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Review

Biocatalytic asymmetric amination of carbonyl functional groups – a synthetic biology approach to organic chemistry

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Transaminases catalyze amino transfer reactions from amino donors such as amino acids or amines to keto acids or ketones to give chiral amino acid or amines in optically pure form. α-Amino acid dehydrogenases catalyze the asymmetric reductive amination of α-keto acids using ammonia as amino donor to furnish L-amino acids. The distinct features and synthetic application of these two enzymes are reviewed in an effort to illustrate their promising and challenging aspects in serving as approaches to the direct asymmetric synthesis of optically pure amines from the corresponding keto compounds, a formidable problem in organic chemistry.

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1 Introduction

Chiral amines are core component of a great number of pharmaceuticals, agrochemicals and other specialty chemicals. Analysis indicates that about 40–50% of optically active drugs are chiral amines [1]. This provides a strong impetus for the technology to make such molecules. There usually are three approaches: (i) the transformation of suitable, naturally occurring optically active amines; (ii) the resolution of racemic amines; and (iii) asymmetric construction of C-N bonds. Obviously, the first technique is limited by the available optically active amine pools, and the downside for the second one is its intrinsic feature of maximum yield of 50%. The methods for asymmetric C-N bond construction from prochiral precursors include asymmetric hydrogenation of imines, stereoselective addition of carbon nucleophiles to imines, addition of nitrogen nucleophiles to C=C bonds and reductive amination of carbonyl compounds. Among these, asymmetric reductive amination of carbonyl compounds represents an attractive approach, but it is still a great challenge in organic chemistry [2, 3]. Therefore, asymmetric reductive amination of carbonyl compounds has been identified as one of the aspirational reactions by the Pharmaceutical Round Table at the Green Chemistry Institute of American Chemical Society, and is currently a very hot research area in organic synthesis.

In traditional organic chemistry, enantioselective reductive amination of carbonyl compounds is performed in two ways (Scheme 1) [2, 3]. One is indirect reductive amination: the carbonyl compound reacts with the appropriate amine to form a stable imine and/or enamine, which is isolated and then enantioselectively reduced to chiral amine. The second approach is direct reductive amination in which the mixture of a carbonyl compound with an amine is reduced directly to chiral amine without the isolation of the intermediates. Recently, progresses have been made for both approaches of enantioselective reductive amination *via* chiral

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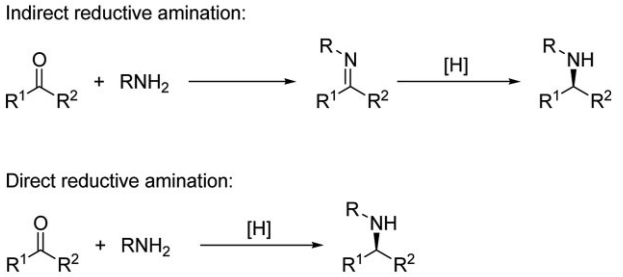
Abbreviations: AAT, aspartate aminotransferase; **ee**, enantiomeric excess; **FDH,** formate dehydrogenases; **LeuDH**, leucine dehydrogenase; **PheDH**, phenylalanine dehydrogenase



Scheme 1.

metal catalysis and organocatalysis [2, 4–13]. However, the indirect reductive amination is limited to the cases where the imine or enamine is stable enough for isolation [4]; the occurrence of a wide range of reducible intermediates in the reaction mixture complicates the reduction step and presents great challenge for a selective reduction in direct reductive amination [3].

In addition, amines are used as amino donor and *N*-alkylated or *N*-arylated amines are thus the products in almost all studies on asymmetric reductive amination of carbonyl compounds with chemical methods. If a primary amine is the desired product, an extra step is needed to remove the *N*-alkyl or *N*-aryl group (Eq. 1). The only exception is described in the report by Kadyrov and Riermeier [14], in which ammonium formate was used as the amino donor in the enantioselective reductive amination of acetophenones with Ru catalysts, but the product was a mixture of free amine, its *N*-formylated form and alcohol (Eq. 2). As such, the asymmetric reductive amination of carbonyl compounds using ammonia as amino donor to synthesize optically pure amines is still an unsolved, formidable problem in organic chemistry.

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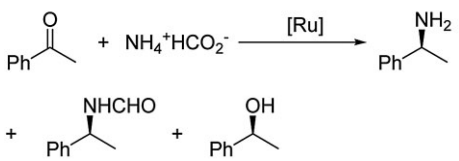
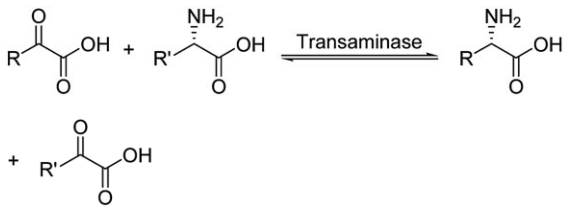


(2)

Since chiral amines are ubiquitous in biological systems, nature has developed various methods for the construction of such moieties. In this review, the distinct features and synthetic application of transaminases and amino acid dehydrogenases are summarized to illustrate their potential and challenges in the asymmetric synthesis of chiral amine molecules from the prochiral keto compounds.

