1

Transamination and Reductive Amination

2.4.1

2.4

Amino Acid and Amine Dehydrogenases

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General Introduction

 α -Keto acids can be reductively aminated to α -amino acids via amino acid dehydrogenase catalysis, with NAD(P)H as cofactor. The nitrogen source for the amine functionality is ammonia, the least expensive source. Regeneration of the co-factor NAD(P)+ back to NAD(P)H is required for synthesis and is commonly afforded via formate dehydrogenase catalyzed oxidation of formate to carbon dioxide or glucose dehydrogenase catalyzed oxidation of glucose to gluconic acid. While the equilibrium position strongly favors formation of amino acids from keto acids, amino acids can also be deaminated to keto acids if the coupled regeneration reactions provide the driving force.

Recently, amine dehydrogenases, which reductively aminate ketones to amines, have been developed via protein engineering. Both amino acid and amine dehydrogenases are exquisitely enantioselective, leading to (S)- or, less frequently, to (R)-amino acids or to (R)-amines.^[1]

2.4.1.1 Amino Acid Dehydrogenases

Enantiospecific reduction of C=N bonds is a promising route for the synthesis of α -amino acids and derivatives, such as amino alcohols or amines. The reduction can be achieved by enzymes following various mechanisms, e.g. by pyridoxal 5'-phosphate (PLP) dependent transaminases (see Section 2.4.3) or by amino acid dehydrogenases (EC 1.4.1.–) using NADH or NADPH as cofactor. The synthetic usefulness of the transaminase reaction is diminished by the position of the equilibrium (K_{eq} is often close to 1), which means that either complex mixtures result that are often laborious to separate, or extra measures are required to shift the equilibrium considerably further toward the products. These can include removal of the keto-containing coproduct through distillation or pervaporation (if it is volatile), or through reactions catalyzed by one to three additional enzymes.

This section focuses on the reduction of C=N bonds by reductive amination with amino acid dehydrogenases (AADHs) (Scheme 1). In an equilibrium process, an α -keto acid can be reductively aminated to an α -amino acid or, vice versa, the α -amino acid can be oxidatively deaminated to the α -keto acid.

Scheme 1 General Reaction for the Formation of α -Amino Acids from α -Keto Acids Using Amino Acid Dehydrogenases

$$\bigcap_{R^1} (CO_2H) + NADH + NH_3 + H^+$$

for references see p 20

1 (1 of 24)

This asymmetric synthesis route to amino acids possesses a number of advantages that are attractive in today's context of seeking highly selective and environmentally benign processes: (i) compact synthesis of the α -keto acid substrates; (ii) extreme enantioselectivity of amino acid dehydrogenases; and (iii) yields of up to 100% with respect to the α -keto acid, with no undesirable enantiomers or other byproducts. In Table 1, the known amino acid amino acid dehydrogenases and their EC numbers are listed.^[2]

 Table 1
 NAD(P)⁺ Dependent Amino Acid Dehydrogenases

EC Number	Enzyme	Coenzyme	Source(s)
1.4.1.1	alanine dehydrogenase	NAD ⁺	bacterium (Bacillus, Streptomyces, Halobacterium)
1.4.1.2	glutamate dehydrogenase	NAD ⁺	bacterium, fungus, yeast, plant
1.4.1.3	glutamate dehydrogenase	NAD(P) ⁺	animal, fungus, Tetrahymena
1.4.1.4	glutamate dehydrogenase	NAD ⁺	bacterium, fungus, yeast, Chlor- ella
1.4.1.7	serine dehydrogenase	NAD ⁺	plant
1.4.1.8	valine dehydrogenase	NAD(P)+	bacterium (<i>Alcaligenes</i> , <i>Strepto-</i> <i>myces</i>), plant
1.4.1.9	leucine dehydrogenase	NAD ⁺	bacterium (Bacillus, Clostridium)
1.4.1.10	glycine dehydrogenase	NAD ⁺	bacterium (Mycobacterium)
1.4.1.11	3,5-diaminohexanoate dehy- drogenase	NAD ⁺	bacterium (Clostridium)
1.4.1.12	2,4-diaminopentanoate dehy- drogenase	NAD ⁺	bacterium (Clostridium)
1.4.1.15	lysine dehydrogenase	NAD ⁺	human, bacterium (<i>Agrobacteri-um</i>)
1.4.1.16	diaminopimelate dehydro- genase	NADP ⁺	bacterium (Bacillus, Corynebacteri- um)
1.4.1.19	tryptophan dehydrogenase	NAD(P) ⁺	plant
1.4.1.20	phenylalanine dehydrogenase	NAD ⁺	bacterium (Brevibacterium, Bacil- lus, Rhodococcus)
1.4.1.21	aspartate dehydrogenase	NAD ⁺	bacterium (Pseudomonas aerugi- nosa)

2.4.1.1.1 Physicochemical, Sequence, and Structure Comparison

There is very little thermodynamic limitation on the reductive amination of keto acids; for the leucine/ α -ketoisocaproate (ketoleucine) reaction at pH 11.0, K_{eq} equals 9×10^{12} ,^[3] while for phenylalanine/phenylpyruvate at pH 7.95 a K_{eq} of 2.5×10^7 has been reported.^[4] Thus, the maximum degree of conversion is very close to 100%. Coupling of the reductive amination reaction of keto acids with cofactor regeneration via the formate dehydrogenase (FDH)/formate or glucose dehydrogenase (GDH)/glucose reaction, which have equilibrium constants even higher than those for amino acid/keto acid, further helps to shift the equilibrium toward the amino acid product.

The mechanism of reductive amination and the geometry of the active center^[4–8] cause the *S*-configured amino acid products of the reaction to be completely enantiomerically pure, an important criterion for large-scale application. The catalytic mechanism of amino acid dehydrogenases (AADHs) has been studied most thoroughly with glutamate dehydrogenase (GluDH) from *Clostridium symbiosum*^[5,9] and with phenylalanine dehydro-

genase (PheDH) from *Rhodococcus* M4.^[4] The reader is referred to these articles for details. The mechanism was found to be remarkably similar in both cases.

