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Review

Revisit of aminotransferase in the genomic era and its application to biocatalysis

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Abstract

Aminotransferases (ATs) have useful applications in the chemical industry because of their capability of introducing amino group into ketones or keto acids as well as their high enantioselectivity and regioselectivity and broad substrate specificity. Abundant protein sequence databases and new powerful tools such as advanced computational structure modeling, multiple sequence analysis, and in vitro evolution have made it possible to understand the detailed reaction mechanisms of various ATs and to isolate and design novel enzymes for unnatural substrates. This, in turn, suggests that developing new integrated approaches to screen ATs are possible, but at the same time poses formidable technical challenges. Here, this paper reviews the use of family profile analysis to find the correlation between the type of ATs and their substrate specificities, the relation between the 3-D structures of ATs and their substrate specificities, and enzyme engineering for the synthesis of unnatural substrates.

Keywords: Aminotransferase; Biosynthesis; Subgroup; Screening; Inhibition

Contents

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1. Introduction

Aminotransferases (ATs) (EC 2.6.1.X) are pyridoxal 5′-phosphate (PLP)-dependent enzymes which are ubiquitous in nature and play an important amino group transferring role in nitrogen metabolism in cells. ATs have many advantages over other systems such as no requirement of external addition of cofactor, high enantioselectivity and regioselectivity, broad substrate specificity, and high reaction rate and stability [1]. Because of these characteristics, ATs have become one of the several industrially used enzymes to produce amino acids, unnatural amino acids, chiral amines, amino alcohols, and amino sugars, which are valuable key intermediates or starting materials for chiral drugs and agricultural products [2–4]. However, the development of an AT reactions for industrial purposes usually has several obstacles that must be overcome such as substrate/product inhibition and expansion of substrate specificity.

Since ATs are essential enzymes for the nitrogen metabolism in cell, most of the organisms have at least several ATs, and a nitrogen fixing microorganism like *Mesorizobium loti* has 50 ATs including putative ATs with unidentified roles [5]. As of July 2005, 77 ATs are listed in BRENDA (http://www.brenda.uni-koeln.de), and the number of the lists will steadily increase. ATs are classified into five subgroups on the basis of multiple sequence alignments and protein structures in Pfam (http://www.sanger.ac.uk/Software/Pfam/). Table 1 lists the notable ATs widely used in biotechnology, their main substrates, and PDB ID codes of the crystal structures of the ATs from different species. To discover new ATs with different substrate specificities compared with the existing ATs, two different approaches are plausible. The first choice of traditional approach would be extensive screening with automation of screening procedure after chemical random mutagenesis, in vitro evolution and/or enrichment culture [6–8]. As the second approach, in parallel, recent development in bioinformatics and computational chemistry enables us *in silico* screening and analysis of target enzymes. Multiple sequence analysis, three-dimensional (3-D) structure modeling, chemical library generation, and high throughput ligand docking become powerful tools to sub-classify enzymes, to understand their detailed reaction mechanisms of the enzymes which belong to the same superfamily, and to screen and design novel enzymes and inhibitors [9–11]. When the above experimental and computational approaches are optimally integrated, very useful and powerful tools to screen target enzymes with small changes in protein structures and substrate specificities can be developed in the genomic era. Related to the integrated approaches, we would like to review recent technology developments and limitations in the applications of ATs to biocatalysis.

2. Reaction mechanism and classification

All PLP-dependent enzymes including ATs, decarboxylases, and lyases, etc., follow the same mechanism in the first half reaction, but their individual functions are determined by different subsequent half reactions depending upon the possibility of the abstraction of the α-proton. They can be divided into four subgroups (α family, β family, D-alanine aminotransferase family, and alanine racemase family) based on their structural information [12]. In the case of AT, all ATs except branched-chain amino acid aminotransferases (BCATs) and D-aminotransferases (DATs) belong to the α family, whereas BCATs and DATs belong to the D-alanine aminotransferase family.

The transamination reaction by AT consists of two half reactions [13,14]. Each half reaction comprises three major steps. The first step is transaldimination, where the internal Schiff base between the PLP and a ε-NH_2 of a lysine residue in the enzyme active site is replaced by a Schiff base between the amino substrate (amino donor) and the PLP, forming an external aldimine. The lysine residue released during this process becomes the catalytic base in the next step. The second step is a 1,3 hydrogen shift involving the abstraction of the α-proton from the external aldimine and reprotonation to the imine of the coenzyme to yield a ketimine intermediate. The final step in the first half reaction is hydrolysis of the ketimine to release the oxo product and the pyridoxamine 5′-phosphate (PMP) enzyme. The second half reaction involving amino acceptor produces the corresponding amino product and regenerates the PLP form.

