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RESEARCH PAPER

The Reductive Amination of Carbonyl Compounds Using Native Amine Dehydrogenase from *Laribacter hongkongensis*

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Abstract Amine dehydrogenases (AmDHs) are one of the key emerging enzymes used in the synthesis of various amines with the expense of only one ammonium ion as an amino donor, thereby, generates only water molecule as a by-product. Currently, most available AmDHs have been created through protein engineering using the existing natural L-amino acid dehydrogenase, and native AmDHs are rarely reported. In this study, a novel native AmDH from *Laribacter hongkongensis* (*Lh*AmDH) was identified based on the GenBank database using a sequence-driven approach. *Lh*AmDH showed a good activity towards various carbonyl compounds such as cyclohexanone (170 mU/mg) and isovaleraldehyde (214 mU/mg). The reductive amination of model substrate, cyclohexanone (up to 100 mM) into cyclohexylamine was successfully performed in *Lh*AmDH and FDH system with > 99% conversion using *Escherichia coli* whole-cell as well as purified enzymes. Furthermore, three enzymes cascade (ω-transaminase, *Lh*AmDH, and FDH) was designed to produce chiral amine from the corresponding ketone using inexpensive ammonium formate as sole sacrificial agent. The active site of *Lh*AmDH residues were predicted using the protein structure homology model building program SWISS-MODEL server. In the docking analysis, cyclohexanone is well-orientated with -5.4 kcal/mol of binding energy and 3.16 Å distance from side chain of E104, which is a key residue for interacting ammonia and substrate. This *Lh*AmDH model can be used as a promising template to produce chiral amines through semi-rational design.

Keywords: amine dehydrogenase, biocatalysts, chiral amine, reductive amination

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1. Introduction

Chiral amines are widely used in agricultural, refined chemicals, biologically active natural products and important pharmaceutical intermediates [1]. Therefore, the efficient synthesis of the enantiopure amines has become an attractive challenge for organic chemists and biologists in recent years [2]. Many biocatalytic methods for chiral amines production have been developed using several biocatalysts, such as amine oxidase, imine reductase, lipase, ω-transaminase, ammonia lyases, Pictet-Spenglerase, barberine bridge enzyme, engineered P450 monooxygenase, and AmDH [3-5].

AmDHs are especially attractive to produce primary amine compared to other enzymes since it requires only one cheap ammonium ion as an amino donor, but the redox reaction requires expensive cofactors, such as NAD(P)H, contributing to the high cost of the reaction. Initially, AmDH was generated through protein engineering of existing L-amino acid dehydrogenase from *Bacillus stearothermophilus*, to accommodate ketone substrates by introducing 2-4 point mutations [6]. Since then, several engineered AmDHs have been developed by evolving L-amino acid dehydrogenases from many different organisms such as *Bacillus badius*, *Rhodococcus* sp. M4, *Exiguobacterium sibiricum*, and *Caldalkalibacillus thermarum* [3]. Tseliou *et al.* generated a different type of amino acid dehydrogenase using ε-

deaminating l-lysine dehydrogenase from *Geobacillus stearothermophilus* [6]. These engineered AmDHs have been found useful for the production of chiral amines; however, the sequence diversity remained limited [7]. Itoh and co-workers first reported native enzymes from *Streptomyces virginiae* which converted non-functionalized ketones to chiral amines with ammonia [8], because of low enantioselectivity, the native AmDH from *Clostridium sticklandii* (4OP-AmDH) catalyzing the amination of 4-oxopentanoic acid (4OP) into (2*R*)-2-amino-4-oxopentanoic acid was utilized for the screening of novel native AmDHs within bacterial biodiversity [9,10]. As results, a family of native AmDHs was discovered and showed high reductive amination activity for various aliphatic and alicyclic ketones. Subsequently, crystal structures of three native AmDHs (AmDH4, *Msme*AmDH, and *Cfus*AmDH) were determined, and AmDHs were further rationally engineered to obtain activity towards pentane-2-one, which is an inert substrate for wild-type enzyme, using 3D structure [9]. Later, to further explore native AmDH biodiversity, 4OP-AmDHs were used in the second sequence-driven approach by the same group [11]. This iterative approach successfully led to the identification and characterization of five native AmDHs. However, reported native AmDH still has low enzyme activity and narrow substrate scope.