As investigations of amino acid sequences and crystal structures of the amino acid dehydrogenases have revealed, most amino acid dehydrogenases are clearly homologues and feature the same type of three-dimensional structure. Most amino acid dehydrogenases possess a hexameric structure, although octamers, tetramers, dimers, and even monomers have also been found. The subunits are usually of similar size; for instance, most bacterial amino acid dehydrogenases are hexamers with a molecular weight of around 49 kDa per subunit. In Table 2, the levels of identity of the amino acid sequence across the most frequently encountered amino acid dehydrogenases are listed.^[10]

Proteinª	LeuDH B. c.	LeuDH B. sph.	LeuDH L. sph.	LeuDH T. i.	PheDH Rh. r.	PheDH L. sph.	PheDH T. i.	PheDH B b.	ValDH S. a.
LeuDH G. st.	83.5	80.0	77.5	80.9	37.1	49.9	47.9	48.2	50.6
LeuDH B. c.	100.0	76.9	79.4	78.6	36.5	47.0	46.3	47.0	48.9
LeuDH B. sph.		100.0	84.3	77.8	36.5	47.0	42.8	45.6	50.0
LeuDH L. sph.			100.0	77.2	35.3	49.5	44.2	50.3	48.9
LeuDH <i>T. i.</i>				100.0	37.1	49.0	45.5	47.4	49.7
PheDH Rh. r.					100.0	33.5	31.0	32.7	36.1
PheDH L. sph.						100.0	51.6	53.8	45.3
PheDH <i>T. i.</i>							100.0	53.8	45.3
PheDH <i>B b.</i>								100.0	43.9
ValDH S. a.									100.0

Table 2 Identities of Protein Sequences of Different Amino Acid Dehydrogenases (as Percentages)^[10]

^a The data was calculated using Clustal2.1; LeuDH G. st. = leucine dehydrogenase from Geobacillus (previously Bacillus) stearothermophilus; LeuDH B. c. = leucine dehydrogenase from Bacillus cereus; LeuDH B. sph. = leucine dehydrogenase from Bacillus sphaericus (IFO3525); LeuDH L. sph. = leucine dehydrogenase from Lysinibacillus sphaericus (ATCC4525); LeuDH T. i. = leucine dehydrogenase from Thermoactinomyces intermedius; PheDH Rh. r. = phenylalanine dehydrogenase from Rhodococcus rhodocrous; PheDH L. sph. = phenylalanine dehydrogenase from Lysinibacillus sphaericus (ATCC4525); PheDH T. i. = phenylalanine dehydrogenase from Thermoactinomyces intermedius; PheDH B b. = phenylalanine dehydrogenase from Bacillus badius; ValDH S. a. = valine dehydrogenase from Streptomyces albus.

The comparison of the sequence of the best researched amino acid dehydrogenase, i.e. that of GluDH from *C. symbiosum*, does not appear in Table 2. Interestingly, it shows the lowest level of identity with all of the amino acid dehydrogenases listed in Table 2; the level of identity with PheDHs (from *R. rhodocrous* and *T. intermedius*) and leucine dehydrogenases (LeuDHs) (from *G. stearothermophilus*, *B. cereus*, and *B. sphaericus*) is only between 12.4 and 14.0%.

In contrast, all five LeuDHs in Table 2 feature very high levels of identity with each other (between 77 and 84%). The valine dehydrogenase (from *Streptomyces albus*) is equidistant in amino acid sequence to each LeuDH, with approximately 50% identity (48.9–50.6%). The four PheDHs are each 51–54% identical to each other, except for the case of the PheDH from *Rhodococcus rhodocrous*, which is only 31–34% identical to the other three.

The analysis of the amino acid sequence of amino acid dehydrogenases suggests that, with the possible exception of GluDH from *Clostridium glutamicum*, all amino acid dehydrogenases feature the same three-dimensional structural type. Table 3 lists the currently (as of September 2014) available X-ray structures for amino acid dehydrogenases.^[5,11-37]

for references see p 20

3 (3 of 24)

3

Biocatalysis 2.4 Transamination and Reductive Amination

Amino Acid Dehydrogenase	X-ray Structure Availability	Ref
leucine dehydrogenase (LeuDH)	Bacillus sphaericus (1LEH)	[11]
	Sporosarcina psychrophila (3VPX)	[12]
phenylalanine dehydrogenase (PheDH)	Rhodococcus rhodocrous (1XBG, 1BW9, 1C1D, 1C1X)	[13,14]
alanine dehydrogenase (AlaDH)	Phormidium lapideum (1PJB, 1PJC, 1SAY)	[15]
	Thermus thermophilus (2EEZ)	[16]
	Mycobacterium tuberculosis (2VHY, 2VHZ, 2VHV, 2VHW, 2VHX)	[17]
glutamate dehydrogenase (GluDH)	Thermotaga maritama (1B26, 1B3B, 2TMG)	[18]
	Clostridium symbiosum (1BGV, 1K89)	[5,19]
	Thermococcus litoralis (1BVU)	[20]
	Thermococcus profundus (1EUZ)	[21]
	Pyrococcus furiosus (1GTM, 1HRD)	[22]
	Pyrobaculum islandicum (1V9L)	[23]
	Plasmodium falciparum (2BMA, 3R3J)	[24,25]
	Peptoniphilus asaccharolyticus (2YFQ)	[26]
	Thermus thermophilis (3AOE, 3AOG)	[27]
	Bacillus subtilis (3K92, 3K8Z)	[28]
	bovine (1HWY, 1HWZ, 1NR1, 1NQT, 1NR7, 3ETE, 3MW9, 3MVO, 3MVQ)	[29–31]
<i>meso</i> -diaminopimelate dehydrogenase (<i>meso</i> -DAPDH)	Corynebacterium glutamicum (1DAP, 2DAP, 3DAP)	[32–34]
	Clostridium tetani E88 (3WGZ, 3WGO, 3WGQ, 3WGY)	[35]
	Symbiobacterium thermophilum (3WB9, 3WBB, 3WBF)	[36,37]

 Table 3
 X-ray Structures of Amino Acid Dehydrogenases^[5,11-36]]

As expected from the sequence similarity pattern, the crystal structures of amino acid dehydrogenases superimpose very well, except for the *meso*-diaminopimelate dehydrogenases. As an example, the RMSD (residual mean square difference) of α -carbons between LeuDH from *Bacillus sphaericus* (1LEH) and PheDH from *Rhodococcus* sp. M4 is 0.234 Å. All amino acid dehydrogenases feature the familiar Rossman-type fold for the NAD(P)H cofactor.