According to the above reaction mechanism, all ATs have a catalytic lysine which forms a Schiff base with the PLP, and aspartate or glutamate which forms a salt bridge with the pyridine N-1 of the PLP in the active site. The phosphate group of the PLP is stabilized by hydrogen bonds with the highly conserved residues such as serine, tyrosine, arginine, aspartate, and threonine in the active site. In terms of 3-D structure, the active site of AT is composed of two binding pockets (Fig. 1). One is large and the other is small. The amino group of the substrate is placed in the middle of the binding pockets toward the PLP.

ATs can be divided into five subgroups based upon their multiple sequence alignments in Pfam database (Table 1). The selected ATs below in the five subgroups in Table 1 are very useful in many industrial applications, so that remarkable structural and chemical properties of the ATs are illustrated in detail.

2.1. Aspartate aminotransferases (AspATs) and aromatic aminotransferases (AroATs)

AspATs and AroATs are the most widely studied ATs. In general, AspATs and AroATs use L-aspartate and L-

Table 1
Subgroups of aminotransferases, their PDB IDs and their main substrates

Subgroup[a]	Enzyme[b]	PDB ID[c]	Main substrates	
			Amino donor	Amino acceptor
I and II	AspAT	1AAT, 1AJS, 1AMA, 1AMQ, BJW, 1J32, 1O4S, 1YAA, 2CST, 5BJ3	L-Aspartate	2-Ketoglutarate
	AlaAT	1HOC, 1VJO	L-Alanine	2-Ketoglutarate
	AroAT	1AY5, 1BWO, 1DJU, 3TAT	L-Phenylalanine	2-Ketoglutarate
	HisPAT	1FG3, 1H1C	L-Histidinol-phosphate	2-Ketoglutarate
III	AcornAT	1DTY	*N*-Acetyl-L-ornithine	2-Ketoglutarate
	OrnAT	1OAT	L-Ornithine	2-Ketoglutarate
	ω-AaAT		β-Alanine	Pyruvate
	GaBaAT	1OHV	4-Aminobutyrate	2-Ketoglutarate
	DapaAT		7,8-Diaminopelargonate	Methylthio-2-oxobutanoate
IV	D-AlaAT	1DAA, 1G2W, 2DAA	D-Alanine	2-Ketoglutarate
	BCAT	1A3G, 1EKF	L-Leucine	2-Ketoglutarate
V	SerAT		L-Serine	Pyruvate
	PSerAT	1BT4	3-Phospho-L-serine	2-Ketoglutarate
DegT_DnrJ_EryC1 (VI)	ArnB	1MDO	L-Glutamate	UDP-2-acetamido-4-keto-2,6-dideoxyglucose
	TylB		L-Glutamate	TDP-3-keto-6-deoxy-D-glucose
	StsC		L-Glutamine	*scyllo*-Inosose

[a] The subgroups follow the classification in Pfam (http://www.sanger.ac.uk/Software/Pfam/).
[b] AspAT: aspartate aminotransferase; AlaAT: alanine aminotransferase; AroAT: aromatic aminotransferase; HisPAT: histidinol-phosphate aminotransferase; AcornAT: acetylornithine aminotransferase; OrnAT: ornithine aminotransferase; ω-AaAT: ω-amino acid aminotransferase; GaBaAT: 4-aminobutyrate aminotransferase; DapaAT: diaminopelargonate aminotransferase; D-AlaAT: D-alanine aminotransferase; BCAT: branched-chain amino acid aminotransferase; SerAT: serine aminotransferase; PSerAT: phosphoserine aminotransferase; ArnB: UDP-2-acetamido-4-amino-2,4,6-trideoxyglucose aminotransferase; TylB: TDP-3-keto-6-deoxy-D-glucose aminotransferase; StsC: L-glutamine-*scyllo*-inosose aminotransferase.
[c] PDB ID of the crystal structures of wild type aminotransferases from different species.