2 Transaminase catalyzed asymmetric amination of carbonyl compounds using amino acid or amine as amino donor

Transaminase (also known as amino acid aminotransferase) is a pyridoxal-5′-phosphate-dependent enzyme that catalyzes asymmetric amino transfer reaction between an amino acid and an α-keto acid, in which the amino acid is converted to the corresponding α-keto acid while the reactant α-keto acid is converted to the corresponding amino acid as shown in Eq. (3). This reaction is important in the biosynthesis of various amino acids in biological systems, and also has been used in the synthesis of optically pure nonproteinogenic amino acids and amines [15–17]



(3)

However, the equilibrium constant of the amino transfer reaction between an amino acid and an α-keto acid is about 1, so that shifting the equilibrium to the product direction is required to assure the efficiency of transaminase used in asymmetric synthesis [18]. Transaminases usually show a broad substrate spectrum. Amines and ketones can also be the amino donor or acceptor, respectively, but the amino transfer from α-amino acid to ketone is more thermodynamically unfavorable. For example, the equilibrium constant for the amino transfer reaction between acetophenone and alanine was reported to be 8.8×10^{-4} [19]. Thus transaminases have often been used in the kinetic resolution of racemic amines [20, 21]. For the asymmetric synthesis of optically pure amines from the corresponding ketones, shifting the equilibrium to the product formation is even more necessary. In addition, inhibition by the keto by-product has been observed to prevent efficient conversion. An unfavorable thermodynamic equilibrium and product inhibition are two major obstacles in the synthetic application of transaminases. Here the recent advances in addressing these problems are outlined to illustrate the potential and challenges of this

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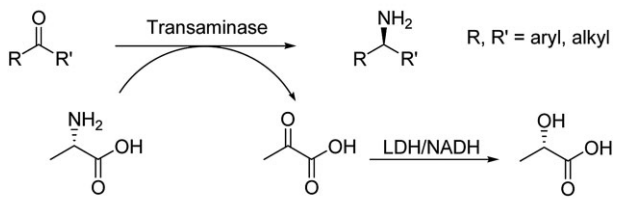
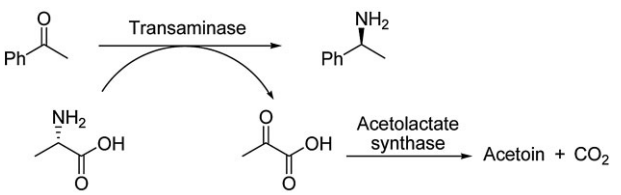
category of enzymes in the asymmetric synthesis of chiral amino acids and amines.

2.1 Shifting a thermodynamically unfavorable equilibrium

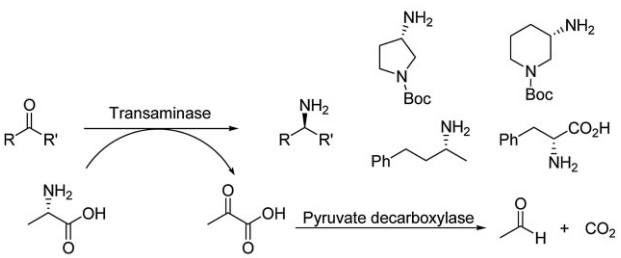
To reach efficient conversion, several approaches have been employed to remove the keto product, thus shifting the unfavorable thermodynamic equilibrium to the desired direction and eliminating the product inhibition. In most cases, α-amino acid is used as the amino donor, and the co-product α-keto acid can be removed by several methods. One approach is to reduce the co-product α-keto acid to α-hydroxy acid to drive the reaction to completion. For example, when alanine is used as the amino donor, lactate dehydrogenase is incorporated to convert pyruvate to lactate (Scheme 2) [22–24]. Acetolactate synthase is also used to convert pyruvate to acetolactate, which decomposes to acetoin and carbon dioxide (Scheme 3) [25]. The equilibrium can be efficiently shifted toward the product direction using pyruvate decarboxylase (PDC) (Scheme 4) [26]. The amino donor can also be recycled using amino acid dehydrogenase, which catalyzes the reductive amination of the by-product α-keto acid to regenerate the amino donor (Scheme 5) [24, 27].