2.4.1.1.2 Substrate Specificity

2.4.1.1.2.1 Specificity of Alanine Dehydrogenase

Alanine dehydrogenase (AlaDH; EC 1.4.1.1) has been isolated and characterized from both mesophilic (*Bacillus subtilis* and *Bacillus sphaericus*)^[38–40] and thermophilic (*Bacillus stearo-thermophilus*)^[41] organisms. Successful cloning and purification has been reported in the literature.^[42] The narrow substrate specificity of AlaDH renders the enzyme useful for the synthesis of L-alanine and analogues, such as L-[¹⁵N]alanine,^[43] 3-fluoro-L-alanine,^[44] and 3-chloro-L-alanine, only.^[45]

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2.4.1.1.2.2 Specificity of Leucine Dehydrogenase

Isolation and characterization of leucine dehydrogenase (LeuDH; EC 1.4.1.9) has been pioneered by Hummel^[46] (from *Bacillus sphaericus*), Schütte^[47] (from *Bacillus cereus*), and by Ohshima and Soda (from mesophilic *B. sphaericus* and from moderately thermophilic *Bacillus stearothermophilus*).^[48–50] The biochemical data of the latter two enzymes, however, does not differ much, as Tables 4 and 5 reveal.^[48]

 Table 4
 Properties of Leucine Dehydrogenase from Bacillus sphaericus and Bacillus stearothermophilus^[48]

Parameter	B. sphaericus	B. stearothermophilus	Ref
M _r (kDa)	245 000	300 000	[48]
subunit (<i>M</i> _r)	41 000 (hexamer)	49000 (hexamer)	[48]
optimum pH: deamination	10.7	11.0	[48]
optimum pH: amination	9.0–9.5	9.0–9.5	[48]
coenzyme	NAD (K _M 0.39 mM)	NAD (K _M 0.49 mM)	[48]

 Table 5
 Substrate Specificities for Leucine Dehydrogenase from Bacillus sphaericus and Bacillus stearothermophilus^[48]

Reaction Type Substrate		Substrat	e Specificity ^a (%)	Ref
		B. sphaericus	B. stearothermophilus	
deamination	L-leucine	100 (1.0)	100 (4.4)	[48]
	L-valine	74 (1.7)	98 (3.9)	[48]
	L-isoleucine	58 (1.8)	73 (1.4)	[48]
	L-norvaline	41 (3.5)	n.r.	[48]
	L- α -aminobutyrate	14 (10)	n.r.	[48]
	L-norleucine	10 (6.3)	n.r.	[48]
	D-leucine	0	0	[48]
amination	α -ketoisocaproate	100 (0.31)	100	[48]
	α -ketoisovalerate	126 (1.4)	167	[48]
	α -ketovalerate	76 (1.7)	86	[48]
	α -ketobutyrate	57 (1.7)	45	[48]
	α -ketocaproate	46 (7.0)	n.r.	[48]

^a Substrate specificity relative to that for L-leucine or α -ketoisocaproate (set as 100%); $K_{\rm M}$ (mM) is given in parentheses; n.r. = not reported.

LeuDH from *B. stearothermophilus*, in comparison to that from *B. sphaericus*, features an extended pH range of activity (5.5–10 vs 6.5–8.5), a higher heat stability [temperature of optimum activity (T_{opt}) is 70 vs 50 °C after heat treatment of 5 min], a longer half-life (several months vs six days at pH 7.2 and 6 °C), and much greater stability toward organic solvents and denaturants.^[48] LeuDH from *B. stearothermophilus* had already been cloned and overexpressed^[49,51] during early studies. Recently, the production of recombinant enzyme from *B. cereus*, even on large scale, has been demonstrated.^[52,53]

The substrate specificity of LeuDHs was reported by Zink and Sanwal in 1962,^[54] and subsequently by Schütte (*B. cereus*),^[47] Ohshima and Soda (*B. stearothermophilus* and *B. sphaericus*),^[2] Nagata (*Bacillus DSM 7330*),^[55] Misono (*Corynebacterium pseudodiphtheriti*-

for references see p 20

cum),^[56] and Bommarius (*B. stearothermophilus*).^[57] In addition to the proteinogenic amino acids valine, leucine, and isoleucine, unnatural amino acids such as *tert*-leucine^[58–60] or L- β -hydroxyvaline^[61] can be synthesized.

LeuDHs from *B. cereus*, *B. sphaericus*, and *B. stearothermophilus* display a remarkably similar substrate spectrum: (i) LeuDHs accept 2-oxo acids with hydrophobic, aliphatic, branched, and unbranched carbon side chains with up to six carbon atoms, as well as some alicyclic keto acids, as substrates; however, a substrate with the adamantyl group as a side chain, where the geometric limit seems to be reached, is not accepted. 2-Oxo-3methylpentanoic acid is the preferred substrate and the preferred chain length is five carbon atoms. (ii) The keto acid substrate should have at least four carbon atoms; pyruvate is only converted at less than 3% compared to α -ketoisocaproate (the standard substrate). (iii) The various amino acid dehydrogenases differentiate substrate side chains mainly based on steric factors at the C3 and C4 position of branched keto acids. (iv) Functionalized keto acids such as ketoglutarate are not accepted (activity <0.1% of base case). Phenylpyruvate, as a model of an aromatic substrate, is inert.^[41]

A study of the LeuDH activity with respect to van der Waals volumes^[62] or hydrophobicities^[63] of side chains with various carbon atom configurations yielded a moderate correlation.^[57,63]