tyrosine (or L-phenylalanine) as an amino donor, respectively, and α-ketoglutarate as an amino acceptor. Until now, three-dimensional structures of 10 AspATs (PDB ID: 1AAT, 1AJS, 1AMA, 1AMQ, 1BJW, 1J32, 1YAA, 1O4S, 5BJ3, and 2CST) and four AroATs (PDB ID: 1AY5, 1DJU, 1BWO, and 3TAT) have been resolved. According to the structure information, all these ATs are homodimeric enzymes, and the active site of the ATs is composed of two binding pockets (Fig. 1A). The carboxylic group of the donor amino acid is placed in the large binding pocket and stabilized by an arginine and an asparagine. Then the side chain of the amino acid is placed in the small binding pocket. The difference between an AspAT and an AroAT is the hydrophobicity of the active site binding pocket [15]. Six mutations in an AspAT from *Escherichia coli* changed it to an AroAT, and the mutant showed higher activity for aromatic amino acids than its parent AspAT by 10^3-fold [16].

AspATs and AroATs have been applied to the synthesis of natural and unnatural amino acids. An AspAT coupled with phosphoenolpyruvate carboxykinase was used to produce L-phenylalanine from phenylpyruvate with 93% conversion yield [17]. AroATs have also been used in the synthesis of L-homophenylalnine (HPA) [18,19], L-2-aminobutyric acid [20], L-thienylalanine [21], and D-β-heterocyclic alanine [22]. Vacca et al. [23] increased the activity of an AspAT from *E. coli* for L-norleucine by 17 times by site-directed mutagenesis. Nowicki et al. [24] and Wu et al. [25] expanded the substrate specificities of an AroAT from *Trypanosoma cruzi* and an phenylalanine aminotransferase (PheAT) from *E. coli* by site-directed mutagenesis, respectively. Chow et al. [26] narrowed the substrate specificities of an AspAT by directed evolution.

2.2. ω-*Aminotransferases (ω-ATs)*

The ATs in the subgroup III such as ω-amino acid aminotransferase (ω-AaATs), ornithine ATs, and 4-aminobutyrate ATs (GaBa ATs) have broad substrate specificities and they are quite useful for preparation of chiral amines and unnatural amino acids such as β-amino acids. Only three crystal structures of ω-ATs (PDB ID: 1OHV, 1OAT, and 1DTY) have been published till now. As the large binding pocket of ω-ATs is bigger than that of the AspATs and AroATs (Fig. 1B), it is possible that ω-ATs accept substrates of larger size than natural amino acids [27]. Comparisons of the protein sequences of ω-ATs again help to understand their substrate specificities, and to screen novel ω-ATs for a given substrate. Using this approach, putative ω-ATs from *Caulabacter crescentus* (cc3143) and *Mesorhizobium loti* (mll1632) were detected as the ones having high homology and similar structures with the ω-AT from *Alcaligenes denitrificans* which shows a high activity to L-β-amino-*n*-butyric acid [28]. All the three enzymes showed high activities toward short-chain aliphatic β-amino acids.

2.3. *Branched-chain aminotransferases (BCATs) and* D-*aminotransferases (DATs)*

In all ATs, PLP cofactor is positioned in the active site by numerous non-covalent interactions so that *si* face of the pyridine