In this study, in order to add the biocatalytic toolbox available for chiral amine synthesis, a novel native AmDH was identified reactive toward various carbonyl compounds from the GenBank database using the sequence-driven approach with *Msme*AmDH. To demonstrate its applicability, herein we described the production of cyclohexylamine through reductive amination of cyclohexanone by AmDH with help of a FDH to recycle the NADH. Also, the coupling reaction of *Lh*AmDH and ω-TA was performed to produce chiral amine, (*S*)-α-MBA from acetophenone.

2. Materials and Methods

2.1. Chemicals and media

All ketones, amines, and nicotinamide cofactors (NADH and NAD^+) were purchased from Sigma-Aldrich (Sigma-Aldrich Korea, Seoul, Korea). Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from EMD Chemicals (San Diego, USA). All the other chemicals and reagents were of analytical grade.

2.2. Screening, expression AmDHs and purification of *Lh*AmDH

For the screening of AmDHs, the Basic Local Alignment Search Tool (BLAST) was performed by using the query-template sequence. Among the screened AmDHs those with less than 70% identity were selected which was based on the template *Msme*AmDH. The AmDH genes were synthesized by Bionics (Seoul, Korea). The genes were cloned into IPTG-inducible pET-24ma vector and recombinant His-tagged proteins were expressed in *Escherichia coli*. The transformants were grown at 37°C in 1 L LB (Lysogeny broth)-containing kanamycin (50 μg/mL). When the OD_{600} reached 0.6, IPTG was added to final concentration of 0.5 mM [12]. After overnight induction at 20°C, the cells were harvested at 4°C centrifugation subsequently washed twice with Tris-HCl buffer (20 mM, pH 9.0) [13]. Lysis buffer (Na_2HPO_4 (50 mM), NaCl (300 mM), Imidazole (5 mM)) was utilized to break the cell membrane with sonication and centrifuged at 16,000 rpm at 4°C for 30 min for separation into soluble and insoluble fractions and both fractions were subjected to SDS-PAGE (12%) [14]. For the purification of AmDHs, Ni-NTA affinity chromatography (GE Healthcare Bio-Sciences, Sweden) was performed using standard protocol [15] and protein concentration was measured by the Bradford method using Bovine Serum Albumin as a standard (Fig. 1). For co-expression of *Lh*AmDH and FDH, the FDH gene was cloned into the pQE-80L vector which is compatible with pET-24ma vector. Two plasmids (pET-24ma-*Lh*AmDH and pQE-80L-FDH) were co-transformed into *E. coli* BL21 with LB media containing ampicillin (100 μg/mL) and kanamycin (50 μg/

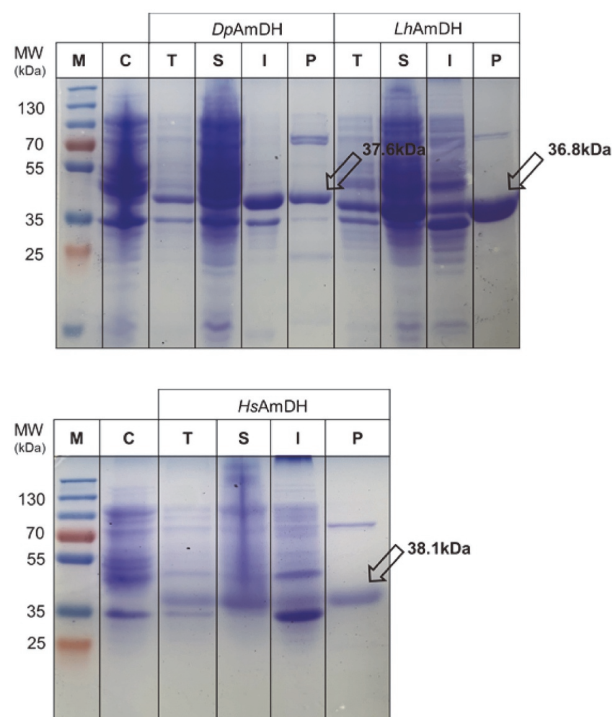

Fig. 1. SDS-PAGE analysis for expression of *Dp*AmDH (37.6 kDa), *Lh*AmDH (36.8 kDa), and *Hs*AmDH (38.1 kDa); Lanes : M, protein markers; C, BL21 (DE3) *Escherichia coli* cells; T, total protein; S, soluble protein; I, insoluble protein fractions; P, purified enzymes.

mL) for the cell growth. Once OD_{600} of the cells reached 0.6-0.8, IPTG was added (0.1 mM final concentration) and cells were cultivated overnight. The cells were harvested and washed twice with Tris-HCl buffer (20 mM, pH 9.0) and used further whole-cell reaction.