2.4.1.1.2.3 Specificity of Valine Dehydrogenase

The valine dehydrogenase (ValDH) from *Streptomyces albus* is a 364 amino acid dimeric protein (38 kDa subunit; 67 kDa dimer) that catalyzes the oxidative deamination of aliphatic and branched-chain amino acids, and the reductive amination of the corresponding keto acids. Upon overexpression of the gene in *E. coli*, the optimal conditions were found to be 37 °C and pH values of 10.5 and 8.0 for dehydrogenase activity with valine and for reductive amination activity with α -ketoisovaleric acid, respectively.^[64] Mutations of the conserved Lys79 and Lys91 residues to alanine revealed their involvement in substrate binding and catalysis, respectively, analogous to the corresponding residues in the homologous leucine dehydrogenase and phenylalanine dehydrogenase. Enzyme specificity has been altered by substituting alanine for glycine at position 124 by site-directed mutagenesis; the Ala124Gly variant displays lower activities toward aliphatic amino acids, but higher activities toward L-phenylalanine, L-tyrosine, and L-methionine compared to the wild-type enzyme.^[65]

2.4.1.1.2.4 Specificity of Glutamate Dehydrogenase

Glutamate dehydrogenase (GluDH) has been investigated by the groups of Engel and Rice since the 1980s; accordingly, more is known about GluDH, especially from *Clostridium symbiosum*, than about any other amino acid dehydrogenase. Although there is no sequence identity with respect to other amino acid dehydrogenases beyond random similarity (see Section 2.4.1.1.1), site-directed mutagenesis of two amino acid residues (to give Lys89Leu and Ser380Val mutants) led to similar activity levels toward glutamate, norleucine, and methionine, and demonstrated the importance especially of the Lys89Leu mutation.^[66,67] To this day, studies on GluDH from *C. symbiosum* act as the standard of knowledge regarding conformational changes of amino acid dehydrogenases upon binding of a keto acid substrate. These conformational changes also seem to be responsible, in part, for substrate specificity.^[18]

2.4.1.1.2.5 Specificity of Phenylalanine Dehydrogenase

An enzyme catalyzing the reductive amination of phenylpyruvate to the desired L-phenylalanine was first found by Hummel^[68] in a strain of *Brevibacterium*, and then later in *Rhodococcus* sp.^[69,70] In Tables 6 and 7, the microbiological and kinetic data of the phenylalanine dehydrogenases (PheDHs) is summarized.^[71]

Table 6	Comparison of Microbiological Data of Phenylalanine Dehydrogenase from Brevi-
bacteriun	n and Rhodococcus Species ^[69,70,71]

Amino Acid Inducer	Enzyme Yie	Ref	
	Brevibacterium ^[69]	Rhodococcus ^[70]	
L-phenylalanine	210	15200	[69,70]
L-histidine	120	1800	[69,70]
L-phenylalaninamide	-	3500	[69,70]
L-isoleucine	0	0	[69,70]
D-phenylalanine	204	0	[69,70]
D,L-phenylalanine	214	0	[69,70]

 $^{\rm a}$ Results of induction with amino acid [enzyme yield (U/L)] after

addition of 1% of the amino acid as an inducer.

 Table 7
 Comparison of Enzymological Data of Phenylalanine Dehydrogenase from Brevibacterium and Rhodococcus Species^[69,70,71]

Parameter		Brevibacterium ^[69]	Rhodococcus ^[70]	Ref
optimum pH	amination	9.0	9.25	[69,70]
	deamination	10	10	[69,70]
<i>К</i> _м (mM)	phenylpyruvate	0.11	0.16	[69,70]
	4-hydroxyphenylpyruvate	0.24	2.4	[69,70]
	3-(1 <i>H</i> -indol-2-yl)-2-oxopropan- oate	8.0	7.7	[69,70]
	4-(methylsulfanyl)-2-oxobutan- oate	3.0	2.1	[69,70]
V _{max} (relative to phenylpyruvate)	phenylpyruvate	100	100	[69,70]
	4-hydroxyphenylpyruvate	96	5	[69,70]
	3-(1 <i>H</i> -indol-2-yl)-2-oxopropan- oate	24	3	[69,70]
	4-(methylsulfanyl)-2-oxobutan- oate	59	33	[69,70]
K _M	NADH	47 μΜ	130 μM	[69,70]
	NH ₄ ⁺	431 mM	387 mM	[69,70]
stability	t _{1/2} when stored at 4°C	4–8 h	10 d	[69,70]
	deactivation (% per day) under operating conditions	26	5	[69,70]

In addition to L-phenylalanine, the homologue L-homophenylalanine, important as a component in angiotensin-converting-enzyme (ACE) inhibitors, can be obtained from 2-oxo-4-phenylbutanoate using phenylalanine dehydrogenase (PheDH).^[72] The substrate

for references see p 20

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Ziegler + Müller, Kirchentellinsfurt · APEX Version: 1.0.1-26

7 (7 of 24)

7

specificity of PheDH from *Bacillus sphaericus* has been investigated by Asano.^[73] In Table 8, the activities of two PheDHs from R. *rhodocrous*^[70] and *B. sphaericus*^[73] for the transformation of aromatic and aliphatic keto acids are compared.

 Table 8
 Substrate Specificity of Phenylalanine Dehydrogenase from Rhodococcus rhodocrous and Bacillus sphaericus^[57,70,73]

Substrate ^a		R.	rhodoc	B. sphaeri- cus ^[73]	Ref	
		V _{max} (U/mL)	<i>К</i> _м (mM)	Relative Activity (%)	Relative Activity (%)	
α-ketoisocaproate	8.0	-	-	4.2	-	[70,73]
4-(methylsulfanyl)-2-oxobutanoic acid	8.5	50	2.1	33	6.0	[70,73]
phenylpyruvate	8.0	150	0.16	100	100	[70,73]
4-hydroxyphenylpyruvate	8.5	7.5	2.4	5	138	[70,73]
3-(1H-indol-2-yl)-2-oxopropanoate	8.5	4.5	7.7	3	n.d. ^b	[70,73]
2-oxo-4-phenylbutanoate	8.0	96	0.01	64	1.9	[70,73]
2-oxo-5-phenylpentanoate	8.0	46	0.65	30	1.5	[70,73]

^a Conditions: 25 °C, concentration of substrate = 0.1 M; for comparison: relative activities for LeuDH from *B. cereus*: 2-oxo-4-methylpentanoic acid = 100%, 2-oxo-4-phenylbutanoate = 0.2%.^[57]

^b n.d. = not determined.