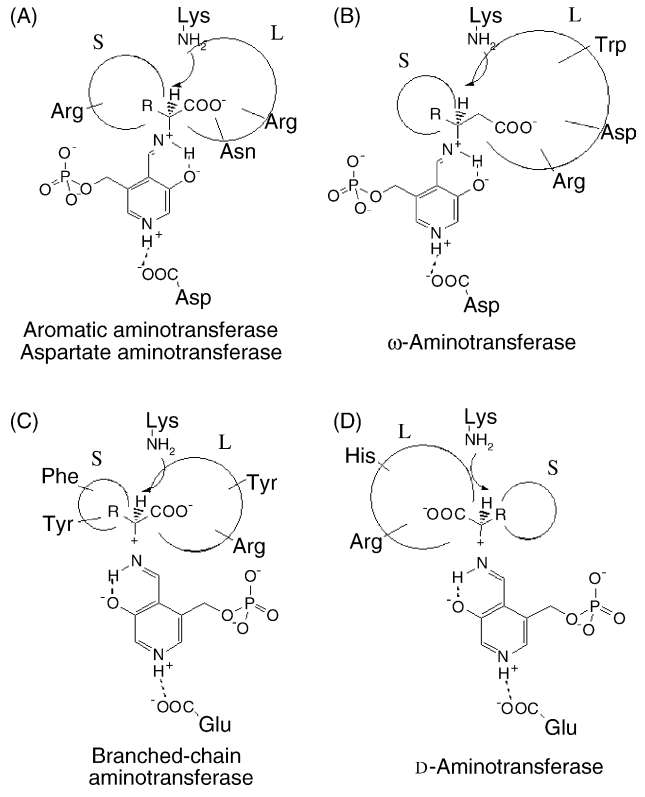

Fig. 1. The schematic diagram of active binding sites of aminotransferases. (A) AroAT or AspAT: the carboxylic group of the substrate is stabilized by arginine and asparagine in the small binding pocket and the pyridoxal ring of the cofactor PLP is stabilized by aspartate; (B) ω-AT: the large binding pocket is larger than that of aromatic or aspartate aminotransferase, and the tryptophan in the large binding pocket can stabilize the aromatic substrate by π–π interactions; (C) BCAT: the abstraction and addition of a proton occurs from the *re* face of the pyridine ring and the pyridoxal ring of the cofactor PLP is stabilized by glutamate; (D) D-AT: the abstraction and addition of a proton occurs from the *re* face of the pyridine ring and the small and large binding pockets are positioned in the opposite orientation compared with BCAT.

ring lies toward solvent and incoming substrates, and *re* face of the pyridine ring lies toward the protein. However, in the case of BCATs and DATs, the abstraction and addition of a proton occurs from the *re* face of the pyridine ring compared with other ATs [29,30]. The major structural difference between BCATs and DATs is the hydrophobicity of the active binding pockets [14]: i.e. the histidine and arginine residues are located at the large pocket in DATs, whereas the tyrosine and phenylalanine residues are at the small pocket in BCATs (Fig. 1C and D). The 3-D structures of two BCATs (PDB ID: 1A3G and 1EKF) and three DATs (PDB ID: 1DAA, 2DAA and 1G2W) have been solved. Another thing to note from the comparison of the 3-D structures is that the relative positions of the small pocket and large pocket in BCATs are reversed in DATs, so that the proton abstraction comes from the opposite side. This would explain the opposite enantioselectivity of the two enzyme groups. L-*tert*-Leucine can be synthesized using BCATs, whereas the synthesis of a number of D-amino acids such as D-2-aminobutyrate, D-alanine [31], and D-phenylalanine [32] were attempted using DATs. Moreover, by increasing the conformational flexibility of the loop region connecting the homodimer, Gutierrez et al. [33] obtained a mutant showing two- to four-fold higher activity toward D-amino acids including D-norleucine and D-norvaline than the wild type from *Bacillus* sp. YM-1. Van Ophem et al. [34] evolved a DAT into a racemase by site-directed mutagenesis.

2.4. Sugar aminotransferases (SATs)

SATs are mostly involved in the amino sugar synthesis occurring in cells producing secondary metabolites. They are specially classified into DegT_DnrJ_EryC1 aminotransferase subfamilies in the Pfam database. SATs are mainly involved in the synthesis of O antigen and the lipopolysaccharides present in the outer membrane of Gram-negative bacteria, and secondary metabolites such as antibiotics [35]. The three dimensional structure of only one SAT (PDB ID: 1MDO) has been revealed until now. Amino acceptors of the SATs are mainly NDP-keto-sugars or *scyllo*-inosose, and amino donors of the SATs are mainly glutamate or glutamine. SATs show nucleotide moiety specificity as well as regio-specificity for amino group transfer in respect of NDP-keto-sugars. Per from *Vibrio cholerae* [36], LmbS from *Streptomyces linchlnensis* [37], ArnB from *Salmonella typhimurium* [38] and WecE from *E. coli* act on the position of C4 of pyranose [39], whereas TylB from *Streptomyces fradiae* [40], DesV from *Streptomyces venezuelae* [41], and MegCII from *Micromonospora megalomicea* [42] act on the position of C3 of pyranose. In terms of the nucleotide specificities of SATs, Per from *V. cholerae* acts on GDP-keto-sugar, ArnB from *S. typhimurium* shows the activity for UDP-keto-sugar, whereas WecE from *E. coli*, TylB from *S. fradiae* and DesV from *S. venezuelae* accept TDP-keto-sugars as substrates. The multiple alignments among SATs showed that they could be divided into three subgroups, VI_α, VI_β, and VI_γ, would act on NDP-4-keto sugars, NDP-3-keto sugars, and *scyllo*-inosose, respectively [39].

