydrogen of PMP between the cofactor and the substrate. This is the first example of an ω-aminotransferase whose stereospecificity for the hydrogen transfer is shown. The three-dimensional structure of human OAT whose primary structure is highly homologous to that of the *Bacillus* sp. YM-2 OAT (Jhee et al., 1995) has been reported to resemble that of AspAT (Shen et al., 1994). The primary structures of aminotransferases, their three-dimensional structures, and their stereochemistry for the hydrogen transfer coincide with one another. The results obtained support the idea that stereospecificities of aminotransferases for the hydrogen transfer reflect their molecular evolution (Dunathan & Voet, 1974).

REFERENCES

Austermuhle-Bertrola, E. (1973) Ph.D. Dissertation No. 5009, ETH, Zurich, Switzerland.

Ayling, J. E., Dunathan, H. C., & Snell, E. E. (1968) *Biochemistry 7*, 4532−4542.

Bailey, G. B., Kusamrarn, T., & Vuttivej, K. (1970) *Fed. Proc. 29*, 857.

Besmer, P., & Arigoni, D. (1969) *Chimia 23*, 190.

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

Dunathan, H. C., & Voet, J. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3888−3891.

Gehring, H. (1984) *Biochemistry 23*, 6335−6340.

Jhee, K.-W., Yoshimura, T., Esaki, N., Yonaha, K., & Soda, K. (1995) *J. Biochem. 118*, 101−108.

Kuramitsu, S., Ogawa, T., Ogawa, H., & Kagamiyama, H. (1985). *J. Biochem. 97*, 993−999.

Lim, Y.-H., Yokoigawa, K., Esaki, N., & Soda, K. (1993) *J. Bacteriol. 175*, 4213−4217.

Martinez-Carrion, M., Hubert, E., Iriarte, A., Mattingly, J. R., & Zito, S. W. (1985) in *Transaminases* (Christen, P., & Metzler, D. E., Eds.) pp 308−316, John Wiley & Sons, New York.

Mehta, P. K., Hale, T. I., & Christen, P. (1993) *Eur. J. Biochem. 214*, 549−561.

Shen, B. W., Burcham, J., Ramesh, V., Gardner, P., Henning, M., Mueller, R., Hohenester, E., & Jansonius, J. N. (1994) in *Abstracts of 9th Meeting Vitamin B6 and Carbonyl Catalysis and 3rd Symposium PQQ and Quinoproteins*, Capri, Italy, p 115.

Sugio, S., Petsko, G. A., Manning, J. M., Soda, K., & Ringe, D. (1995) *Biochemistry 34*, 9661−9669.

Sukhareva, B. S., Dunathan, H. C., & Braunstein, A. E. (1971) *FEBS Lett. 15*, 241−244.

Tanizawa, K., Yoshimura, T., Asada, Y., Sawada, S., Misono, H., & Soda, K. (1982) *Biochemistry, 21*, 1104−1108.

Tanizawa, K., Masu, Y., Asano, S., Tanaka, H., & Soda, K. (1989a) *J. Biol. Chem. 264*, 2445−2449.

Tanizawa, K., Asano, S., Masu, Y., Kuramitsu, S., Kagamiyama, H., Tanaka, H., & Soda, K. (1989b) *J. Biol. Chem. 264*, 2450−2454.

Voet, J. G., Hindenlang, D. M., Blanck, T. J. J., Ulevitch, R. J., Kaljen, R. G., & Dunathan, H. C. (1973) *J. Biol. Chem. 248*, 841−842.

Yasuda, M., Misono, H., Soda, K., Yonaha, K., & Toyama, S. (1979) *FEBS Lett. 105*, 209−212.

Yoshimura, T., Nishimura, K., Ito, J., Esaki, N., Kagamiyama, H., Manning, J. M., & Soda, K. (1993) *J. Am. Chem. Soc. 115*, 3987−3900.

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