Sequencing, cloning, and heterologous expression of PheDH from *Rhodococcus* was first described by Brunhuber and co-workers.^[74] A double mutation Gly124Ala/Leu307Val was created by site-directed mutagenesis of PheDH from *B. sphaericus* to change the substrate specificity to be closer to that of a leucine dehydrogenase (LeuDH). This led to a mutant with decreased activity toward L-phenylalanine and enhanced activity toward almost all aliphatic amino acid substrates, thus confirming the predictions made from molecular modeling.^[75]

PheDH differs markedly from all LeuDHs, as it can convert not only aromatic substrates but also the aliphatic substrates typical for LeuDHs. Because of the high intrinsic specific activity of PheDH from *Rhodococcus*, the enzyme in many cases actually registers higher specific activity than LeuDH with sterically demanding α -keto acid substrates. The substrate specificities of PheDH from *Rhodococcus rhodocrous* and *Bacillus sphaericus* seem to exhibit more variation with respect to each other than do the specificities of LeuDHs from various species. PheDH from *B. sphaericus* mainly converts (substituted) phenylpyruvates, whereas the enzyme from *Rhodococcus* sp. displays a rather high degree of activity in the presence of a phenylalkyl group in the substrate.

2.4.1.1.2.6 Specificity of *meso*-Diaminopimelate Dehydrogenase

meso-Diaminopimelate dehydrogenase (*meso*-DAPDH) is one of the first enzymes in the lysine biosynthesis pathway, and the only dehydrogenase. It catalyzes the reversible, NAD(P)H dependent oxidative deamination of the *R* stereocenter of *meso*-diaminopimelate (*meso*-2,6-diaminoheptanedioate) to generate L-2-amino-6-oxopimelate and is usually absolutely specific for the *meso* stereoisomer of DAP. The 320–326 amino acid, 35 kDa dimeric enzyme has been isolated and characterized from *Corynebacterium glutamicum*,^[76] *Brevibacterium lactofermentum*,^[77] *Bacillus sphaericus*,^[78] *Brevibacterium flavum*,^[79] *Symbiobacterium thermophilum*,^[29,37] and *Ureibacillus thermosphaericus* strain A1,^[80] with about 50% amino acid sequence identity across the sequences of the latter three organ-

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9

isms. The wild-type enzyme from *Symbiobacterium thermophilum* is not very active on midsize keto acids. A single variant, His227Val, found via site-saturation mutagenesis of four sites in the vicinity of the active site, increased the specific activity 35-fold to 2.4 U/mg.^[37]

Starting from the *meso*-DAPDH from *C. glutamicum*, three rounds of site-directed and random mutagenesis led to a variant that no longer accepted diaminopimelate, but did accept several aliphatic, including branched-chain, p-amino acids as substrates for oxidative deamination. The best variant contained five mutations (Gln151Leu/Asp155Gly/ Thr170Ile/Arg196Met/His245Asn) and was found to reduce keto acids to the corresponding p-amino acids with excellent enantioselectivity and >95% conversion (Scheme 2).^[81] Some years later, five point mutations (Gln154Leu/Asp158Gly/Thr173Ile/Arg199Met/His249Asn) in equivalent positions around the active site were again necessary to switch the specificity of the protein *U. thermosphaericus* strain A1 from diaminopimelate as substrate (for the wild-type) to various aliphatic 2-oxo acids for the pentavariant.^[80] Interestingly, the pentavariant is still completely stable at 65 °C, and is thus considerably more stable than the wild-type.



2.4.1.1.2.7 Specificity of Other Amino Acid Dehydrogenases

Only two L-aspartate dehydrogenases (L-AspDH; EC 1.4.1.21) have been discovered so far. An open reading frame (ORF) encoding a putative AspDH from *Pseudomonas aeruginosa* PAO1 has been cloned and overexpressed in *E. coli* to yield, after purification to homogeneity, a 28 kDa subunit dimer (*PaeAspDH*) with 127 and 147 U/mg specific activity for L-aspartate and oxaloacetate, respectively.^[82] *PaeAspDH* is promiscuous for NADH and NADPH, with similar $K_{\rm M}$ values. The enzyme is greatly stabilized by the addition of either 0.4 M NaCl or 0.3 M glycerol, with a melting temperature ($T_{\rm m}$) increase from 48 to 60 °C.

L-Aspartate is obtained in 89% molar yield and 625 mM product concentration from fumarate in a fed-batch operation with a three-enzyme system: *PaeAspDH*, *Bacillus subtilis* malate dehydrogenase, and *E. coli* fumarase.^[83]

Proline dehydrogenase (ProDH; EC 1.5.5.2) plays a role in the metabolic pathway from proline to glutamate. The gene encoding ProDH from the *Pseudomonas fluorescens* pf-5 strain was cloned into *E. coli* and the His-tagged 40 kDa protein was expressed and purified to reveal optimum performance at pH 8.5 and 30 °C; V_{max} and K_{M} values were 160 U/mg and 20 mM, respectively, with L-proline as a substrate.^[84]

After finding *N*-methyl-I-amino acid dehydrogenase activity in various bacterial strains, Mihara and co-workers cloned the gene from *P. putida* ATCC12633 into *E. coli*. The enzyme (purified to homogeneity) catalyzes the NADPH dependent reductive amination of α -oxo acids, such as pyruvate, phenylpyruvate, and hydroxypyruvate, with alkylamines, such as methylamine, ethylamine, and propylamine, but not ammonia, to form *N*-alkyl-I-amino acids.^[85] NADPH is more than 300 times more effective as a cofactor than NADH. Analysis of the amino sequence of the *N*-methylamino acid dehydrogenase revealed no sequence homology with conventional NAD(P) dependent amino acid dehydrogenase (EC 1.4.1.1), and thus is the first member of a new NAD(P) dependent oxidoreductase superfamily.

for references see p 20

9 (9 of 24)