2.3. Enzyme assay

The activities of AmDH and FDH were determined using an NADH-dependent (340 nm, λ_{340} = 6,220 $M^{-1}cm^{-1}$) spectrophotometric assay. The stoichiometric reduction of NAD^+ or the oxidation of NADH, as defined by the change in absorbance over time, is used to measure activity. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 mol of NAD^+ or NADH in 1 min [3]. The standard solution for the AmDH assay contained 0.2 mM NADH (or NAD^+) and an appropriate amount of the enzyme.

2.4. Quantitative analysis of amines

The analysis of cyclohexylamine was performed using a gas chromatography instrument with a flame ionization detector (GC/FID) fitted with an AOC-20i series autosampler injector (GC 2010 plus Series, Shimadzu Scientific Instruments, Kyoto, Japan) [13]. According to a previously reported study, HPLC technique was used for the quantitative analysis of (*S*)-α-MBA at 210 nm with a Crownpak CR column (Daicel Co., Japan) [16].

3. Results and Discussion

3.1. Screening of AmDHs

A family of native AmDHs (AmDH4, *Msme*AmDH, and *Cfus*AmDH) can catalyze the reductive amination of ketones and aldehydes with ammonia and methylamine [9]. The structure of *Msme*AmDH was closely related to AmDH4 with the monomers of each enzyme trapped in the closed form, provide further evidence for this conformation as crucial in the catalytic cycle of this dehydrogenase class. *Msme*AmDH can catalyze the reductive amination of various carbonyl compounds such as cyclohexanone (196 mU/mg), 3-methyl-butan-2-one (129 mU/mg), and 3-hydroxybutanone (177 mU/mg). Interestingly, the enzyme showed reactivity towards benzaldehyde (< 3 mU/mg). It suggests that the enzyme can be used for the sequence-driven approach to screen novel AmDHs for the synthesis of amines from the various carbonyl compounds. Therefore, Basic Local Alignment Search Tool (BLAST) was performed using *Msme*AmDH as the query sequence [17], and the first top 100 rank candidates were collected.

The first top 100 rank candidates were divided into subgroups through phylogenetic analysis. ClustalX aligned amino acid sequences and the trees were generated in PhyML using the maximum-likelihood method [18]. For the sequence diversities of candidates, at least one enzyme was selected from every subgroup. The enzyme showing the higher sequence identity with *Msme*AmDH may give similar functionality with *Msme*AmDH. Therefore, the enzymes having more than 70% identity were excluded. Finally, 5 candidates showing a similarity of 30-60% with *Msme*AmDH were selected (Table 1). The codon optimized genes encoding corresponding AmDH were cloned in pET-24ma. Each of the C-terminal His-tagged enzymes was expressed and purified using a Ni-NTA affinity column chromatography. As a result, 3.8, 0.6, 1.5, 7.3, and 0.6 mg of purified enzymes from 500 mL culture were obtained for *Hs*AmDH, *Nj*AmDH, *Dp*AmDH, *Lh*AmDH, and *Vt*AmDH, respectively (Table 1 and Fig. 1). In the case of *Nj*AmDH and *Vt*AmDH, the expression level of the enzyme was very poor. Since the cyclohexanone and benzaldehyde were active substrates for natural amine dehydrogenase in the previous study [9], the AmDH activity assay was performed with 0.5 mg/mL of purified enzyme in the presence of 10 mM cyclohexanone 0.2 mM NADH, 0.5 M ammonium formate buffer. Among them, only *Lh*AmDH showed considerable activity for cyclohexanone (40 mU/mg) and benzaldehyde (10 mU/mg), while other screened enzymes showed negligible activity (< 1 mU/mg) for both substrates. The *Lh*AmDH displayed higher activities towards benzaldehyde than previously reported *Msme*AmDH (< 3 mU/mg). Moreover, due to high expression level and considerable activity, *Lh*AmDH was further characterized.

Table 1. AmDHs identified by sequence-driven approach

Entry	Organism	Enzyme ID No	Abbreviation	Identity with *Msme*AmDH	Purified enzyme (mg)[a]	Reference
1	*Mycobacterium smegmatis*	A0A0D6I8P6	*Msme*AmDH	100	-	[9]
2	*Hydrogenoanaerobacterium saccharoVorans*	WP_092753241.1	*Hs*AmDH	39.21	3.8	In this work
3	*Nocardia jiangxiensis*	WP_040831253.1	*Nj*AmDH	60.36	0.6	In this work
4	*Desulfosporosinus* sp.BRH_c37	KUO76656.1	*Dp*AmDH	40.12	1.5	In this work
5	*Laribacter hongkongensis*	WP_088861355.1	*Lh*AmDH	40.96	7.3	In this work
6	*Vulcanisaeta thermophila*	WP_069807543.1	*Vt*AmDH	29.52	0.6	In this work

[a]For 500 mL of the culture.