3. Use of bioinformatics tools for studying ATs

Development of bioinformatics and functional genomics makes it possible to screen many desirable ATs with the help of computational techniques from the AT databases [43]. We can predict approximate functions and substrate specificities of putative enzymes using BLAST search, multiple sequence alignment, family profile analysis, 3-D structure modeling, and computer simulation of substrate docking into the active site of the enzyme. The five subgroups of the AT superfamily are quite distinctively differentiated by family profile analysis, and a subfamily profile of each subgroup among the main five groups can be also uniquely determined by the same iterative analysis.

3.1. Family profile analysis

As abundant genomic databases are accumulated from genomic projects, correct calling and identification of the functions of unknown and putative ATs in cell or biosynthesis pathways have become a new research subject. An advanced technique in multiple sequence alignment called “family profile analysis” can be used for the subclassification of AT, so

that ATs can be more precisely classified into the five subgroups identified (Table 1). AspATs and AroATs (which belong to aminotransferase subgroup I and II), ω-ATs (subgroup III), BCATs and DATs (subgroup IV), phosphoserine aminotransferases (subgroup V), and TylB and StsC (subgroup VI) are well-known representative ATs for industrial applications. The sequence similarities between ATs appear to be closely related with their substrate specificities. For example, Hartmann et al. [44] screened by BLAST and identified an aminotransferase which was related with L-lysine biosynthesis. WecE from *E. coli* was expected to be an SAT by BLAST searches and family profile analysis, and its function was experimentally proven [39]. The profile analysis of 50 ATs including putative ATs from *M. loti* [5] was carried out. We could find that 25, 19, 3, 1, and 2 belonged to AT subgroup I and II, AT subgroup III, AT subgroup IV, AT subgroup V, and AT subgroup VI, respectively.

3.2. Computational methods

Rational protein design by site-directed mutagenesis is one of the most effective strategies to isolate improved enzymes [45]. If a good 3-D structure of a protein is available, various trials for improving the properties of the protein can be carried out, such as reshaping a substrate-binding site to change substrate specificity [46], cofactor binding affinity [47], enantioselectivity [48], and stability [49]. Recent advancement in molecular modeling techniques allows us to develop a reasonably accurate protein model using sequence homology and protein fold recognition. Using the model, comparison of the target proteins for substrate specificity and rational approaches can be performed.

To build up the 3-D structure of a target protein, homology modeling and threading techniques are commonly used depending upon the degree of protein sequence homology. Homology modeling uses already known PDB coordinates of the most homologous proteins (usually >35% homology) as a template, whereas threading compares, generates 3-D structures of the similar folds from protein databases, and connects them for constructing the 3-D structural model [50,51]. In the case of various ATs, homology modeling approach is relatively easy and useful compared to other methods as their sequence homology is very high and 28 PDB coordinates from different species are already revealed (Table 1). However, threading is becoming more powerful and realistic as the techniques in computational chemistry technology are rapidly advancing. The comparison of the 3-D structures of several AroATs revealed that Ser297 plays an important role in the enzyme function of PheAT from *E. coli* [52]. Onuffer and Kirsch [16] succeeded in redesigning the substrate specificity of *E. coli* AspAT into that of AroAT by homology modeling and site-directed mutagenesis.

4. Engineering of the ATs for bioprocess development

4.1. Screening technologies

As ATs have broad substrate specificities, screening novel ATs for the production of derivatives of natural amino acids, chiral amines, unnatural amino acids, and amino sugars is possible and in great demand. For successful high throughput screening (HTS) of novel enzymes using random *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) mutagenesis or in vitro evolution such as gene shuffling and error-prone PCR, above all it is essential to develop well-defined detection methods that can discriminate the desired AT reactions. The three physical or chemical methods elaborated below are commonly used to screen target ATs.

4.1.1. Enrichment culture

Improved enzyme activity can be directly correlated to higher growth rates of cells by changing dilution rate in continuous culture. ATs can be screened using this popular enrichment cultivation technique where an amino donor is used as a sole nitrogen source in the culture medium. For example, ω-ATs from *Vibrio fluvialis* showing enzyme activity for (*S*)-aromatic amines [53] and from *A. denitrificans* showing the enzyme activity for (*S*)-aliphatic beta amino acids [28] were isolated using enrichment culture. An AroAT from *Enterobacter* sp. bacterium for the production of L-HPA [18] and a thermostable DAT from thermophilic *Bacillus* sp. [31] were also isolated using the same technique.

