Transaminases for Chiral Amine Synthesis

Andrew Gomm, Elaine O'Reilly*

School of Chemistry, University of Nottingham, University Park, Nottingham, NG7 2RD

Corresponding author: Elaine O'Reilly (elaine.oreilly@nottingham.ac.uk)

Abstract

Amine transaminases are important biocatalysts for the synthesis of chiral primary amines. Unlike many enzymes that have been employed for the synthesis of optically active amines, amine transaminases are capable of asymmetric synthesis and do not rely on costly cofactors that must be regenerated *in situ*. However, their application as general catalysts for the preparation of amines is hampered by a limited substrate scope, substrate and (co)product inhibition and difficulties associated with displacing challenging reaction equilibrium. There has been important progress made to overcome these challenges, including the development of enzymes with broader substrate scope and the design of methodology to effectively displace the reaction equilibrium. Amine transaminases are also being applied in an increasing range of (chemo)enzymatic cascades and immobilized for applications in flow.

Introduction

Chiral amines are prevalent in active pharmaceutical ingredients, agrochemicals and bioactive natural products and are important pharmaceutical building blocks. As such, the development of broadly applicable biocatalytic strategies for their synthesis is of great interest. A number of enzymes have been employed for the synthesis of chiral amines, including transaminases, amine dehydrogenases, imine reductases, ammonia lyases, monoamine oxidases [1] and the more recently discovered reductive aminase [2**] (Figure 1). Transaminases (TAs) belong to fold types I and IV of pyridoxal 5'-phosphate (PLP) dependent enzymes and catalyse the reversible transfer of an amino group from a suitable donor to a carbonyl acceptor. Two types of PLP-dependent TAs have been identified and are grouped according to the type of substrate they convert [3]. α-TAs exclusively convert α-amino and α-keto acids whereas ω-TAs can accept substrates with a distal carboxylate group. Importantly, a subgroup of ω-TAs, known as amine TAs (ATAs) are capable of accepting substrates that completely lack a carboxylate group and these enzymes have received considerable attention in recent years due to their potential for the synthesis of chiral primary amines from the corresponding prochiral ketones.

Despite the enormous potential of ATAs, challenges associated with substrate and co-product inhibition, difficulties displacing reaction equilibria and substrate restrictions have contributed to their slow uptake by both the academic and industrial communities. There are a number of research groups addressing these challenges through enzyme engineering and process development and this review will focus on the most significant developments in the field over the past two years.
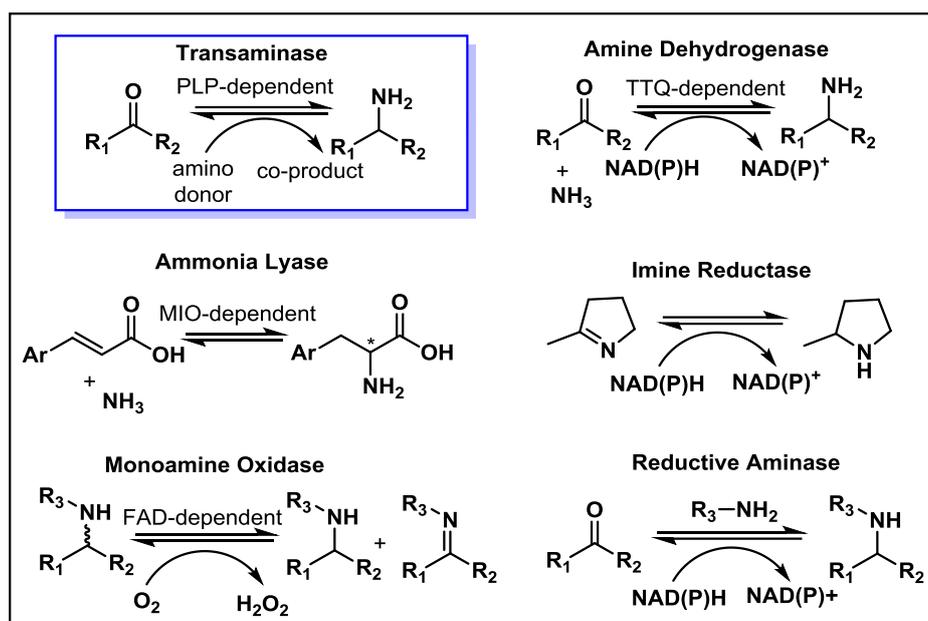
Figure 1. A selection of enzymes that can be used for the synthesis of chiral amines