3.2. Characterization of *Lh*AmDH

The effect of concentration of substrate ammonia on enzyme activity was examined in the presence of 40 mU/mg cyclohexanone at pH 9.0. Since ammonium formate buffer itself supplies ammonia to the enzyme, the enzyme activity was determined with 0.5, 1.0, and 2.0 M ammonium formate buffer. The activity of *Lh*AmDH was linearly increased with the increase of buffer concentration, suggesting that the Km value of enzyme towards ammonia is very high (Fig. 2). After then, the optimal pH of the enzyme was examined with 2.0 M ammonium formate buffer. The *Lh*AmDH showed the highest activity at pH 9.0. This slightly alkaline pH for the optimal activity was also observed with reported native AmDHs [9], which was likely due to the increase in the concentration of the deprotonated ammonia that enhances the formation of imine. To explore the applicability of *Lh*AmDH for reductive amination of various carbonyl compounds, the substrate specificity of *Lh*AmDH was examined (Table 2). Among them, *Lh*AmDH showed the highest reactivity for isovaleraldehyde (364 mU/mg) and cyclohexanone (170 mU/mg). Linear aliphatic carbonyl compounds such as 3-heptanone, 2-pentanone were not reactive substrates. The enzyme showed a considerable reactivity towards the aromatic substrate, benzaldehyde (18 mU/mg), but its activity towards acetophenone and propiophenone is very poor, which may be due to the presence of side chains, which cause steric hindrance in the active site of the enzymes (see 3.6.

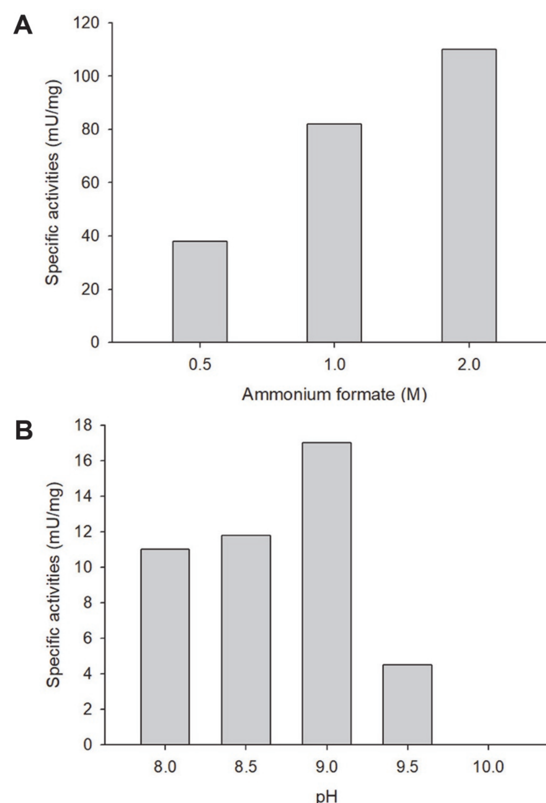

Fig. 2. The effect of ammonium formate buffer concentration (A) and pH (B) on the activity of *Lh*AmDH. Reaction conditions: 0.2 mL final volume, 10 mM substrate, ammonium formate buffer, 0.2 mM NADH, 0.5 mg/mL *Lh*AmDH, 25ºC, reaction time 2 min.

Table 2. Substrate specificities of *Lh*AmDH

Substrate	Relative activity(%)[a]	Substrate	Relative activity(%)[a]
Cyclopentanone	3.4	Phenylacetate	n.d.
Cyclohexanone[b]	100	Phenoxy-2-propanone	n.d.
Benzaldehyde	10.45	2-butanone	5.46
Acetophenone	0.27	2-pentanone	n.d.
Propiophenone	0.39	2-hexanone	n.d.
Butyrophenone	n.d.[c]	3-heptanone	n.d.
4-phenyl-2-butanone	n.d.	Isovaleraldehyde	214

[a]Reaction conditions : 10 mM substrates, 1 mM NAD^+, 0.05-0.5 mg/mL *Lh*AmDH, 2 M ammonium formate buffer, pH 9.0, room temperature. [b]The specific activity of the *Lh*AmDH (170 mU/mg) for cyclohexanone was taken as 100%. [c]No detected activity.

