D-AMINO ACID RESEARCH: MY RETROSPECT AND PERSPECTIVE

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Introduction

D-Amino acids were once regarded as unnatural compounds, but that is not the case. It is now highly probable that D-amino acids prebiologically occurred in the primordial soup. The L-amino acids of the racemates synthesized on the primeval Earth were selectively incorporated into proteins leading to the production of enzymes and eventually the birth of the first organisms. Accordingly, the first organisms were quite likely emerged in the presence of racemic or D-amino acids. In fact, D-amino acids are found in both free and petidyl forms in hyperthermophilic archaea, which are presumed to be the most primitive organisms. Various D-amino acids are now demonstrated not only microorganisms, but also in higher plants, insects, marine invertebrates and mammals (1-3). Only recently, it was reported that D-histidine and D-lysine react with respective aminoacyl-tRNA synthetases of *Escherichia coli* (4) : D-amino acids may be incorporated into usual proteins to produce unusual proteins, though very slowly.

In the late 1950's when we began to study D-amino acid biochemistry, only a few scientists were interested in D-amino acids, and we were engaged in enzymological study of microbial L-amino acid aminotransferases. Only several kinds of L-amino acids were commercially available, and expensive. Accordingly, we synthesized the racemates chemically, and resolve them by means of aminoacylase isolated from mammalian tissues or fungi to prepare L-amino acids. D-Amino acids were produced as side-products. We first studied microbial aminotransferases by using L-amino acids prepared, and found a few new aminotransferases such as L-lysine ε -aminotransferase(5) and taurine aminotransferse(6). We undertook screening the enzymes participating in D-amino acid metabolism as well. I describe here a brief account of my biochemical research on D-amino acids with a perspective.

Results and Discussion

We found that various species of *Bacillus* abundantly produce D-amino acid aminotransferase, and purified and characterized the enzymes from *B. sphaericus*(7) and a moderate thermophile, *Bacillus* sp. YM-1 isolated from a dust of sauna(8). The enzyme from the thermophile is thermostable. We showed the structure, enzymological properties, reaction mechanism, suicide-substrate reaction mechanism and stabilization mechanism by using the enzyme produced by the clone cells of *E. coli* (9). The stereospecificity of enzyme is absolute: D-amino acids are the exclusive amino donors and the products from the amino acceptors. However, the structural specificity is very low: a variety of D-amino acids and 2-oxo acids are the amino donors and acceptors, respectively. The primary structure of D-amino acid aminotransferase is significantly homologous to that of branched-chain

L-amino acid aminotransferase of *E. coli*, though both enzymes fall into the same subgroup of aminotrqnsferases on the basis of the primary structure, and show no similarity to all other aminotransferases(10). The circular dichroism spectra of various L-amino acid aminotransferases such as aspartate aminotransferase show positive bands at the wavelengths where their internal Schiff base intermediate absorbs, but that of D-amino acid aminotransferase shows the negative band at 430 nm.

The aminotransferase reaction proceeds through an external Schiff base (Fig. 1). D-Amino acid aminotransferase and branched-chain L-amino acid aminotransferase show the unique stereospecificity of hydrogen transfer between C-1 of the amino acid moiety of aldimine Schiff base intermediate and C-4' of the coenzyme moiety of ketimine Schiff base intermediate through the carbanionic intermediate. Both catalyze the *pro-R* specific hydrogen transfer in contrast to other aminotransferases such as aspartate aminotransferase and ornithine δ -aminotransferase as shown in Fig. 2 (11). The three- dimensional structure of D-amino aminotransferase is peculiar. The catalytically important amino acid residues including the internal Schiff base lysine 145 are located on the *re*-face side in contrast to aspartate aminotransferase, in which the residues are shown on the *si*-face side(12). Thus, D-amino acid aminotransferase is peculiar in the substrate specificity, the primary structure, the stereostructure of the active center and the stereospecificity for the hydrogen transfer.