Expanding their substrate scope

The substrate scope of ATAs can be considered relatively broad, as they are capable of catalysing the amination of a wide range of aldehydes and ketones. However, the active site of these enzymes contains a small and large binding pocket and therefore wild-type enzymes are restricted to ketones bearing at least one 'small' substituent (methyl or ethyl typically). Considerable effort has been directed towards increasing the capacity of the small binding pocket to enable these enzymes to accept ketones with two bulky substituents. The most high-profile example of such an engineering effort was reported in 2010, where an evolved (R)-selective ω-TA replaced a rhodium-catalysed hydrogenation step in the conversion of bulky ketone **1** (Figure 2) to the anti-diabetic drug, Sitagliptin [4]. The biocatalytic route offers dramatic improvements in yield, purity and selectivity compared to the traditional metal-catalysed approach and this landmark example has inspired subsequent engineering projects (Figure 2). Bornscheuer and co-workers have made substantial progress towards developing ATAs that accept substrates with two sterically demanding substituents. They recently reported an (S)-selective ω-TA variant from *Ruegeria sp.* TM1040 (3FCR) that is capable of converting bulky ketones (including **2** and **3**) to the corresponding chiral amines with good to excellent yields and enantiomeric excess [5**]. Interestingly, the variant contains only four mutations and the approach has been shown to be transferrable to other fold class I ω-TA templates with similar sequence identities. Despite the impressive activity of this variant towards bulky substrates, relatively modest substituent changes are not tolerated well by the mutant and this highlights the challenges associated with developing TAs with broad activity. Additionally, the variants have not been shown to work effectively at high concentrations. The ATA from *Ruegeria sp.* TM1040 has also been engineered to accept the sterically demanding bridged ketone, exo-3-amino-8-aza-bicyclo[3.2.1]oct-8-yl-phenylmethanone **4** [6], and variants of an AT from *Vibrio fluvialis* (PDB-ID: 4E3Q) showed activity towards branched-chain bulky-bulky ketones [7]. Moody and co-workers also developed a variant of 43EQ capable of mediating the conversion of the bulky substrate, 2-acetylbiphenyl **5**, having identified no residual activity in the wild-type enzyme [8]. Targeting mutations in the small binding pocket to increase capacity is not the only effective approach. Shin and co-workers reported that mutation of the L57 residue in the

large binding pocket of a TA from *Ochrobactrum anthropi* dramatically enhanced activity towards bulky arylalkylamines and alkylamines [9].
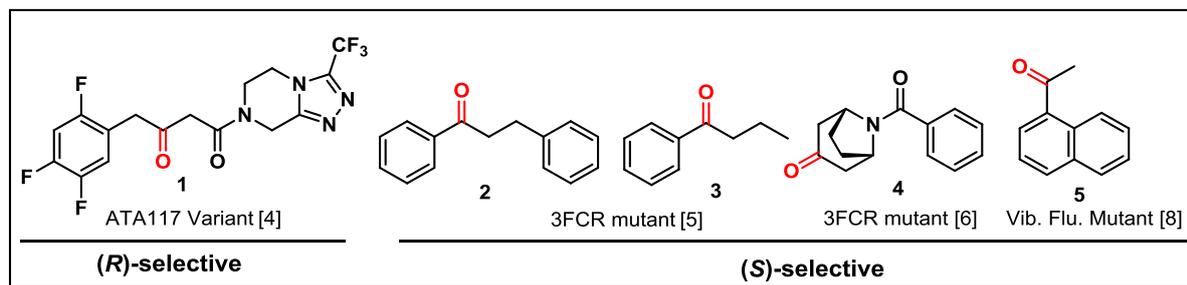
Figure 2. Engineered ATAs that act on bulky substrates **1-5** [4,5,6,8]