Homology modeling of *Lh*AmDH). However, it is notable that the activity for benzaldehyde offers a promising protein engineering template to enhance substrate range aromatic ketones containing side chain such as acetophenone, and butyrophenone, had very low or no detectable activity for any of reported enzymes [9].

3.3. Reductive amination of Cyclohexanone using purified *Lh*AmDH and FDH

The development process of cyclohexylamine synthesis, which is a common industrial intermediate, has become a key hotspot [19]. Industrially it is produced by the amination of cyclohexanone and widely utilized as one of the most important raw materials in agriculture, rubber, food, oil, pharm, petroleum, and textile industries [19]. To produce cyclohexylamine from cyclohexanone using *Lh*AmDH, the efficient recycling system of the cofactor, NADH is required (Fig. 3). Glucose dehydrogenase (GDH) and FDH are generally used for cofactor regeneration. GDH utilizes glucose, and coproduct gluconolactone is irreversibly hydrolyzed into gluconic acid, providing a strong driving force for NADH regeneration. However, the main disadvantage of the GDH system is the pH drops in the reaction medium due to the formation of gluconic acid. While formate is converted to carbon dioxide FDH, NADH is formed. In the case of FDH, formate is converted to carbon dioxide by FDH and NADH is formed. The involvement of FDH has advantages like the use of cheap formate as an electron source, the removal of the coproduct carbon dioxide (a favorable thermodynamic equilibrium) and negligible pH-change of the reaction solution. Also, formate can be supplied from ammonia formate buffer. Therefore, *Lh*AmDH was combined with FDH from *Candida boidinii* source in order to recycle the cofactor [20]. In the coupled *Lh*AmDH/FDH reaction, the reaction condition should be carefully determined to balance the activities of two enzymes. The pH dependence and the effect of the ammonium formate concentration buffer for FDH were examined. The effect of buffer concentration was examined at pH 9.0 (the optimal pH for AmDH). In 2 M ammonium formate buffer, FDH gave 1.3 U/mg which is much higher than AmDH (0.2 U/mg), suggesting that FDH activity is enough to recycle NADH in the *Lh*AmDH/FDH reaction (Fig. 4A). The reductive amination of cyclohexanone using *Lh*AmDH/FDH was performed with 10, 50, and 100 mM substrate in 0.5 mL of

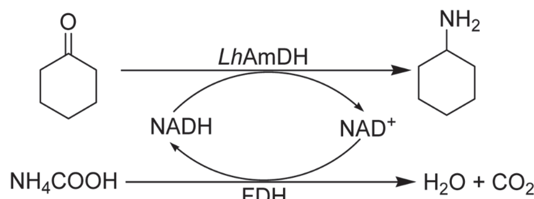

Fig. 3. The reductive amination of a carbonyl compound (*e.g.* cyclohexanone) by native AmDH in combination with FDH. Carbonyl compound (*e.g.* cyclohexanone) was reductive aminated into the corresponding amine by AmDH utilizing ammonia and NADH. Additionally, NADH is regenerated by FDH.

