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

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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. [5-^{3}H]L- and D-ornithines were prepared by incubation of L- and D-ornithines with the *B.* sp. YM-2 ornithine 5-aminotransferase in $^{3}H_2O$, respectively. The C-5 of the tritiated L-and D-ornithine was proved to have the *S*-configuration with L-ornithine 5-aminotransferase of a mesophile, *Bacillus sphaericus*, catalyzing the stereospecific abstraction of pro-*S* hydrogen from C-5 of L-ornithine and amino acid racemase with low-substrate specificity of *Pseudomonas putida* . When apo-form of the enzyme was incubated with pyridoxamine 5'-phosphates 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. These results suggest that the *B.* sp. YM-2 ornithine 5-aminotransferase stereospecifically abstracts the pro-*S* hydrogen from C-5 of L- and D-ornithine. The hydrogen abstracted is then transferred to C-4' of the cofactor moiety stereospecifically on the *si* face of the external Schiff base intermediate irrespective of the C-2 configuration of amino donor.

Keywords: Stereochemistry/ Ornithine transaminase/ Pyridoxal phosphate

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. The hydrogen abstracted is transferred to C-4' of the cofactor, and consequently the pyridoxamine 5'-phosphate (PMP) form of enzyme and keto acid are produced through the ketimine

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Scope of research

Structure and function of biocatalysis, in particular, pyridoxal enzymes, NAD enzymes, and enzymes acting on xenobiotic compounds are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of selenium and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of thermostable enzymes and their application are under investigation.

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intermediate. Three stereochemical possibilities exist for the hydrogen transfer: the stereospecific transfer on the *si*- or *re*-face, alternatively the non-stereospecific transfer on both faces of the plane of the π-electron system of the intermediate. The stereospecificities of various α-aminotransferases for the hydrogen transfer have been examined. However, no information is available about the stereospecificity of ω-aminotransferases such as ornithine 5-aminotransferase (OAT, EC 2.6.1.13). The stereospecificity reflects the active-site structure of the enzyme, especially the geometrical relationship between the catalytic base of enzyme for the hydrogen transfer and the bound cofactor. Thus, we determined the stereospecificity for the hydrogen transfer catalyzed by the thermostable OAT from *Bacillus* sp. YM-2 (1). The enzyme is unique in acting on both enantiomers of ornithine, although D-ornithine is poorer as an amino donor than the L-enantiomer (2).

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. At first, we determined the stereospecificity for the hydrogen abstraction from C-5 of L- and D-ornithine catalyzed by the thermostable OAT from *B.* sp. YM-2. When L- and D-ornithines were incubated with the *B.* sp. YM-2 OAT in $^{3}H_2O$, they were tritiated. The specific radioactivities of L- and D-ornithines were 3.9 x 10^{2} and 3.7 x 10^{4} (dpm/mmol), respectively. The rate of the tritium incorporation to D-ornithine was about 1 % of that to L-ornithine. The tritium was probably incorporated into C-5 of L- and D-ornithine. Because, ^{1}H-NMR spectral change of L-ornithine during the incubation with the *B.* sp. YM-2 OAT in $^{2}H_2O$ demonstrated that the enzyme catalyzes the exchange of one of the two hydrogen atoms at C-5 with a solvent deuteron in the half reaction of transamination.

When 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 (3), 78.5 % of the initial radioactivity was 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. 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, tritium was only little released into the solvent in the absence of the amino acid racemase. The amino acid racemase does not act on C-5 of ornithine. 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 *B.* sp. YM-2 OAT stereospecifically abstracts a pro-*S* hydrogen from C-5 of both D- and L-ornithines.

Then, we studied the stereospecificity of the *B.* sp. YM-2 OAT for the abstraction and addition of hydrogen at C-4' of the cofactor in the half and overall reactions according to the method of Yoshimura et al (4). When (4'*S*)-[4'-^{3}H] PMP or (4'*R*)-[4'-^{3}H] PMP was incubated with the apo-form of the *B.* sp. YM-2 OAT in the presence of 2-oxoglutarate, tritium was exclusively released from (4'*S*)-[4'-^{3}H] PMP into the solvent. 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 apo-enzyme 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. 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 *B.* sp. YM-2 OAT irrespective of the C-2 configuration of amino donor.

In the α-transaminase reactions, the intramolecular hydrogen transfer between C-2 of the substrate and C-4' of the cofactor was observed (5). 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 the transamination catalyzed by the *B.* sp. YM-2 OAT. The stereospecificity for the hydrogen transfer is not dependent on the configuration of 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 the same. The C-2 moiety of D-ornithine is probably bound to the same binding-site as that for L-ornithine.

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