4.1.2. Spectrophotometric methods

As the properties of the substrates and products in an AT reaction are quite similar, it is not easy to differentiate between the products and the substrates. However, when the substrate has some special chemical properties, or a selective coupled enzyme system can be found, the AT reaction can be monitored using a spectrophotometer. For example, in the case of the ω-AT reaction, amino donors such as amine or β-amino acid and amino acceptors such as normally α-keto acid are converted into the corresponding ketone or β-keto acid, and α-amino acid, respectively. When a staining solution such as $CuSO_4$/MeOH is added to the reaction mixture, the resulting α-amino acid forms a complex with copper ions, which produces a blue color. Thus, the reaction can be easily quantified using a spectrophotometer (Fig. 2A) [54].

The coupling system with a dehydrogenase can be used to determine BCAT activity (Fig. 2B) [55]. AspAT [56] and tyrosine aminotransferase (TyrAT) [57] activities were identified using glutamate dehydrogenase and hydroxyisocaproate dehydrogenase coupling systems, respectively. Using leucine dehydrogenase, Cooper et al. [58] have developed a high throughput 96-well plate spectrophotometric assay for BCAT.

4.1.3. Use of auxotroph

The method of using amino acid auxotroph of *E. coli* strain is very classical and widely employed for the HTS of AT mutagenesis and evolution. Yano et al. [59] and Que et al. [60] succeeded in the directed evolution of AspAT to change its substrate specificity to branched-chain amino acids using a combination of DNA shuffling and subsequent selection with an *ilvE*-deficient *E. coli* auxotroph strain. Rothman and Kirsh [61] also succeeded in the directed evolution of AspAT to change it into TyrAT.

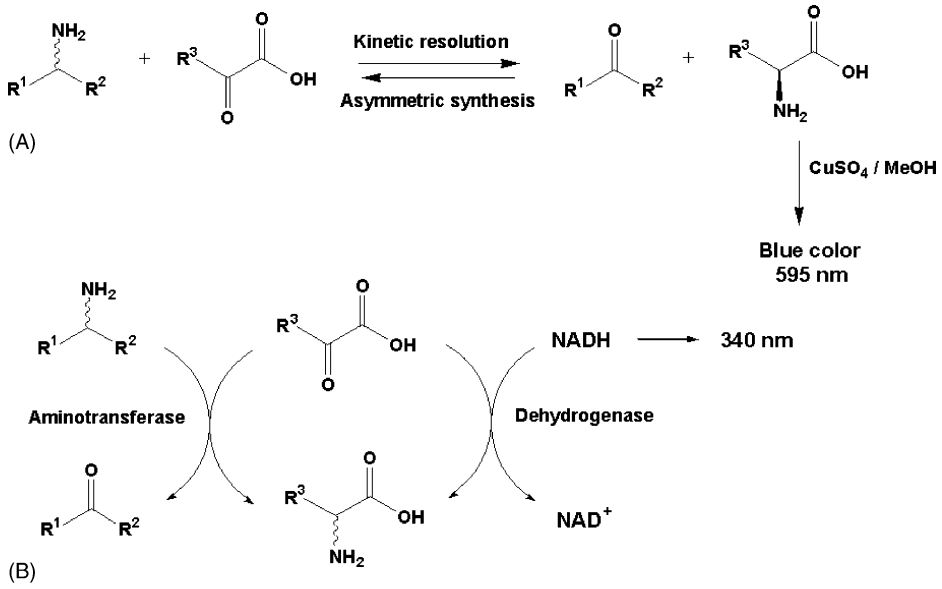

Fig. 2. The spectrophotometric screening methods for aminotransferase activity assay. (A) When the staining solution such as $CuSO_4$/MeOH is added to the reaction mixture, the α-amino acid forms a complex with the copper ion, resulting blue color, which can be easily quantified using a spectrophotometer at 595 nm; (B) the AT activity can be quantified through measuring the NADH formation by the coupled dehydrogenase using a spectrophotometer at 340 nm.

4.2. How to overcome substrate/product inhibitions

Although ATs have many advantages over other enzymatic systems for the application to industrial process, there are still some problems to be overcome. One of the biggest problems in the industrial use of ATs is the inhibition by substrate/product. In the case of the ω-AT from *V. fluvialis*, the enzyme showed very distinct enantioselective substrate inhibition [62]. The problem of substrate inhibition can be rather easily handled by developing a strategic feeding protocol for the substrate. Intermittent addition of the substrate and continuous precipitation of the product in the reaction media could make it possible to obtain a high concentration of L-HPA [63]. In addition, the substrate inhibition of DAT could be somewhat relieved by the addition of salts and the reduced form of nicotinamide adenine dinucleotide [64].