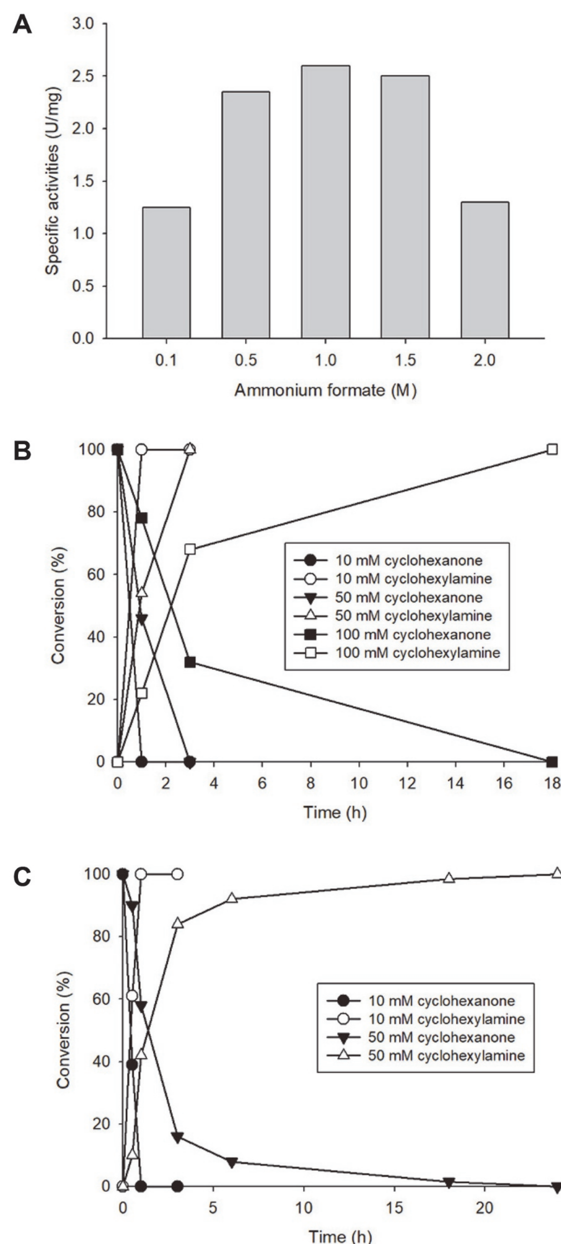

Fig. 4. (A) The effect of ammonium formate concentration on FDH. Reaction conditions : 0.2 mL final volume, 1 mM NAD^+, 0.005 mg/mL FDH and NH_4COOH buffer (0.1-2 M, pH 9.0), 25°C. (B) Production of cyclohexylamine using *Lh*AmDH with FDH. Reaction conditions: cyclohexanone (10, 50, or 100 mM), 1 mM NAD^+, *Lh*AmDH (4 mg/mL), FDH (0.81 mg/mL), 2 M NH_4COOH buffer (pH 9.0), 25°C, 170 rpm. (C) Reductive amination of cyclohexanone using *Lh*AmDH-FDH whole cell system. Reaction conditions: cyclohexanone (10 or 50 mM), *Lh*AmDH-FDH cells (36 mg_{CDW}/mL), 2 M NH_4COOH buffer (pH 9.0), 25°C, 170 rpm.

2 M ammonium formate buffer (pH 9.0) containing 1 mM NADH. 10 mM cyclohexanone was converted into cyclohexylamine with 95% conversion for 30 min (Fig. 4B). In the 50 mM substrate reaction, 24 mM cyclohexylamine was produced for 1 h and all of the substrate was converted into cyclohexylamine for 3 h (Fig. 4B). After getting an excellent conversion, this result encouraged us to perform a higher substrate concentration reaction (100 mM) and the product was obtained with > 99% conversion (Fig. 4B). The successful reductive amination catalyzed by *Lh*AmDH suggested their potential use for the production of cyclohexylamine form various cyclic carbonyl compounds.

3.4. Production of cyclohexylamine using whole-cells co-expressing *Lh*AmDH and FDH

Biocatalytic cascades using whole-cells offer a highly potent alternative to standard biochemical synthesis to establish cheap, selective, and efficient production processes [21]. The reported whole-cell biotransformation is about 10 times more cost-effective than the purified enzyme system [22,23]. Cell-free extracts can also be used, but because of the disadvantage of being unstable, whole-cells expressing the enzyme of interest are preferred [24]. Therefore, recombinant *E. coli* cells co-expressing *Lh*AmDH and FDH were used as biocatalysts. The recombinant *E. coli* system expressing both enzymes was developed by cloning of *Lh*AmDH and FDH genes into pET-24ma and pQE-80L vectors, respectively. The cells were harvested, washed twice with Tris-HCl buffer (20 mM, pH 9.0), and used further whole-cell reaction (see 2. Materials and Methods).

The reductive amination of 10 mM cyclohexanone carried out in 2 M ammonium formate buffer (5 mL, pH 9.0) with whole-cell (36 mg_{CDW}/mL) without adding cofactor NADH resulted complete conversion into cyclohexylamine in 3 h (Fig. 4C). The whole-cell catalysts converted 50 mM cyclohexanone into the product in 24 h, suggesting that the whole-cell catalyst co-expressing both enzymes, is applicable for the large-scale production of cyclohexylamine (Fig. 4C).