2.4.1.1.3 Synthetic Applications

2.4.1.1.3.1 Synthesis of (S)-tert-Leucine

Non-proteinogenic amino acids, such as optically pure amino acids with bulky side chains, are of great value as synthons in the pharmaceutical industry, and biocatalytic routes have been developed to allow their synthesis. In particular, (*S*)-*tert*-leucine is a valuable building block for various active pharmaceutical ingredients (APIs), such as the hepatitis C antiviral NS1 protease inhibitors telaprevir^[86] (Incivek, Incivo; Vertex, Janssen) and bocepravir (Victrelis; Merck), or the HIV protease inhibitor atazanavir^[87] (Reyataz; Bristol-Myers Squibb). Process options for the synthesis of (*S*)-*tert*-leucine have been discussed in refs^[88–91]. Enantiomeric salt separation is cumbersome, especially when attempting to reach the 99.5% enantiomeric excesses required by the FDA.

Several biocatalytic routes cannot be applied to *tert*-leucine; in neither aminoacylasebased processes nor a hydantoinase/carbamoylase process do the respective substrates undergo reaction.^[92] However, both the resolution of racemic *tert*-leucine with penicillin G acylase,^[93,94] as well as transamination with ω-transaminase, lead to a successful synthesis of (*S*)-*tert*-leucine.^[95] Resolution of racemic *tert*-leucine with L-leucine dehydrogenase (LeuDH)/NADH oxidase results in 50% each of trimethylpyruvate and (*R*)-*tert*-leucine.^[96] However, the amino acid dehydrogenase catalyzed reductive amination of trimethylpyruvate to (*S*)-*tert*-leucine has received the most attention.^[92-94,97] Both LeuDH and phenylalanine dehydrogenase (PheDH) present broad substrate specificity. While LeuDH accepts 2-oxo acids with hydrophobic, aliphatic, branched, and unbranched carbon chains (up to a straight chain of eight carbon atoms), and some alicyclic keto acids as substrates, PheDH additionally accepts aromatic substrates.^[4,91,98] Maximum conversion often approaches 100% (at high substrate concentrations, however, various limiting factors need to be taken into consideration^[57]).

Coupling the amino acid dehydrogenase with a cofactor recycling system such as formate dehydrogenase (FDH)/formate (ammonium formate, also used as source of ammonium for the reductive amination) allows large-scale industrial production of L-amino acids such as *tert*-leucine (Scheme 3). The continuous reduction of trimethylpyruvate to (*S*)-*tert*leucine has also been carried out in an enzyme-membrane reactor (EMR)^[99] while other approaches include the use of whole-cell biocatalysts.^[100–102] High yields (>70%) and productivities (>600 g·L⁻¹·d⁻¹) are reported.^[103]

Scheme 3 Leucine Dehydrogenase Catalyzed Reductive Amination of Trimethylpyruvic Acid to *tert*-Leucine Using Formate Dehydrogenase as Cofactor Regeneration System^[91]



Even in the last few years, the synthesis of (*S*)-*tert*-leucine from trimethylpyruvate has seen a flurry of activity. Both the LeuDH gene from *Bacillus cereus* and the FDH gene from *Candida boidinii* were co-cloned into *E. coli* "designer cells", and both proteins were expressed to afford asymmetric reductive amination of trimethylpyruvate to (*S*)-*tert*-leucine.^[101] Neither purification of the enzymes nor addition of exogenous cofactors was necessary. (*S*)-*tert*-Leucine was formed in a batch reactor with high conversion of ca. 90% and an enantiomeric purity of >99% ee.

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A novel LeuDH from *Exiguobacterium sibiricum* (*Es*LeuDH) was overexpressed in *E. coli* BL21, purified to homogeneity, and characterized to show good thermostability, with a half-life of 3.1 hours at 60 °C.^[90] The broad substrate specificity of *Es*LeuDH ranges from many aliphatic α -keto acids and L-amino acids, to some aromatic α -keto acids and α -amino acids, such as α -phenylpyruvate and (*S*)-phenylglycine. The *Es*LeuDH was successfully coexpressed with *Bacillus megaterium* glucose dehydrogenase (*Bm*GDH) in *E. coli* BL21 for the production of (*S*)-*tert*-leucine on a 100-g scale. A substrate concentration of 0.6 M (78.1 g·L⁻¹) on 1-L scale with 99% conversion after 5.5 hours, resulted in 80.1% yield and >99% ee.

The LeuDH gene from *Lysinibacillus sphaericus* CGMCC 1.1677 encoding LeuDH has been cloned and coexpressed with NAD⁺ dependent FDH from *C. boidinii* for NADH regeneration.^[93] The batch reaction conditions, with cell-free extracts of recombinant *E. coli*, for the synthesis of (*S*)-*tert*-leucine were systematically optimized for both intermittent and continuous substrate feeding modes to alleviate substrate inhibition, and thus improve the space-time yield. The continuous-feeding process was performed at an overall substrate concentration up to 1.5 M, with both conversion and enantiomeric excess values of >99% and a space-time yield of 786 g·L⁻¹·d⁻¹. Furthermore, the route was successfully scaled up to a 1-L scale.

In the last few years, several patents have been filed on the process to generate (*S*)-*tert*-leucine by reductive amination.^[104–109] In a typical embodiment, LeuDH, FDH, and NAD were measured, and 5.0 M ammonium formate solution (200 mL) and deionized water (430 mL) were added. 2.0 M trimethylpyruvic acid solution was added in the sequence of 150 mL at the start and after 2 hours, and 70 mL after 4 hours. After 24 hours total reaction time, the solution was heated for 1 to 2 hours at 60 °C to deactivate the enzymes, diatomaceous earth was added, the mixture was filtered, and the remaining solution was vacuum-concentrated to obtain crude product. After twice stirring the solid in methanol followed by distillation to remove solvent, a refined product with purity of >98% and an optical purity of >99% ee was obtained. The invention is claimed to have the advantages of affording high purity and yield of product, of being a simple process that has no specific requirement of facilities, and being suitable for large-scale production.^[107]

2.4.1.1.3.2 Synthesis of (S)-L-6-Hydroxynorleucine

(*S*)-L-6-Hydroxynorleucine [(*S*)-2-amino-6-hydroxyhexanoic acid], a chiral intermediate required in the synthesis of omapatrilat (Vanlev; Bristol-Myers Squibb), a vasopeptidase inhibitor and thus an antihypertensive drug, has been obtained via two different routes.