Product inhibition could be solved by selective product removal using following methods: i.e. coupling enzymatic reaction [20,65], solvent extraction [66], selective adsorption [67], selective product degradation or modification [19,68], vacuum evaporation [69], and so on. When the ω-AT from *V. fluvialis* was used with high concentrations of α-methylbenzylamine, the product inhibition by ketone by-product was quite severe. The product inhibition can be overcome by an alcohol dehydrogenase coupling reaction to convert the produced ketone into other non-inhibitory compound [70], and by using a two-liquid phase reaction to reduce product ketone concentration in the reaction mixture [65]. AlaAT coupling reactions can be used to overcome product inhibition of ω-AT reaction by converting the product of ω-AT into the amino acceptor of ω-AT [18]. Shin and Kim succeeded in overcoming product inhibition of ω-AT reaction using a packed-bed reactor [71] and a membrane reactor [72].

Substrate/product inhibition could be also overcome by in vitro evolution of target ATs. Rothman et al. could obtain a mutant A293D of AspAT from *E. coli* showing 10-fold lower inhibition after a single round of directed evolution using a tyrosine auxotroph as a selection marker [73]. Enrichment culture was applied as a tool to select mutants resistant to product inhibition [74]. Site-directed mutagenesis was used in parallel to make a DAT mutant which was relieved from substrate inhibition [75].

5. Concluding remarks

Above we have discussed examples of AT reactions for the production of valuable chiral intermediates and products, such as natural and unnatural amino acids, chiral amines, and amino sugars. In the future, ATs are likely to become a more valuable enzyme group for the chemical and pharmaceutical industry. For such applications, amino alcohols and heterocyclic amino acids are interesting compounds and especially vicinal amino alcohols with double chiral centers are good candidates for investigation (Fig. 3). As more diverse substrates and their derivatives are needed, new ATs should be screened, evolved and designed accordingly. To achieve such goals, various new biological and engineering strategies such as screening of novel ATs from unexplored nature and metagenome [76], and virtual screening of desired enzymes from the genomic databases would be beneficial and complementary (Fig. 4). Although traditional screening methods based on enrichment and random mutagenesis are still dominant in industry, recent progress of directed evolution techniques and virtual screening of novel enzymes using computer modeling makes them quite attractive and valuable approaches in the coming genomic era [77]. To make ATs more practical and attractive enzymes, expansion of substrate specificity, reducing cost of amino donors and acceptors, improvement in enzyme stability, and process development to overcome substrate and product inhibitions would be the primary concerns