3.5. Asymmetric synthesis of chiral amine by combining ω-TA and *Lh*AmDH

ω-TAs catalyze the stereoselective transfer of an amino group between an amino donor and a prochiral ketone substrate, which are useful biocatalytic tools for the production of optically pure chiral amines [25-32]. One of the main disadvantages of ω-TA reaction is the requirements of expensive amino donors in excess compared to the target substrate. To overcome it, a multi-enzymatic cascade comprising ω-TA, *Lh*AmDH, and FDH was designed to produce chiral amines (Fig. 5). Whereas amino donor (cyclohexylamine) of ω-TA reaction is recycled via reductive amination of deaminated product (cyclohexanone) by

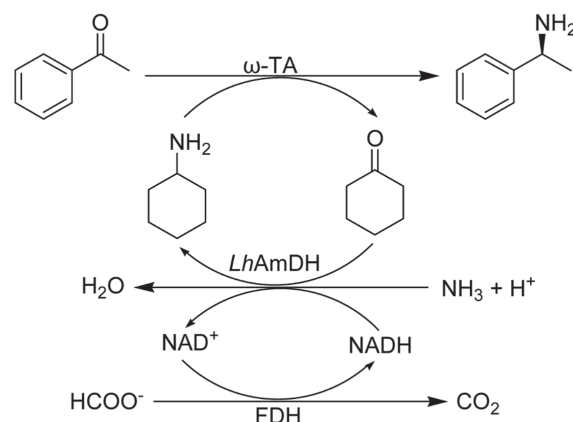

Fig. 5. Biocatalytic asymmetric cascade synthesis of (*S*)-chiral amine ((*S*)-α-MBA) catalyzed by ω-TA, *Lh*AmDH, and FDH, driven through cheap ammonium formate as both amino donor and reducing agent.

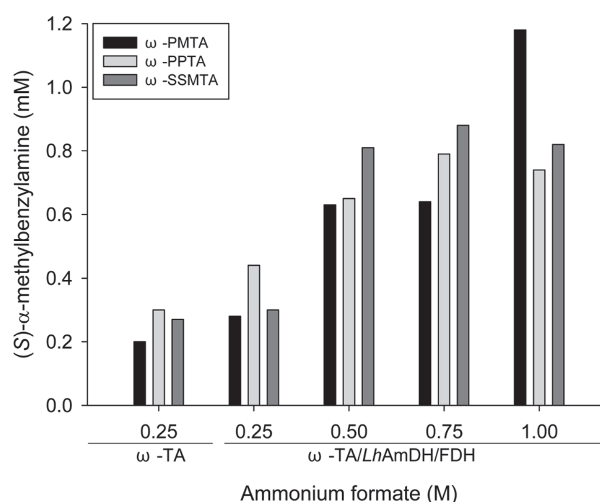

Fig. 6. Comparison of ω-TA and ω-TA/*Lh*AmDH/FDH systems for the synthesis of (*S*)-α-MBA using various ω-TAs. Reaction conditions: NH_4COOH buffer (0.25-1 M, pH 9.0), 10 mM acetophenone, 100 mM cyclohexylamine, ω-TA cells (9 mg_{CDW}/mL), cells (4.5 mg_{CDW}/mL), 25°C, 170 rpm for 2 h.

AmDH using inexpensive ammonium formate as sole sacrificial agent. Also, the recycling of cyclohexanone will reduce the production inhibition by cyclohexanone since ω-TA suffers from severe product inhibition by ketone product. As a proof of concept, we tried to produce (*S*)-α-MBA from acetophenone (Fig. 6). In order to screen ω-TAs utilizing cyclohexylamine as an amino donor from a small in-house library of ω-TAs, the asymmetric synthesis of (*S*)-α-MBA from 10 mM acetophenone in 1 M ammonium formate buffer (pH 9.0) with 100 mM cyclohexylamine was performed. ω-TAs from *Silicibacter pomeroyi* (ω-PMTA), *Phaeobacter porticola* (ω-PPTA), and *Shimia marina* (ω-SSMTA) gave 1.2, 0.7, and 0.8 mM (*S*)-α-MBA for 2 h respectively (Fig. 6). The multi-enzymatic cascade

reaction was performed with screened active TAs at various concentration of ammonia formate buffer. The product formation is increased by recycling cyclohexylamine as an amino donor using *Lh*AmDH/FDH/ω-TA system compared to the ω-TA alone reaction. For instance, (*S*)-α-MBA from 10 mM acetophenone was produced from ω-PPTA by multi-enzymatic cascade reaction which is 6.0-fold higher than that of alone ω-TA reaction. Although only 0.45 mM product was produced this result showed the potential of designed cascade works well. However, when ω-TA is screened which shows a good activity to use cyclohexylamine as an amino donor, the designed cascade will be applicable for efficient ω-TA mediated amination of various ketones, resulting in high conversion with water and CO_2 will be the only waste products.