Bovine liver glutamate dehydrogenase (GluDH) was the best catalyst for the reductive amination of 6-hydroxy-2-oxohexanoic acid sodium salt to (*S*)-L-6-hydroxynorleucine.^[110] The reaction of 95 mM of substrate (2:1 mixture of 6-hydroxy-2-oxohexanoic acid sodium salt in equilibrium with 2-hydroxytetrahydro-2*H*-pyran-2-carboxylic acid) was complete within 3 hours, resulting in the amino acid product in 89–92% chemical yield and with >99% optical purity.

Because the keto acid substrate is very cumbersome to synthesize, a method that proceeds by deracemization of racemic 6-hydroxynorleucine, via treatment with D-amino acid oxidase [either porcine kidney D-amino acid oxidase (DAAO) and beef liver catalase or *Trigonopsis variabilis* whole cells] and catalase, seems attractive. In the second step [after obtaining unreacted enantiopure (S)-L-6-hydroxynorleucine], the reductive amination procedure with bovine liver GluDH/NADH is used to convert the mixture containing 6-hydroxy-2-oxohexanoic acid and (S)-L-6-hydroxynorleucine into just (S)-L-6-hydroxynorleucine (>98% ee) with yields of 97% from racemic 6-hydroxynorleucine (starting

for references see p 20

11 (11 of 24)

12

with 100 g·L⁻¹) (Scheme 4).^[110,111] Both formate dehydrogenase (FDH)/formate and glucose dehydrogenase (GDH)/glucose were employed for cofactor regeneration. D,L-6-hydroxy-norleucine can be prepared easily from 5-(4-hydroxybutyl)imidazolidine-2,4-dione.





2.4.1.1.3.3 Synthesis of (S)-2-Amino-5-(1,3-dioxolan-2-yl)pentanoic Acid

Phenylalanine dehydrogenase (PheDH) from *Thermoactinomyces intermedius* ATCC 33205 was recently used to synthesize (*S*)-2-amino-5-(1,3-dioxolan-2-yl)pentanoic acid (allysine ethylene acetal) from the corresponding keto acid, with regeneration of NAD⁺ cofactor by formate dehydrogenase (FDH)/formate (Scheme 5);^[112] the specific activity toward the keto acid was 16% compared to the standard substrate phenylpyruvate.





The system was used in three different configurations: (i) A system with heat-dried cells from *T. intermedius* (PheDH) and *C. boidinii* (FDH) yielded on average only 84 mol% and could not be scaled up owing to lysis of the *T. intermedius* cells. (ii) A similar system using recombinant PheDH from *E. coli* improved the yield to 91 mol%. (iii) Heat-dried *Pichia pastoris* containing endogenous FDH and expressing recombinant PheDH from *T. intermedius* yielded 98 mol% with an optical purity of >98% ee. Altogether, more than 200 kg of (*S*)-2-amino-5-(1,3-dioxolan-2-yl)pentanoic acid was produced.

cpgp.BommariusAu.210cck.3d・2014-10-22・Rev: 1
Ziegler + Müller, Kirchentellinsfurt・APEX Version: 1.0.1-26

2.4.1.1.3.4 Synthesis of (S)-3-Hydroxyadamantan-1-ylglycine

Another interesting application of amino acid dehydrogenases concerns the preparation of (*S*)-3-hydroxyadamantan-1-ylglycine from 2-(3-hydroxyadamantan-1-yl)-2-oxoacetic acid using a modified form of a recombinant phenylalanine dehydrogenase (PheDH), cloned from *Thermoactinomyces intermedius* and expressed in *Pichia pastoris* or *Escherichia coli* (Scheme 6).^[113] The non-proteinogenic amino acid is a key intermediate required for the synthesis of saxagliptin, a dipeptidyl peptidase IV inhibitor sold by Bristol-Myers Squibb under the tradename Onglyza for the treatment of type 2 diabetes mellitus. *E. coli* coexpressing PheDH and formate dehydrogenase (FDH) from *P. pastoris* for cofactor regeneration allowed the successful reductive amination of the keto acid on several-kg scale (40 kg in an 800-L vessel and with 99% conversion).





2.4.1.1.3.5 Synthesis of (S)-1-Cyclopropyl-2-methoxyethanamine

(*S*)-1-Cyclopropyl-2-methoxyethanamine was sought by Bristol-Myers Squibb as a key chiral intermediate for the synthesis of a corticotropin-releasing factor-1 (CRF-1) receptor antagonist. Initially, resolution of the racemic amine was attempted but this only resulted in 53% ee (at 38% yield) after transamination with (*S*)-transaminase from *Vibrio fluvalis*, or in 91% ee at 35% yield by lipase-catalyzed resolution. In contrast, reductive amination of 2-cyclopropyl-2-oxoacetic acid, catalyzed by leucine dehydrogenase (LeuDH) from *Thermoactinomyces intermedius*, accompanied by NADH cofactor recycling by formate dehydrogenase (FDH) from *Pichia pastoris*, gives (*S*)-cyclopropylglycine with no observable *R*-enantiomer (Scheme 7).^[114] The *N-tert*-butoxycarbonyl derivative was converted into the target amine in a three-step procedure (reduction, methylation, and deprotection) to give (*S*)-1-cyclopropyl-2-methoxyethanamine in 62% overall yield from cyclopropylglyoxylic acid. The 2-cyclopropyl-2-oxoacetic acid precursor was obtained by permanganate oxidation of cyclopropylketone.





for references see p 20

cpgp.BommariusAu.210cck.3d · 2014-10-22 · Rev: 1
Ziegler + Müller, Kirchentellinsfurt · APEX Version: 1.0.1-26

2.4.1.1.3.6 Synthesis of (R)-D-Cyclohexylalanine

A recent report of a broad substrate range, nicotinamide-cofactor dependent, and highly stereoselective D-amino acid dehydrogenase, which was created using both rational and random mutagenesis performed on the enzyme *meso*-diaminopimelate D-dehydrogenase (DAPDH), is highly interesting.^[81] Because D-amino acid dehydrogenases are not ubiquitous in nature, as opposed to their L-counterparts, this opens up new possibilities for the synthesis of D-amino acids. The mutant is capable of producing D-amino acids via the reductive amination of the corresponding α-keto acids with ammonia. For example, the mutant catalyzes the amination of 3-cyclohexyl-2-oxopropanoic acid with ammonia to produce D-cyclohexylalanine (Scheme 8). The cofactor, NADPH, was recycled using glucose and glucose dehydrogenase (GDH). The glucose oxidation product, gluconolactone, spontaneously hydrolyzes irreversibly to gluconic acid driving the reaction to completion.