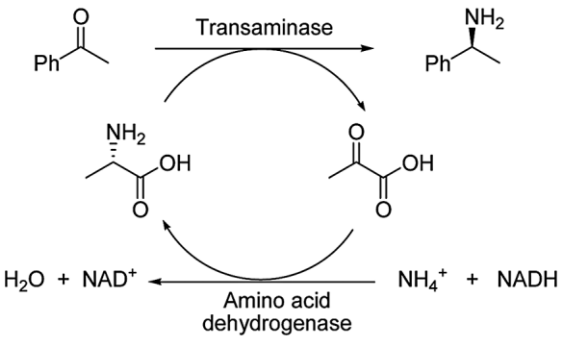
Scheme 2.



Scheme 3.



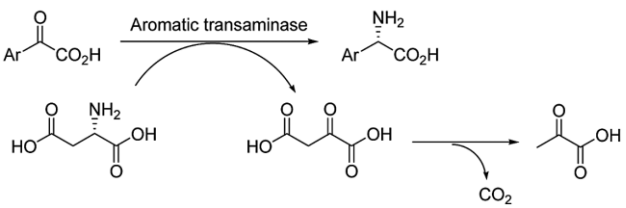
Scheme 4.



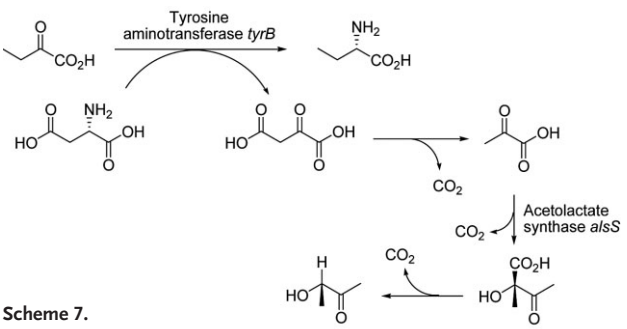
Scheme 5.

Aromatic amino acid transaminases generally show broad substrate specificity and are active towards aromatic and dicarboxylic substrates. Although L-glutamate and L-aspartate can be used as amino donor, the latter is preferred because the deamination product oxaloacetate undergoes spontaneous decarboxylation to pyruvate, driving the equilibrium in the desired direction for a better product yield (Scheme 6) [18, 28]. Acetolactate synthase is often introduced into the reaction system to convert the resultant pyruvate to acetolactate, which decarboxylates to 3-oxo-2-butanol (Scheme 7) [29, 30].

A double mutant, R292E/L18H, of *Escherichia coli* aspartate aminotransferase (AAT) effectively catalyzes the asymmetric conversion of 2-oxo-4-phenylbutanoic acid to L-homophenylalanine using L-lysine as amino donor. In this reaction, the spontaneous nonenzymatic cyclization of the transamination product of L-lysine, 2-keto-6-aminocaproate, to Δ^1-piperidine-2-carboxylic acid in



Scheme 6.



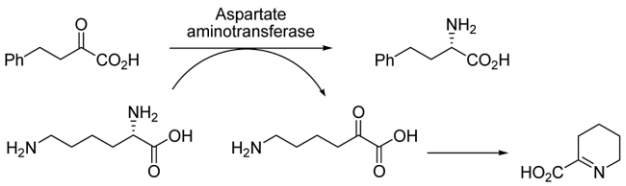
Scheme 7.

the reaction mixture together with the low solubility of L-homophenylalanine drives the reaction completely toward product formation (Scheme 8) [31]. Ornithine-δ-aminotransferase catalyzes the reversible transfer of the δ-amino group of L-ornithine to 2-ketoglutaric acid, affording L-glutamic acid semialdehyde and L-glutamic acid. The former is cyclized under the reaction conditions to give Δ^2-pyrroline-5-carboxylate, driving the reaction to the completion. As such, coupling of ornithine-δ-aminotransferase with *E. coli* tyrosine aminotransferase or branched chain aminotransferase has been used in the production of L-aminobutyric acid or L-*tert*-leucine from the corresponding keto acids, respectively, with dramatically increased product yields (Scheme 9) [32].

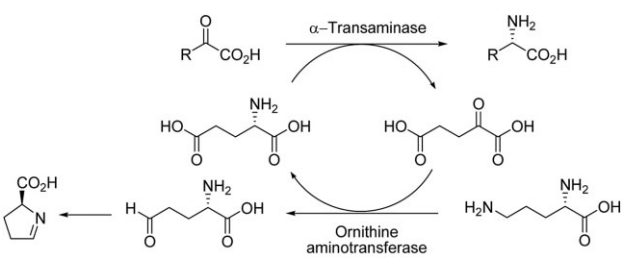
Cysteinesulfinic acid (CSA) has also been used as an amino donor in the enzymatic synthesis of glutamic acid analogues catalyzed by glutamic oxalacetic transaminase (GOT) [33–35] or AAT from pig heart or *E. coli* [36–38]. The co-product keto acid spontaneously decomposes into pyruvic acid and SO_2, leading to equilibrium shift to the product formation (Scheme 10). Four stereomers of the constrained cyclic glutamic acid analogue, L-2-(2-carboxycyclobutyl)glycine, have been synthesized using AAT or branched-chain aminotransferase (BCAT) from *E. coli* [39, 40]. In the case of BCAT, glutamic acid or L-leucine is used as amino donor, which is recycled by glutamic acid dehydrogenase or leucine dehydrogenase (LeuDH) and ammonia as the ultimate amino donor (similar to Scheme 5) [39, 41]. BCAT has a broad substrate spectrum, complimentary to that of AAT [41].