Amino acid racemases are unique in the substrate stereospecificity and mode of the reaction. They apparently catalyze a simple reaction, namely the removal of α -hydrogen from either enantiomer of a substrate amino acid, and subsequently a non-stereospecific reprotonation occurs to attain equilibrium between both enantiomers. Almost all the enzymes catalyze various reactions with only one of an enantiomeric and diastereomeric pair of chiral compounds, but the racemases lack the stereospecificity. Most of them require pyridoxal phosphate as a coenzyme, but some others such as glutamate racemase and proline racemase require no coenzymes and no metals: they are diverse in the coenzyme requirement. Almost all the racemases show practically the absolute substrate specificity. Glutamate racemase acts exclusively on D- and L-glutamates. In contrast, arginine racemase and amino acid racemase with low substrate specificity from *Pseudomonas* bacteria found in our

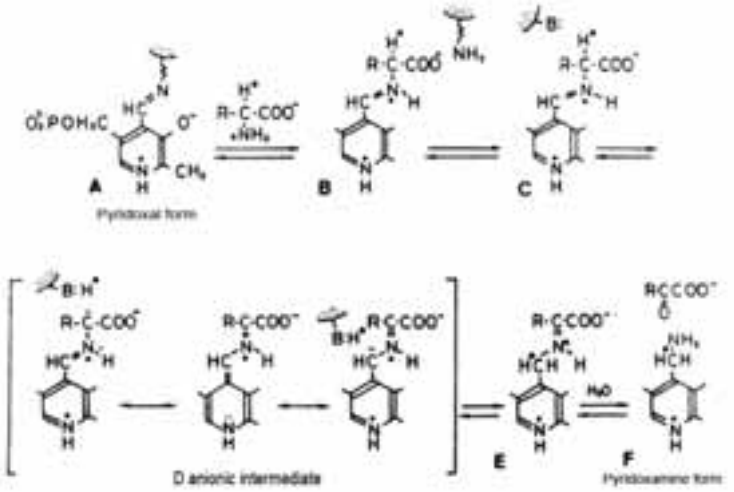

Fig. 1. Reaction Mechanism of Aminotransferase (Half-reaction)

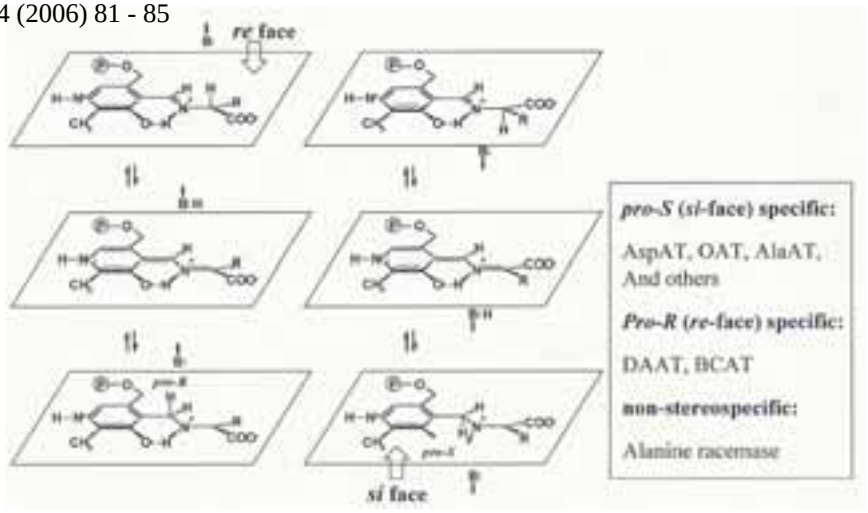

Fig. 2. Stereochemistry of Hydrogen Transfer by Aminotransferase.
AspAT; Aspartate Ainotransferase OAT; Ornithine δ-Aminotransferase AlaAT; Alanine Aminotransferase DAAT; D-Amino Acid Aminotransferase BCAT; Branched-Chain Aminotransferase

laboratory catalyze the racemization of a variety of amino acids except for acidic and aromatic amino acids (13). We showed that the hydrogen is transferred non-stereospecifically between the amino acid C-1 of the aldimine Schiff base intermediate and the coenzyme C-4' of the ketimine intermediate in the transamination catalyzed as a side reaction by alanine racemase (Fig 2).