Displacing reaction equilibria

A significant obstacle to the application of TAs for the synthesis of chiral amines is the reversible nature of the enzymatic reaction [10]. Achieving good conversion of ketone to amine requires removal of the carbonyl co-product in order to prevent the reverse reaction from competing and compromising conversion/yield (Figure 3) [11]. The most common amine donors employed are alanine and isopropyl amine, leading to pyruvate and acetone co-products. While there are well established methods for pyruvate removal, high equivalents of alanine in combination with complex and expensive co-product removal/recycling strategies are necessary. Isopropyl amine must also be employed in high concentrations and the acetone co-product removed in situ to achieve good conversions with challenging substrates and this often requires the use of engineered enzymes that can tolerate high substrate loadings. As neither of these strategies can be considered ideal, there have been a number of studies focused on developing novel methodology for displacing the reaction equilibrium effectively.

Berglund and co-workers reported the application of 3-aminocyclohexa-1,5-dienecarboxylic acid as a TA amine donor [12]. The resulting ketone co-product is highly unstable and tautomerises to give the more stable isomer, 3-hydroxybenzoic acid. While this effectively displaces the reaction equilibrium, the amine donor is not easily accessible and therefore its practical use is limited. Our group have an ongoing interest in the development of 'smart' amine donors and recently demonstrated that commercially available *o*-xylylenediamine can be employed in combination with some of the most widely used ATAs and enables high conversions to be achieved with challenging ketone substrates [13]. Additionally, the black polymer co-product formed means that the methodology also enables effective high-throughput screening. As this amine donor is expensive and unlikely to be employed for large-scale industrial synthesis the methodology has been extended to include other smart diamine donors and has recently been shown to work with a number of ATAs [14,15].

The amines *cis*-1,4-but-2-ene-diamine and *trans*-1,4-but-2-ene-diamine have also been shown to shift the reaction equilibria of TA reactions via cyclization and tautomerization of their co-products [16]. Kroutil and co-workers assessed a small panel of 1,2-diamines that were expected to spontaneously dimerize and oxidize to form a stable co-product [17]. However, the slow dimerization limits the utility of these donors and the methodology has not been shown to work effectively with challenging ketones.
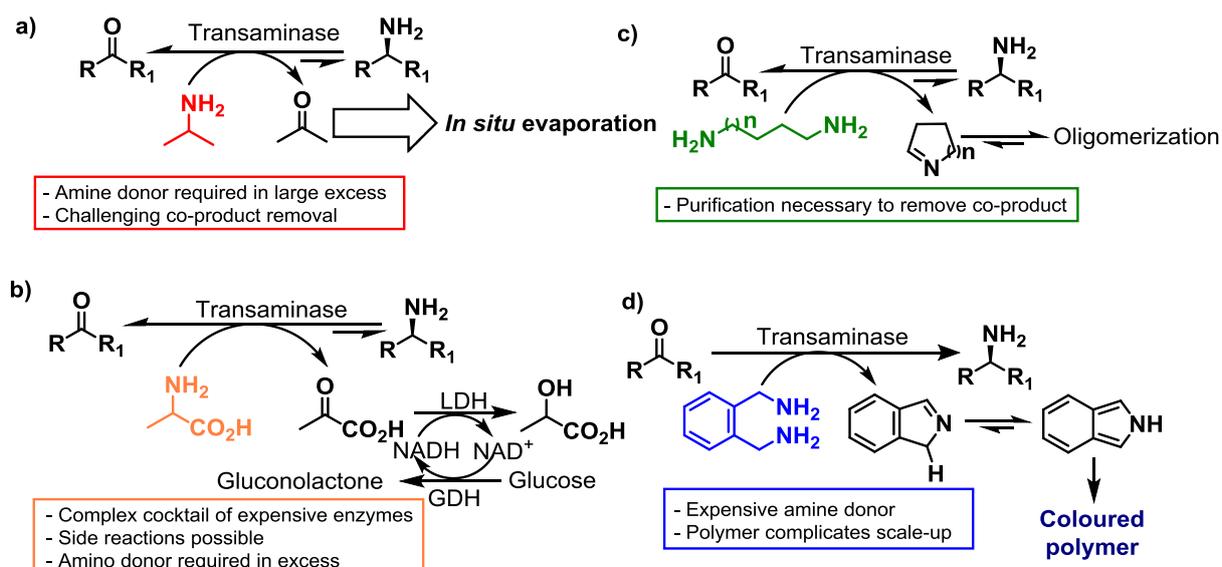
Figure 3. Selected examples of strategies to displace the reaction equilibria of TA reactions