3.6. Homology modeling of *Lh*AmDH

The molecular docking simulation performs homology modeling based on a search of another ligand that has extensively simulated possible binding pose like target [33]. Since the crystal structure of *Lh*AmDH was not revealed, starting from the crystal structure of *Cfus*AmDH (PDB ID 6IAU) from native AmDH complexed with $NADP^+$ and the substrate cyclohexylamine. Based on this we have constructed a three-dimensional structure using the SWISS-MODEL server (http://www.swissmodel.expasy.org) [34]. A homology model was generated using *Cfus*AmDH (PDB ID 6IAU) as a reference which is the most similar PDB structure of *Lh*AmDH has a homo-dimeric structure and the amino acid composition at the active site was similar to *Cfus*AmDH, especially Y168 and E108 (Fig. 7A). The active sites of *Cfus*AmDH, Y168 resembled that of AmDH, whereas cage is formed by the nicotinamide substrate ring $NADP^+$ and an aromatic residue. Also, the coordination of the amine group to E108 holds the tetrahedral carbon bearing the amine, suggests a role for the activation of ammonia by this residue [9]. Thus, docking analysis was performed using cyclohexanone (or cyclohexylamine) with good activity and acetophenone (or α-MBA) with poor activity for whether the tertiary structure was well-formed. As mentioned above, glutamate is a key residue for interacting amino donors and even substrate. As shown in Fig. 7, cyclohexanone is well-orientated and the distance from the side chain of E104 was 3.16 Å with -5.4 kcal/mol of binding energy. However, inert substrate (*S*)-α-MBA was located at a rate of 4.52 Å away from the side chain of E104 residue, indicating that it is difficult to react. These results suggested that the 3D homology model of *Lh*AmDH was constructed well. This *Lh*AmDH model can be utilized for enzyme engineering, such as semi-rational design to obtain enhanced activity with broaden substrate specificity.

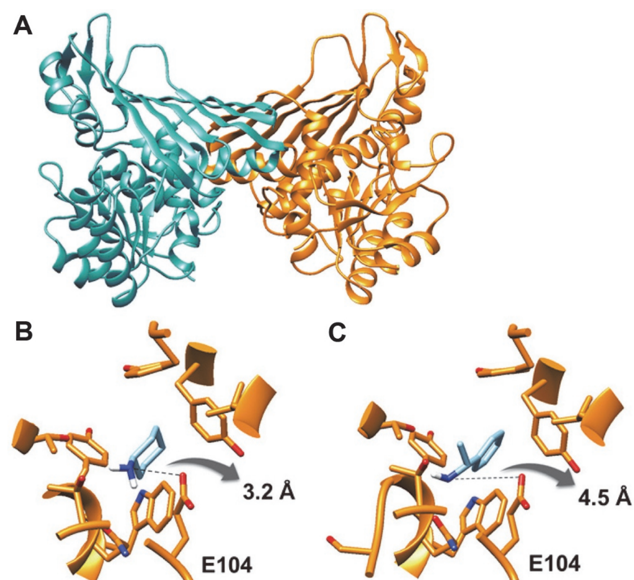

Fig. 7. (A) Overall homology model structure of *Lh*AmDH dimer form colored in cyan and orange, and representative structures of the interaction between the side chain of Glu104 with cyclohexylamine (sky blue) (B), and (*S*)-α-MBA (sky blue) (C).

4. Conclusion

In this study, we identified novel *Lh*AmDH in the GenBank database using a sequence-driven approach and *Lh*AmDH was cloned, functionally expressed in *E. coli* and characterized using a purified enzyme. The applicability of the enzyme for the reductive amination of carbonyl compounds was demonstrated with multi-enzyme cascades such as *Lh*AmDH/FDH and ω-TA/*Lh*AmDH/FDH. Considering that (i) currently most of reported AmDHs have been created from L-amino acid dehydrogenase through protein engineering (the diversity of engineered amine dehydrogenase is limited) and (ii) a limited number of native AmDHs have been reported, newly identified *Lh*AmDH offers a new sequence space and catalytic features for the synthesis of chiral amines. After enzyme engineering of *Lh*AmDH, it would be used for the synthesis of chiral amines from corresponding carbonyl compounds, requiring only one cheap ammonium ion as an amino donor.

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Neither ethical approval nor informed consent was required for this study.

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