Glutamate racemase is a typical coenzyme-independent amino acid racemase. We characterized the racemase from a lactic acid bacterium, *Pediococcus pentosaceus* (14). The enzyme consists of a single subunit with molecular weight of about 29,000, and contains an essential cysteine residue at the active center, which probably serves as a catalytic base to abstract the substrate α-hydrogen. It shows no structural similarity to other enzymes, but the structure of active center is closely similar to the heme binding site of hemoglobin and myoglobin. Hemine stoichiometrically binds to the enzyme active center to inhibit the activity.

Several species of *B. subtilis*, in particular the producers of Japanese and Korean soybean fermented food, Natto and Chungkookjang, respectively produce viscous poly-γ-glutamate in abundance, which is a polycation composed of D-glutamate and L-glutamate with the molar ratio of about 80:20. The ratio depends on the species and the growth conditions. We showed the occurrence of two isozymes of glutamate racemase, Glr and YrpC. Glr participates in the poly-γ-glutamate synthesis, and YrpC is produced only on cell division, and involved in the synthesis of petidoglycan of cell wall by a supply of D-glutamate, an essential constituent.

Poly-γ-glutamate synthetase catalyzes synthesis of the polymer from D- and L-glutamates in the presence of ATP(15). The γ-carboxyl group of a C-terminal glutamate residue of the growing poly-γ-glutamate is first activated to form a phosphate ester with hydrolysis of ATP. An amino group of a substrate glutamate attacks the activated carboxyl group as a nucleophile to elongate the polymer: this is an ATP and glutamate-dependent γ-ligase reaction. We showed that poly-γ-glutamate synthetase consists of three components. Component PgsB, which is found inside the cell membrane, catalyzes the ATP and glutamate-dependent γ-ligase reaction probably in association with PgsC. This component occurs in the hydrophobic region of

cell membrane. Component PgsA is not involved in the poly-γ-glutamate chain elongation directly, but severs as a kind of transporter, and protects the polymer formed from the enzymatic and non-enzymatic depolymerization. The synthetase reaction is peculiar. The D,L- ratio of the poly-γ-glutamate is not regulated by the enzyme, but depends on the D,L-ratio of substrate glutamate, which is controlled by glutamate racemase Glr. The three dimensional structure of synthetase and the detailed reaction mechanism have not yet been elucidated.

Perspective of the Future

Great strides have been made in D-amino acid research since I was first engaged in the research. However, we are not yet systematic in the biochemistry of D-amino acids in comparison with that of L-amino acids for various reasons. For example, analytical techniques for the specific and accurate determination of D-amino acids have not been fully advanced. The development of effective analytical methods for free and bound D—amino acids will lead to elucidation of the occurrence and metabolism of D-amino acids in various organisms. Only several kinds of enzymes involved in D-amino acid metabolism have been studied in detail, and various other important enzymes such as D-glutamate cyclase, D-amino acid dehydrogenase and hydoxyproline epimerase have been little investigated. The detailed information of D-amino acid metabolizing enzymes will result in elucidation of metabolic and physiological functions of D-amino acids. The easy availability of pure D-amino acids uncontaminated with the L-enantiomers is also desired to get the accurate information of D-amino acid biochemistry.

The elucidation of physiological roles of D-amino acids will open a new field of bioscience and biotechnology. Some of D-amino acids such as D-tryptophan taste very sweet, and may be used as a sweetener or a medicine in bound forms, if more effective methods for production of D-amino acids are developed and industrialized. The research of molecular evolution of D-amino acid -metabolizing enzymes and D-amino acids also will stimulate biochemistry and molecular biology.