Continuous flow biotransformations

The application of enzymes in continuous flow is not a recent development, yet there have been limited examples of the use of ATAs. Optimized biotransformations in flow are particularly attractive for large scale reactions as they are typically more reproducible, able to tolerate substrates with low solubility and are significantly more productive and economic than those performed in batch. Effective enzyme immobilization is an essential component of successful flow biotransformations and there have been a number of strategies recently reported for TAs.

Whole *E. coli* cells expressing an (*R*)-selective ω-TA from *Arthrobacter* have been immobilized on methacrylate beads and biotransformations performed in organic solvent to minimize leaching of both the enzyme and PLP co-enzyme [18]. This system allowed the synthesis of a number of α-alkoxy- and α-aryloxy acetone derivatives with good conversions and yields. Paradisi and co-workers immobilized an interesting wild-type ATA (HEWT) from the moderate halophile Halomonas elongate on epoxy Sepabeads [19]. The enzyme was capable of transforming a series of aldehydes to the corresponding amine in excellent conversion and isolated yield. Most impressive was the rapid reaction time and high conversions achieved compared to those performed in batch. For example, the system allowed for complete conversion of *para*-nitro-benzaldehyde to *para*-nitro-benzylamine in 2 minutes under flow conditions whereas the corresponding batch conditions took 210 minutes to achieve the same conversion.

An interesting article has recently been reported where the authors demonstrate a strategy for the dynamic ionic absorption of phosphorylated co-factors (PLP and NADH) on a porous material [20]. The authors demonstrated that a transamination (and asymmetric reduction of a ketone with ADH/NADH) can be performed without the need for externally supplied PLP, due to the co-enzyme shifting from an associated to disassociated state without being leached into the bulk aqueous media. The authors chose the kinetic resolution of Rac-methylbenzylamine to showcase their methodology rather than an asymmetric biocatalytic transamination and therefore it remains to be seen how well the system would perform for the asymmetric synthesis of chiral amines.

Sans and co-workers have recently demonstrated the first example of modified 3D printed devices for the immobilization and application of enzymes in continuous-flow [21]. The

authors use the benchmark conversion of (S)- and (R)-methylbenzylamine to acetophenone in the presence of pyruvate, using both (S)- and (R)-selective ATAs to showcase the technology. The approach has the potential to enable rapid screening of immobilization strategies and reaction conditions that can be easily transferred to large-scale continuous flow bioreactors.

Application in synthesis

Lipases have relatively broad substrate scope and predictable activity, and can be successfully applied for efficient kinetic resolution chemistry. For these reasons, they have secured their place in synthetic chemistry laboratories in both academia and industry [22]. In recent years, there has also been increased interest from industry in the application of TAs for the synthesis of chiral amines.