In addition to α-amino acids, amines are also used as the amino donor. For example, *sec*-butylamine has proven to be a good amino donor in the reductive amination of 3,4-dimethoxyphenylacetone catalyzed by two soil isolates, *Arthrobacter* sp. KNK168 and *Pseudomonas* sp. KNK425, to yield the (*R*)- or (*S*)-isomer of 3,4-dimethoxyamphetamine (DMA) with >99% enantiomeric excess (ee) (Scheme 11), respectively [42]. A large excess of isopropylamine has been used as amino donor to drive the transamination to the product direction in transaminase-catalyzed synthesis of chiral methyl benzylamine [24].

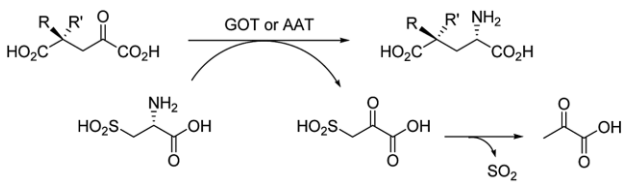
The above-described approaches utilize the further reactions of the keto products to shift the unfavorable thermodynamic equilibrium of transamination to product formation and to avoid the product inhibition of keto products. Process engineering is also useful to achieve these goals in some cases. The large solubility difference between some substrates and products at a given reaction pH and high temperature has successfully been used to shift the reaction equilibrium toward rapid product formation. 2-Oxo-4-phenylbutyrate and phenylglyoxylate are converted to L-homophenylalanine and L-phenylglycine in optically pure form (>99% ee), respectively. These transaminations are catalyzed by a thermophilic aromatic amino acid transaminase at 60°C using L-glutamate as an amino donor. Because of the low solubility at the given reaction pH, the product amino acids continuously precipitate from the reaction solution to drive the reaction to completion [43, 44]. The controlled release of substrate *via* the use of Amberlite (IRA 400)-adsorbed benzoylformate has proven to be a useful technique to circumvent substrate inhibition. This results in a fourfold improvement in product concentration, giving a final D-phenylglycine concentration of 10.25 mg/mL [45]. Intermittent addition of solid-state 2-oxo-4-phenylbutyrate into the reaction mixture was attempted to prevent substrate inhibition in the synthesis of L-homophenylalanine catalyzed by aromatic amino acid transaminase, the final concentration of 840 mM was reached with 94% conversion yield and >99% optical purity of L-homophenylalanine [44].



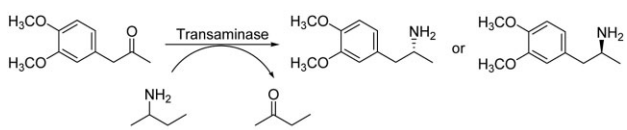
Scheme 8.



Scheme 9.



Scheme 10.

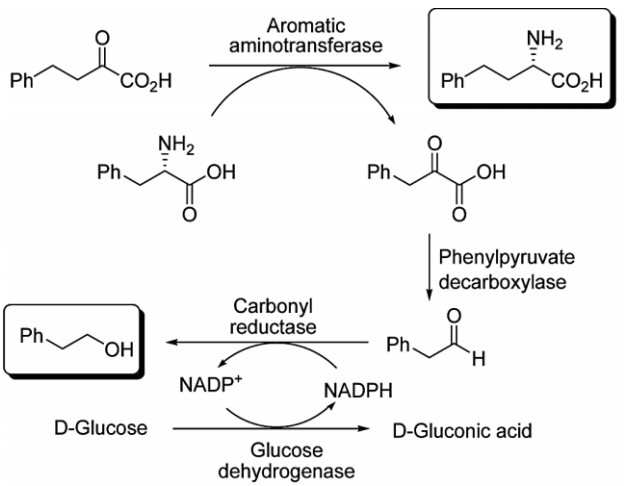


Scheme 11.