References

(1) Meister, A. (1965) *Biochemistry of the Amino Acids*(2nd edition)113-118, New York: Academic Press.

(2) Jolles, P. (ed) (1998) D-Amino Acids in Sequences of Secreted Peptides of Multicellular Organisms, Basel, Birkhauser Verlag

(3) Fujii, N., Saito, T., (2004) Homochirality and life. *Chem Rec 4*, 267-278

(4) Takayama, T., Ogawa, T., Hidaka, M., Shimizu, Y., Ueda, T., Masaki, H. (2005) Esterification of Eschericia coli tRNAs with D-histidine and D-lysine by aminoacyl-tRNA synthetases. *Biosci. Biotechnol. Biochem.* 69, 1040-1041

(5) Soda, K., Misono,H. (1968) L-Lysine-α-Ketoglutarate Aminotransferase. II. Purification, Crystallization and Properties. *Biochemistry*, 7, 4110-4119

(6) Toyama, S., Soda,K., (1972) Occurrence of Taurine: α-Ketoglutarate Aminotransferae in Bacterial Extracts. *J. Bacteriol.* 109, 533-538

(7) Yonaha, K., Misono, H., Yamamoto, T., Soda,K.,(1975) D-Amino Acid Aminotransferae of *Bacillus sphaericus*: Enzymological and Spectrophotometric Properties. *J. Biol. Chem.* 250, 6983-6989

(8) Tanizawa, K., Masu,Y., Asano, S., Tanaka, H., Soda, K., (1989) Thermostable D-Amino Acid Aminotransferase from a Thermophilic *Bacillus* Species. *J. Biol. Chem.* 264, 2445-2449

(9) Sugio, S. Petsko, G. A., Manning, J. M., Soda, K., Ringe, D. (1995) Crystal Structure of a D-

Amino Acid Aminotransferase:How the Protein Controls Stereospecificity. *Biochemistry,* 34, 9661-9669

(10) Tanizawa, K., Asano,S., Masu., Kuramitsu, S., Kagamiyama, H., Tanaka, H. Soda.,K. (1989) The Primary Structure of Thermonstable D-Amino Acid Aminotransferase from a Thermophilic *Bacillus* Species and Its Correlation with L-Amino Acid Aminotransferase. *J. Biol. Chem.*264, 2450-2454

(11) Yoshimura T., Nishimura, K., Ito, J., Esaki, N., Kagamiyama, H., Manning, J.M., Soda, K. (1993) Unique stereospecificity of D-amino acid aminotransferase and branched-chain L-amino acid aminotransferase for C-4'hydrogen transfer of the coenzyme. *J. Amer. Chem. Soc*. 115. 3897-3900

(12) Yorifuji, T., Misono, H., Soda, K., (1971) Arginine Racemase of *Pseudomonas graveolens*. Part I. Purification, Crystallization and Properties. *J. Biol. Chem.*, 246, 5085-5092

(13) Soda, K.,Osumi, T. (1969) Crystalline Amino Acid Racemase with Low Substrate Specificity. *Biochem. Biophys. Res. Commun.* 35, 363-366

(14) Choi, S. –Y., Esaki, N., Yoshimura, T., Soda,K. (1991) Overproduciton of Glutamate Racemase of *Pediococcus pentosaceus* in *Escherichia coli* Clone Cells and Its Purification. *Protein Expression and Purification* 2, 90-93

(15) Ashiuchi, M., Soda, K., Misono, H. (1999) a Poly-γ-glutamate synthetic system of *Bacillus subtilis* IFO 3336: Gene Cloning and Biochemical Analysis of Poly-γ-glutamate produced by *Eschrichia coli* clone cells. *Biochem. Biophys. Res. Commun.* 263, 6-1