Researchers at Pfizer reported a chemo-enzymatic route for the synthesis of key chiral intermediates of a gamma secretase inhibitor with potential anti-tumour activity, employing a transaminase and an alcohol dehydrogenase (Scheme 1c) [23]. ATA-47 from CLecta was selected following the screening of a relatively large enzyme library and the team demonstrated that they could employ the enzyme for the conversion of a substituted tetralone to the corresponding (S)-amine with excellent selectivity. An impressive feature of the work was the large scale at which the reaction was performed (24.8 Kg of 6,8-difluorotetralone), enabling the preparation of almost 40 Kg of material, in 94% isolated yield. The HEC pharm group reported the application of an (R)-selective ATA for the synthesis of pharmaceutically relevant (3R)-3-aminoazepane [24], which is an important motif in a class of epidermal growth factor receptor antagonists that were developed at Novartis. This challenging substrate required extensive reaction optimisation to afford a good yield but the inclusion of the biocatalytic step resulted in a simplified reaction work-up, circumvented the need for precious metal catalysts and provided the desired chiral amine with excellent stereoselectivity.

There have been some recent examples of performing ATA reactions on a preparative scale to access chiral primary amines [25, 26] and a handful where the enzyme is employed to synthesise more complex chiral targets, which highlight their potential for multi-step synthesis. The Natural product (+)-xenovenine and its isomer were synthesised via a chemoenzymatic approach involving a key ATA-mediated amination of tricarbonyl 6 (Scheme 1a) [27]. As expected, the enzyme was completely selective for the methyl ketone and spontaneously cyclised afforded cyclic imine 7, which was subsequently converted to the target compound 8 in two further steps. Ryan *et. al.* recently reported the preparative-scale synthesis of a series of chiral piperidines, starting from easily accessible pro-chiral ketoenones [28*]. Following transamination, a spontaneous thermodynamically favourable aza-Michael type reaction occurs and means that only two equivalents of isopropylamine are required to achieve excellent conversions and isolated yields. The work also highlights an interesting example of how the reversibility of the biocatalytic reaction negates the need for regio-selectivity in the Synthesis of the natural product, (-)-pinidinone 10, starting from dimethyl ketoenone 9 (Scheme 1b) highlights an interesting example of how the reversibility of the biocatalytic reaction negates the need for regio-selectivity and can be exploited to shuttle the amine across the molecular framework.
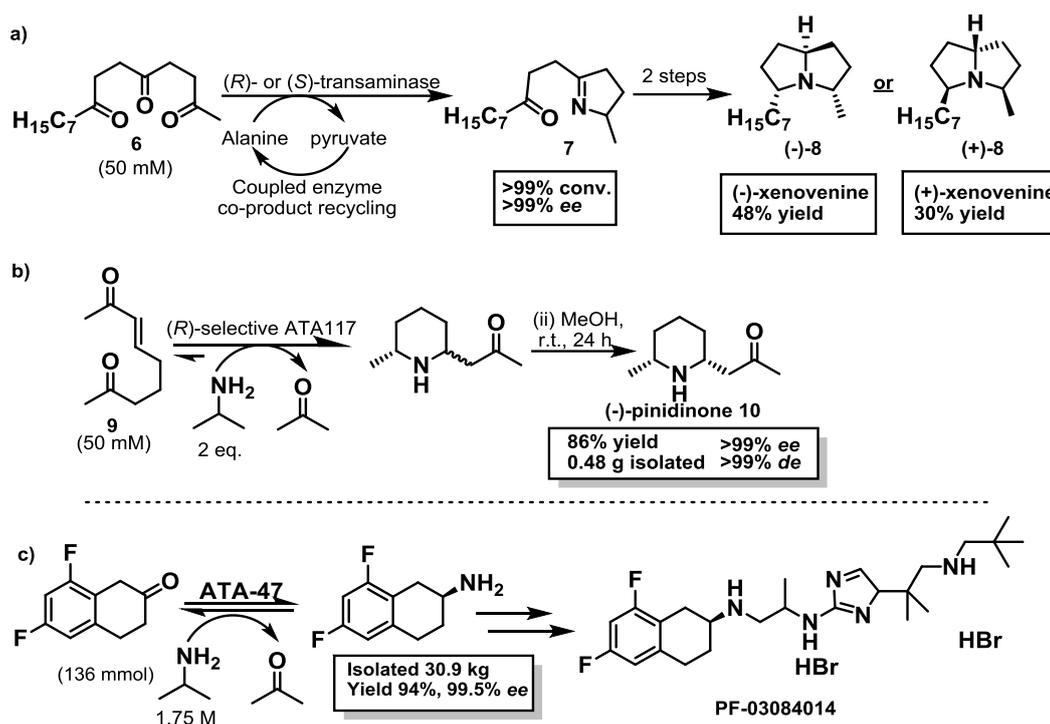
Scheme 1. Selected examples of TAs being applied for the synthesis of more complex products [23, 27, 28]