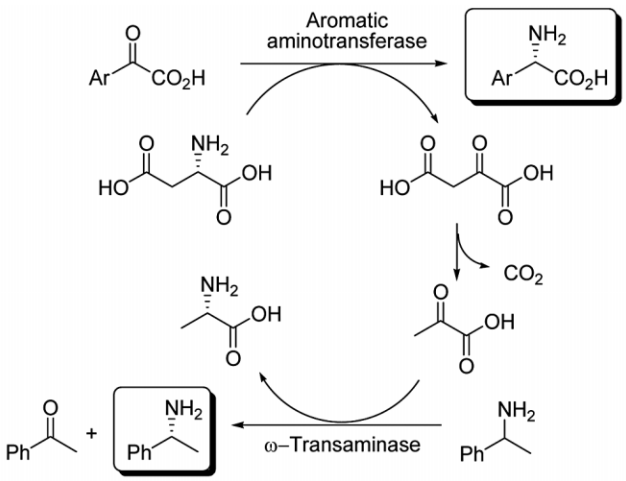
2.2 Coupling with another reaction for the synthesis of multiple products

As discussed above, transaminases have shown great potential as versatile biocatalysts in the asymmetric synthesis of optically pure α-amino acids and amines. The transamination has also been coupled with other useful reactions to produce other compounds in addition to the chiral amines. Hwang *et al*. [46] coupled an aromatic transaminase with the Ehrlich pathway of yeast to simultaneously synthesize L-homophenylalanine and 2-phenylethanol (Scheme 12).

Aromatic aminotransferases such as tyrosine aminotransferase are coupled with ω-transaminase to simultaneously synthesize aromatic L-amino acids and kinetically resolve the racemate of chiral amines (Scheme 13) [47, 48]. A two-liquid phase system is used to prevent the product inhibition by removing product acetophenone into the organic phase [48]. Similarly, optically pure L-aminobutyric acid and (R)-α-methylbenzylamine are simultaneously obtained by coupling α/ω-transaminases [47, 48].



Scheme 12.



Scheme 13.

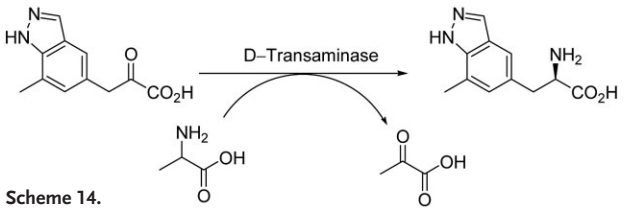
2.3 Enzyme modification

Despite the demonstrated usefulness of transaminases in the production of optically pure amines and L-α-amino acids from the prochiral keto starting materials, their abilities of recognizing substrates are limited or far from being fully explored. As such, effort has been recently made to further expand the application potential of transaminases in the asymmetric synthesis of target chiral amines with diverse structures. Kim and co-workers [49] constructed a structure model of aromatic L-amino acid transaminase from *Enterobactor* sp. BK2K-1 by homology modeling. Structurally based site-directed mutagenesis results in an Y66L variant that is able to synthesize L-diphenylalanine from diphenylpyruvate with 23% conversion yield in 10 h using L-phenylalanine as amino donor, whereas the wild-type enzyme is inactive for the transamination between diphenylpyruvate and L-phenylalanine. Site-directed mutagenesis has also been performed to change substrate specificity of *E. coli* AAT [31].

2.4 Other new transaminases

D-Amino acids are useful precursors for the synthesis of antibiotics, bioactive peptides, and other physiologically active compounds. D-Transaminase-catalyzed aminotransfer reactions are particularly interesting in the enzymatic production of D-amino acids. As such, a multi-enzyme system composed of glutamate racemase, D-amino acid amino transferase, formate dehydrogenase, and glutamate dehydrogenase has been developed for the production of D-phenylalanine and D-tyrosine with 100% optical purity from the corresponding substrate, phenylpyruvate or hydroxyphenylpyruvate, respectively [50, 51]. In a recent example, D-aminotransferase was successfully used in the synthesis of (*R*)-2-amino-3-(7-methyl-1 *H*-indazol-5-yl)propanoic acid, a key intermediate for synthesis of antagonists of calcitonin gene-related peptide receptors, from the corresponding keto acid using excess amount of racemic alanine as amino donor (Scheme 14) [52].

Although transaminases have been widely studied in the asymmetric synthesis of versatile chiral amines, including unnatural α-amino acids, their application in the synthesis of optically pure β-amino acids has been much less explored. An ω-



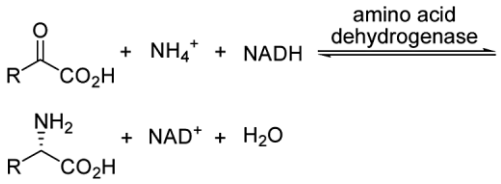
Scheme 14.

transaminase from *Alcaligenes denitrificans* has been shown to catalyze mainly the transamination between aliphatic β-amino acids and pyruvate [53]. The enzyme shows high enantioselectivity in the kinetic resolution of racemic β-aminobutyric acid, producing D-aminobutyric acid with 99% ee and 53% conversion. More recently, Kim *et al*. [54] cloned and characterized a novel β-transaminase from *Mesorhizobium* sp. strain LUK, which was used for the asymmetric synthesis of enantiomerically pure (*S*)-amino-3-phenylpropionic acid from the ketocarboxylic acid substrate using 3-aminobutyric acid as amino donor (Scheme 15).

