Non-stereospecific Transamination Catalyzed by Pyridoxal Phosphate-dependent Amino Acid Racemases of Broad Substrate Specificity

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Pyridoxal 5’-phosphate-dependent amino acid racemases of broad substrate specificity catalyze transamination as a side-reaction. We studied the stereospecificities for hydrogen abstraction from C-4’ of the bound pyridoxamine 5’-phosphate during transamination from pyridoxamine 5’-phosphate to pyruvate catalyzed by three amino acid racemases of broad substrate specificity. When the enzymes were incubated with (4’S)- or (4’R)-[4’-^3H]-pyridoxamine 5’-phosphate in the presence of pyruvate, tritium was released into the solvent from both pyridoxamine 5’-phosphates. Thus, these enzymes abstract a hydrogen non-stereospecifically from C-4’ of the coenzyme in contrast to the other pyridoxal 5’-phosphate-dependent enzymes so far studied which catalyze the stereospecific hydrogen removal. Amino acid racemase of broad substrate specificity from Pseudomonas putida produced D- and L-glutamate from α-ketoglutarate through the transamination with L-ornithine. Because glutamate does not serve as a substrate for racemization, the enzyme catalyzed the non-stereospecific overall transamination between L-ornithine and α-ketoglutarate. The cleavage and formation of the C-H bond at C-4’ of the coenzyme and C-2 of the substrate thus occurs non-stereospecifically on both sides of the plane of the coenzyme-substrate complex intermediate. Amino acid racemase of broad substrate specificity is the first example of a pyridoxal enzyme catalyzing non-stereospecific transamination.

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Although enzymatic racemization of amino acid is apparently simple, consisting of a non-stereospecific rearrangement of the substrate α-hydrogen, several different types of amino acid racemases are found in microorganisms. Aspartate racemase and glutamate racemase are independent of cofactors. Alanine racemase in several microorganisms, and amino acid racemases of broad substrate specificity of Pseudomonas putida depend on pyridoxal 5’-phosphate (PLP). The reaction of amino acid racemase is initiated by transaldimination. In this step, PLP bound with the active-site lysyl residue through an internal Schiff base (Scheme I, A) reacts with a substrate to form an external Schiff base (B). The subsequent α-hydrogen abstraction results in the formation of a resonance-stable anionic intermediate (C). If the reprotonation occurs at C-2 of the substrate moiety on the...
The absorption spectral change has demonstrated that ketoglutarate as well.

Overall transamination between L-ornithine and transamination catalyzed by the amino acid racemases (1).

Removal from C-4' of PMP occurs randomly on both faces of the plane of the enantiomeric intermediate. Amino acid racemases are reported to catalyze the transamination as a side reaction. The transamination catalyzed by amino acid racemases can be attained through a sequence either A → B → F (or G) → H, or A → B → C → F (or G) → H (Scheme I). An equivalent route can be delineated for the antipode: E → D → F (or G) → H, or E → D → C → F (or G) → H. In transamination, mutual hydrogen transfer between the substrate and C-4' of the cofactor occurs. In all previous studies of transaminations catalyzed by aminotransferases as well as other pyridoxal enzymes, the hydrogen transfer between substrate and cofactor occurs strictly stereospecifically on the si or re face of the plane of the anionic intermediate. However, if the transamination catalyzed by amino acid racemases proceeds as depicted in Scheme I, the hydrogen transfer should occur non-stereospecifically on both faces of the planar intermediate. We were therefore interested in the stereospecificity for the hydrogen transfer during transamination catalyzed by amino acid racemases as a side-reaction, and provide the first evidence that hydrogen removal from C-4' of PMP occurs randomly on both faces of the substrate-cofactor imine plane during half transamination catalyzed by the amino acid racemases (1). We also show that the enzyme catalyzes non-stereospecific overall transamination between L-ornithine and α-ketoglutarate as well.

The absorption spectral change has demonstrated that the amino acid racemase of broad substrate specificity from Ps. putida catalyzes the transamination between PMP and pyruvate. When the PMP-form of an enzyme is converted to the PLP form by transamination with keto acid, one of the two hydrogens at C-4' of PMP is usually transferred stereospecifically to Cα of the keto acid. We studied the stereospecificity of amino acid racemase for hydrogen abstraction from C-4' of PMP by measurement of the radioactivity of 3H released from the PMPs which are stereospecifically tritiated at C-4' using the method described previously (2). Each 5 nmol of apo-amino acid racemase was incubated with 1 nmol of (4'R)- or (4'R)-[4'-3H]PMP and 5 nmol of sodium pyruvate. We deduce that PMP was completely converted to PLP because the PMP-form of the amino acid racemase from Ps. putida recovered 100% of the activity theoretically expected. Tritium was released equally from both (4'S)- and (4'R)-[4'-3H]PMPs in the presence of amino acid racemases. The amount of tritium released from each PMP was about 50% of that which initially existed. The control experiment with D-AAT and AspAT showed that they catalyzed the stereospecific removal of tritium from (4'R)- or (4'S)-[4'-3H]PMP, respectively. These results confirm the stereospecific tritium labelling of both PMPs. Thus, the amino acid racemases catalyze the non-stereospecific abstraction of hydrogen from C-4' of PMP. They are the first class of pyridoxal enzyme catalyzing the hydrogen removal on both sides of the plane of a substrate-cofactor complex during transamination.

If the hydrogen is introduced non-specifically to C-2 of the keto acid moiety of the anionic intermediate on both sides of the planar intermediate during the half reaction of transamination, racemic amino acid is formed from the keto acid (Scheme I; H → F (or G) → C → B → A, or H → F (or G) → C → D → E). We studied the stereochemistry of glutamate formed from α-ketoglutarate by transamination with L-ornithine catalyzed by the amino acid racemase of Ps. putida. After the reaction, the products were derived to diastereomers with Marfey’s reagent, and subjected to HPLC. Both enantiomers of glutamate and ornithine were found. The amino acid racemase from Ps. putida catalyzes the racemization of ornithine, but glutamate is inert as a substrate for the racemase reaction. Thus, both enantiomers of glutamate were directly formed by transamination, not by racemization of one enantiomer produced through transamination. The amino acid racemase from Ps. putida is the first example of a pyridoxal enzyme catalyzing non-stereospecific transamination.

References