Biocatalytic cascades

Enzymatic cascades involving the combination of several concurrent biocatalytic steps in one-pot can enable access to complex chiral molecules starting from simple building blocks and avoids the need for costly intermediate purification steps or protecting group manipulations. There have been a number of recent reports of biocatalytic cascades involving TAs in combination with aldolases [29, 30], laccases [31], transketolases [32], pictet-spenglerase [33], alcohol dehydrogenases [34*, 35*, 36], acyl transferases [37] and ene-reductases [38].

An elegant three enzyme cascade has recently been reported for the synthesis of mono- and di-substituted piperidines and pyrrolidines starting from keto acids, by applying a carboxylic acid reductase (CAR), an ω-TA and an imine reductase (IRED) in one-pot [39]. The strategy relies on the selectivity of the commercially available TA (ATA113 from codexis) for the aldehyde over the bulky ketone. Subsequent IRED-mediated reduction of the imine afforded the product piperidines or pyrrolidines. The cascade was also performed in a whole-cell system by Flitsch and co-workers [40].

Both *et. al*. reported an impressive whole-cell biocatalytic cascade for the conversion of 4-substituted ethylbenzenes to 4-substituted phenylethylamines [34*]. The strategy relied on four heterologously expressed enzymes to achieve the transformation, with the required co-factors also supplied by the host. A catalytically self-sufficient cytochrome P450 monooxygenase generated a benzylic alcohol, which was subsequently oxidised to the corresponding ketone using an alcohol dehydrogenase (ADH). Two stereo-complementary ADHs where expressed to ensure complete oxidation in the event of poor selectivity in the oxidation step. Finally, ATA117 from *Arthrobacter* sp catalysed a highly selective transamination to afford the 4-substituted (*R*)-phenylethylamines in excellent *e.e.* and extremely impressive isolated yield.

An outstanding example of a multi-enzyme whole-cell cascade has recently been reported using up to eight carefully selected enzymes, including styrene monooxygenase, epoxide hydrolase, alcohol dehydrogenase and catalase, for the conversion of styrenes to

substituted amino alcohols and amino acids with surprisingly high conversions achieved [35*].

As the substrate scope of ATAs expand, there will be further opportunities to apply them in cascade reactions for the synthesis of complex molecules with multiple stereocentres.

Conclusions

Despite the growing 'toolbox' of enzymes available for biocatalytic amine synthesis, TAs continue to fill an important role for the asymmetric preparation of chiral primary amines. The application of an engineered TA for the industrial synthesis of sitagliptin [4] demonstrates the power and efficiency of chemo-enzymatic synthesis using these important biocatalysts. However, while there has been significant progress made towards expanding the substrate scope of TAs and developing strategies to displace the challenging reaction equilibria, the vast majority of enzymes still lack suitably broad substrate scope and activity and are thus unlikely to be considered as routine catalysts for synthetic chemistry applications. It is necessary to address the challenge of substrate and (co)product inhibition and develop TAs that are not restricted to narrow substrate sets, before these enzymes can start to truly impact on synthetic design strategies and be considered alongside more traditional chemical catalysis [41].

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