Aminotransferase is also involved in the biosynthesis of aminoglycoside antibiotics. For example, genes from *Streptomyces fradiae* [55], *Streptomyces tenebrarius* [56], *Streptomyces griseus* [57] and *Bacillus circulans* [58, 59] have been shown to encode proteins which catalyze the amino transfer reaction from L-glutamine to 2-deoxy-*scyllo*-inosose to give 2-deoxy-*scyllo*-inosamine (Scheme 16), an intermediate in the biosynthesis of 2-deoxystreptamine. These enzymes should find wide applications in the production of aminoglycosides, a large class of clinically important antibiotics, in the near future.

3 Amino acid dehydrogenase catalyzed asymmetric reductive amination of carbonyl compounds using ammonia as amino donor

α-Amino acid dehydrogenases catalyze reversible amination of α-ketoacids to the corresponding L-amino acids in the presence of NADH or NADPH, as shown in Eq. (4). The equilibrium for the reaction lies far to the amination side, with the K_{eq} values in the range 10^{14}–10^{18} [60]. The asymmetrical synthesis of α-amino acids from the corresponding prochiral α-keto acids and ammonia is thermodynamically favorable. As such, amino acid dehydrogenases have considerable potential for the asymmetric synthesis of novel non-proteogenic amino acids of pharmaceutical importance.

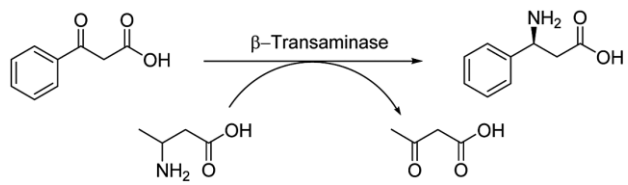


(4)

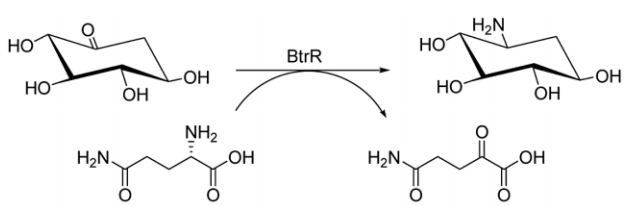
The members of this family of enzymes usually show distinct substrate specificities. Glutamate dehydrogenase accepts glutamate in preference to all other amino acids; leucine and valine dehydrogenases catalyze the oxidative deamination of aliphatic amino acids only; and phenylalanine dehydrogenase has a marked preference for aromatic amino acids as substrate, although it also recognizes smaller hydrophobic amino acids with less activity. For example, LeuDH from *Bacillus sphaericus* catalyzes the deamination of aliphatic α-amino acid [61–63], while phenylalanine dehydrogenase of same origin and its mutants have shown activity toward homophenylalanine and substituted phenylalanines [64–66] as well as phenylalanine. The synthetic application of α-amino acid dehydrogenases is thus dictated by their distinct substrate specificities. As such, the recent advances in the reductive amination of α-keto acids catalyzed by amino acid dehydrogenases, and its application in the asymmetric synthesis of novel nonproteogenic amino acids, are presented and organized by the subcategory of amino acid dehydrogenases.

3.1 Phenylalanine dehydrogenases

Phenylalanine dehydrogenase (EC 1.4.1.20, PheDH) is one of the few amino acid dehydrogenases studied for the application in the asymmetric synthesis of α-amino acids. In their early works, Hummel *et al*. [67, 68] developed an enzyme membrane reactor system containing PheDH (from *Rhodococcus*



Scheme 15.



Scheme 16.

M4 or *Brevibacterium* sp.), yeast formate dehydrogenase (FDH) and polyethylene glycol (PEG)-NADH for the synthesis of L-phenylalanine *via* reductive amination of phenylpyruvate. A similar approach was also used in the reductive amination of 6-(1′,3′-dioxolan-2′-yl)-2-keto-hexanoic acid to 6-(1′,3′-dioxolan-2′-yl)-2S-aminohexanoic acid with time-space-yield of 100 g day^{-1} L^{-1} [69].