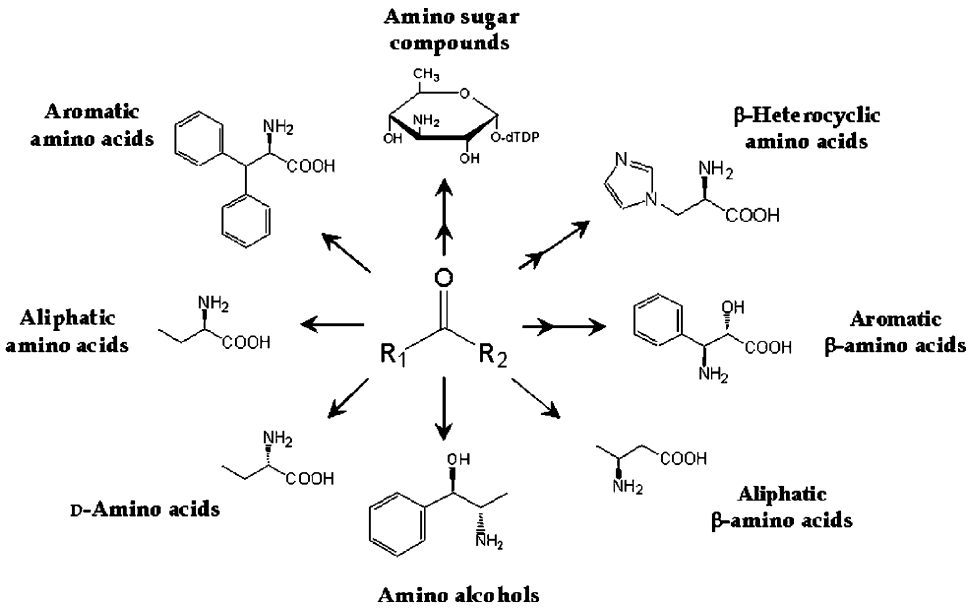

Fig. 3. Aminotransferase technology in chemical and pharmaceutical industry. Aromatic amino acids such as L-diphenylalanine, aliphatic amino acids such as L-2-aminobutyric acid, D-amino acids such as D-2-aminobutyric acid, amino alcohols with dual chiral centers such as L-norephedrin, aliphatic β-amino acids such as L-3-aminobutyric acid, aromatic β-amino acids such as L-phenylisoserine, β-heterocyclic amino acids such as L-thienylalanine, and amino sugar compounds such as TDP-3-amino-4,6-dideoxy-D-glucose can be synthesized by various aminotransferases.

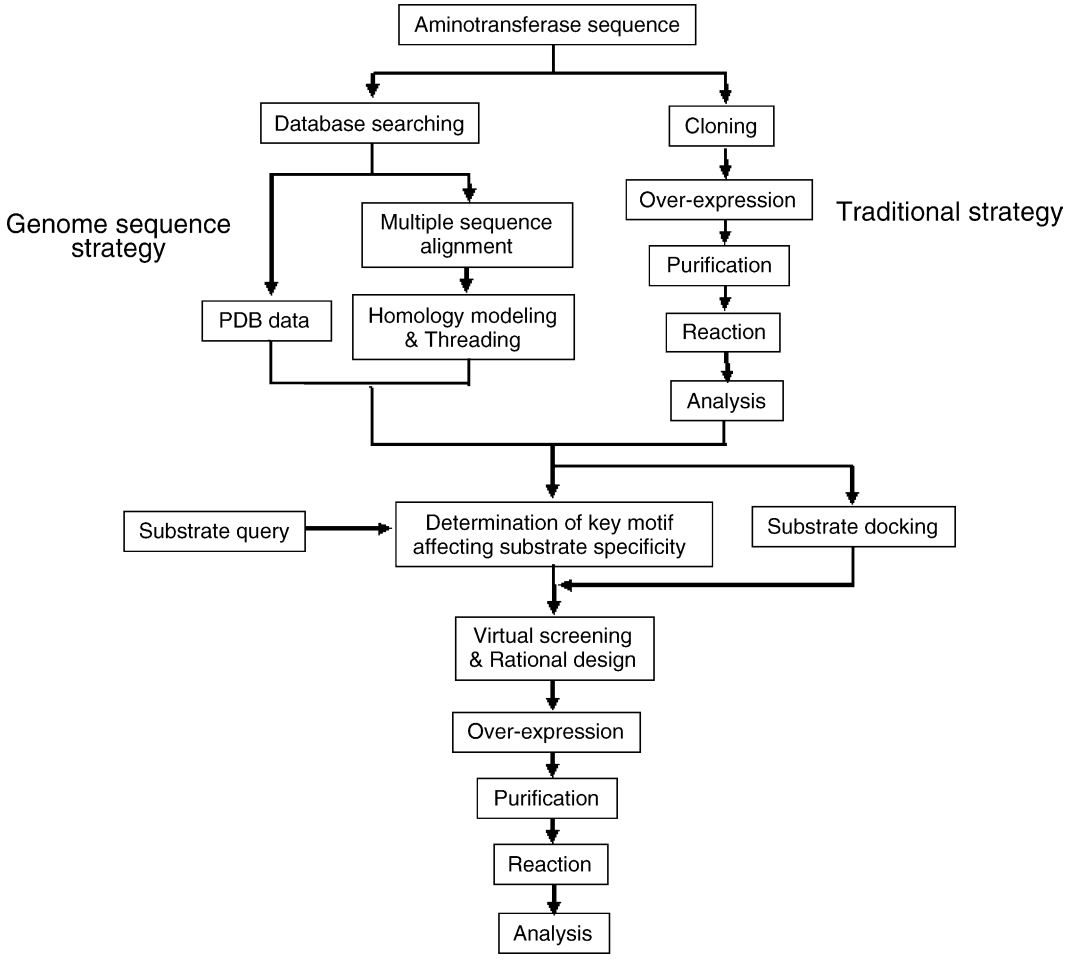

Fig. 4. The schematic diagram of rational design and virtual screening of aminotransferase.

for real applications of AT in industry. Screening and development of thermophilic, psychrophilic, halophilic, and/or organic solvent resistant ATs, and development of deracemization process for AT reaction may add new dimensions of the application of ATs in organic synthesis.

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