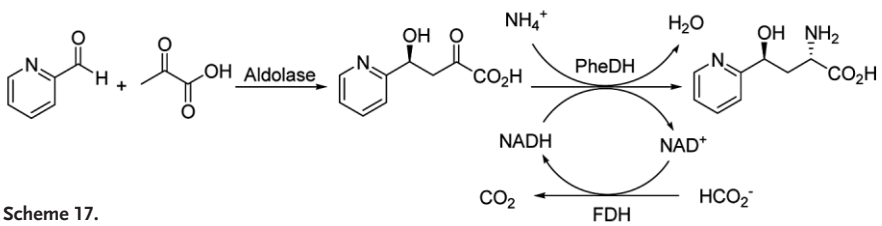
Asano and co-worker [64, 65] have examined the synthetic potential of *Bacillus sphaericus* phenylalanine dehydrogenase, and have found that this enzyme is active toward 3-substituted pyruvic acids with bulky substituents in the reductive amination reaction. Optically pure (*S*)-phenylalanine and several analogues are synthesized from the corresponding α-keto acids using this PheDH and the FDH from *Candida boidinii* for regeneration of NADH. The PheDH from *Bacillus sphaericus* has also been successfully used in the synthesis of the *N*-terminal amino acid portion of nikkomycins K_X and K_Z in a two-step enzymatic process, in which a pyruvate aldolase-catalyzed aldol reaction is followed by a PheDH-catalyzed reductive amination (Scheme 17) [70]. In addition to aromatic α-amino acids, some highly fluorinated leucine analogues such as (*S*)-5,5,5,5′,5′,5′-hexafluoroleucine and (*S*)-5,5,5′,5′-tetrafluoroleucine have recently been synthesized with high enantiomeric purity using commercially available PheDHs and FDHs [71].

Although PheDHs were successful in the above examples, they usually show lower activity toward the non-native substrates, and modification of wild-type enzymes is often required to achieve the desired catalytic efficiency. Site-specific mutagenesis of PheDH from *B. sphaericus* based on homology modeling resulted in several mutant enzymes with altered substrate profiles [66, 72–74], some of which have been shown to be useful biocatalysts for the asymmetric synthesis of *para*-substituted phenylalanine analogues [66, 75, 76]. The mutant enzymes are also used in the kinetic resolution of racemic non-natural α-amino acids to afford D-amino acids such as D-*para*-chlorophenylalanine [77]. The mutant PhenDH N145A supported on Celite is remarkably robust, even in the presence of high concentrations of polar or non-polar organic solvents such as acetone, methanol, *n*-hexane, toluene and methylene chloride. The immobilized biocatalyst has been successfully applied to the reductive amination of poorly water-soluble *p*-NO_2-phenylpyruvic acid with methanol and ethanol as co-solvent, resulting in *p*-NO_2-phenylalanine in optically pure form [78].

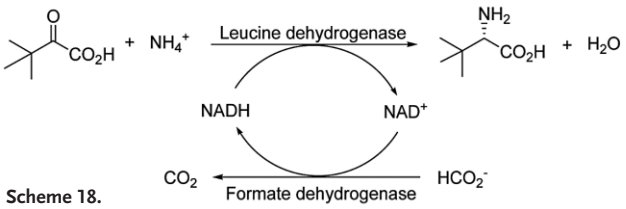
Recently, 2-(3-hydroxy-1-adamantyl)-2-oxoethanoic acid was converted to 2-(3-hydroxy-1-adamantyl)-(2*S*)-aminoethanoic acid by reductive amination using a modified PheDH from *Thermoactinomyces intermedius* and an FDH for recycling of NADH. The modified PheDH includes two amino acid changes at the C terminus and a 12-amino acid extension of the C terminus, and is more effective toward 2-(3-hydroxy-1-adamantyl)-2-oxoethanoic acid than the wild-type enzyme, but less effective for phenylpyruvate, the natural substrate. Extracts of *Pichia pastoris* containing the modified PheDH and endogenous FDH were used for the multi-kilogram batch production of 2-(3-hydroxy-1-adamantyl)-(2*S*)-aminoethanoic acid, a key intermediate required for the synthesis of Saxagliptin, a dipeptidyl peptidase IV inhibitor under development for treatment of type 2 diabetes mellitus [79].

3.2 Leucine dehydrogenases

The survey of substrate specificity of LeuDHs from different sources has revealed the striking similarity. These enzymes recognize aliphatic α-amino acids and α-keto acids as the substrates for oxidative deamination and reductive amination [80–83]. The most prominent example for the synthetic application of LeuDHs is the synthesis of L-*tert*-leucine, an important chiral building block for the pharmaceutical industry [81, 84]. Optically pure L-*tert*-leucine is produced on a tons scale by the reductive amination of trimethylpyruvate catalyzed by LeuDH. The cofactor NADH is regenerated with the use of FDH from *C. boidinii*, as shown in Scheme 18. Recently, a whole-cell biocatalyst bearing an LeuDH from *Bacillus cereus* and an FDH from *C. boidinii* has been developed for the synthesis of optically pure L-*tert*-leucine to further improve the process efficiency by eliminating the use of isolated enzymes and addition of cofactor [85].



Scheme 17.



Scheme 18.

Several other aliphatic L-amino acids, such as L-valine [81], L-leucine [62, 81, 86] L-2-amino-4,4-dimethyl-pentanoic acid [81, 87], L-β-hydroxyvaline [88], L-selenomethionine [89] and L-[^{15}N]leucine [90] have also produced from the corresponding α-keto acids with LeuDH, FDH and NADH. To improve the space-time yields and facilitate control of reaction conditions, a continuous process has been developed by containing LeuDH, FDH and NADH covalently bound to water-soluble PEG in a reactor with an ultrafiltration membrane [91]. LeuDH from *B. cereus* has also been applied to obtain D-*tert*-leucine with an excellent ee of >99% *via* the oxidative resolution of the racemic mixture of DL-*tert*-leucine. An irreversible NAD^+-regenerating step by NADH oxidase from *Lactobacillus brevis* is coupled with the oxidation of L-*tert*-leucine to drive the unfavorable equilibrium toward product formation [92].

The substrate specificity of LeuDH from *B. stearothermophilus* has been altered by protein engineering. Based on molecular modeling, the residues L40, A113, V291, and V294, which are predicted to be involved in recognition of the substrate side chain, were mutated to mimic the substrate specificities of PheDH, glutamate dehydrogenase (GluDH), and lysine dehydrogenases (LysDH). The A113G and A113G/V291L mutants, imitating the PheDH active site, display activities toward L-phenylalanine and phenylpyruvate. Another two sets of mutants, L40K/V294S and L40D/V294S, substituting L40 and V294 of LeuDH with the corresponding residues of GluDH and LysDH, respectively, indicate that the two corresponding residues in the active site are important for discrimination of the hydrophobicity/polarity of the aliphatic substrate side chain [93].

3.3 *N*-Methyl-L-amino acid dehydrogenase

Some amino acid dehydrogenases catalyze the formation of *N*-alkyl-L-amino acids from the corresponding α-oxo acids and alkylamines (*e.g.*, methylamine, ethylamine, and propylamine). These enzymes are clearly distinct from conventional NAD(P)-dependent amino acid dehydrogenases. For example, ammonia is inert as a substrate for the enzyme from *Pseudomonas putida*. NADPH is more than 300 times more efficient than NADH as a hydrogen donor in the enzymatic reductive amination and shows no sequence homology to conventional NAD(P)-dependent amino acid dehydrogenases and opine dehydrogenases [94]. *N*-Methyl-L-amino acid dehydrogenase from *P. putida* has been used in the production of *N*-methyl-L-phenylalanine from phenylpyruvic acid [95] and the synthesis of enantiomerically pure cyclic amino acids [96].

4 Summary and future perspectives

Amino transfer reactions from amino donors such as amino acids or amines to α-keto acids catalyzed by transaminases, and the reductive amination catalyzed by amino acid dehydrogenases, have been clearly demonstrated to be promising approaches to the direct asymmetric synthesis of optically pure amines from the corresponding keto compounds. These two processes or enzymes possess complementary features. First, transaminases exhibit a broad substrate spectrum and are applied to construct various kinds of chiral amine moieties, while α-amino acid dehydrogenases limit their suitable substrates to α-keto acids for the reductive amination and α-amino acids for the oxidative deamination. Second, the reductive amination requires NADH or NADPH as cofactor, which is not needed in amino transfer reaction. Third, the amino transfer from amino acid to ketone is thermodynamically unfavorable and shifting the equilibrium to the product formation is required; the thermodynamics of amino acid dehydrogenase-catalyzed reaction favors the reductive amination. Finally, the amino donor in transaminase-catalyzed amino transfer reaction is the amino acid or amine, of which the suitable enantiomer is required; amino acid dehydrogenase uses ammonia as amino donor, which is much less expensive. Since NADH/NADPH can be effectively regenerated, amino acid dehydrogenase-catalyzed reductive amination should be a more favorable choice for achieving effective asymmetric synthesis of optically pure amines from the corresponding prochiral keto compounds, a formidable task in organic chemistry. However, this is limited by the strict substrate specificity and stereospecificity of α-amino acid dehydrogenase. Extensive efforts are needed to expand the substrate from α-keto acids to other keto compounds (even simple ketones), and to invert their stereospecificity [97]. Because of the unprecedented advances in molecular biology, amine dehydrogenases with desired substrate specificity and stereoselectivity are expected to appear in the coming years.

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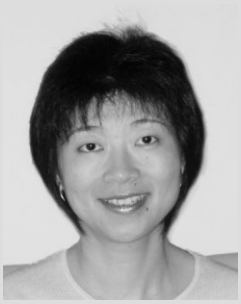

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