2.4.1.1.3.7 Synthesis of (*R*)-5,5,5-Trifluoronorvaline

(R)-5,5,5-Trifluoronorvaline [1; (R)-2-amino-5,5,5-trifluoropentanoic acid] was a target as an intermediate for a γ -secretase inhibitor (BMS-708163). The route to this target from the corresponding keto acid, employing D-amino acid transaminase with L-alanine as the amino donor, required two additional enzymes, lactate dehydrogenase (LDH) and formate dehydrogenase (FDH), to remove the coproduct pyruvate from the equilibrium and complete the reaction. In a simplified process, a D-amino acid dehydrogenase (AADH) was obtained via mutation of the D-diaminopimelate dehydrogenase (DAPDH) gene from *Bacillus sphaericus* (see Section 2.4.1.1.2.6).^[115] Both the modified D-DAPDH gene and that from glucose dehydrogenase (GDH) from *Gluconobacter oxydans* were cloned and the proteins expressed in *E. coli*. The target product was obtained with 100% ee and converted into 2-amino-N-[(4-chlorophenyl)sulfonyl]-5,5,5-trifluoropentanamide en route to the γ -secretase inhibitor development candidate (Scheme 9).

cpgp.BommariusAu.210cck.3d • 2014-10-22 • Rev: 1
Ziegler + Müller, Kirchentellinsfurt • APEX Version: 1.0.1-26



Scheme 9 Preparation of (*R*)-5,5,5-Trifluoronorvaline from the Corresponding Keto Acid via D-Amino Acid Dehydrogenase Catalysis^[115]

(R)-5,5,5-Trifluoronorvaline (1); Typical Procedure:[115]

 H_2O (800 mL) was added to a 2-L reactor and stirred magnetically. NH_4Cl (26.75 g, 500 mmol), glucose (62.5 g, 347 mmol), and 5,5,5-trifluoro-2-oxopentanoic acid (50 g, 294 mmol) were added, followed by 10 M NaOH (29.5 mL). Additional NaOH was added dropwise with continued stirring to bring the pH to 9. NADP (382 mg, 0.5 mmol) and a soln containing the D-AADH (1250 U) and GDH (75800 U), both from cell-free extract, were added to start the reaction. The pH was maintained at 9.0 with 5 M NaOH using a pH stat, and the reaction temperature was kept at 30 °C. After 22 h, the pH was adjusted to 2.0 with concd HCl (64 mL). The final mixture (1100 mL) contained 44.5 g (260 mmol; 88.5% soln yield) of (R)-5,5,5-trifluoronorvaline (98.9% ee), and was filtered to remove precipitated protein. The filtrate was adjusted to pH 7.0 with NaOH, diluted with BuOH (to prevent foaming and bumping), and concentrated under reduced pressure to a wet solid (286 g). This was mixed with MeOH (1430 mL), and the mixture was refluxed briefly. The hot mixture was filtered, and the solids were washed with a little MeOH. The solids (123 g after drying) contained 22 g of (R)-5,5,5-trifluoronorvaline. The solids were mixed with MeOH (500 mL) and refluxed, and the hot mixture was filtered and washed with MeOH (100 mL). The remaining solids were extracted with another portion of MeOH (500 mL) in the same way. The resulting MeOH insoluble solids (106 g), contained 7 g of residual (R)-5,5,5-trifluoronorvaline. The combined MeOH filtrates were concentrated to dryness under reduced pressure, and the residue (92 g) was dissolved in H_2O (370 mL) at boiling point. To remove a small quantity of precipitated protein, the hot soln was filtered, rinsing with hot H₂O (50 mL). Crystallization proceeded as the filtrate cooled. The mixture was cooled to 4 °C and filtered, washing with ice-cold H₂O (40 mL). Drying under reduced pressure at rt gave (R)-5,5,5-trifluoronorvaline as nacreous platelets; yield: 21.4 g (42.5%); >99.8% ee. Recrystallization (H₂O) gave (R)-5,5,5-trifluoronorvaline; mp 289 °C (dec).

2.4.1.1.3.8 Synthesis of Free Amines from Ketones

The asymmetric transformation of a ketone into an amine formally counts as a stereoselective reductive amination. The production reaction can be catalyzed by an ω -transaminase to afford a primary amine with a stereocenter at the α -position. The coproduct, pyruvate, is regenerated via catalysis with alanine dehydrogenase (AlaDH) and formate dehydrogenase (FDH), with formate as the ultimate reducing agent. This cascade only requires formate and ammonia as cosubstrates, besides the ketone (Scheme 10).^[116]

for references see p 20

cpgp.BommariusAu.210cck.3d • 2014-10-22 • Rev: 1
Ziegler + Müller, Kirchentellinsfurt • APEX Version: 1.0.1-26

15 (15 of 24)



2.4.1.1.3.9 Synthesis of Amino Acid Enantiomers from Racemic α-Hydroxy Acids

Deracemization of racemic mandelic acid (2-hydroxy-2-phenylacetic acid) to afford optically pure nonnatural 1-phenylglycine has been achieved via a redox-neutral biocatalytic cascade using three enzymes.^[117] Thus, optically pure 1-phenylglycine is obtained with >97% ee and 94% conversion without the requirement for any additional redox reagents in stoichiometric amounts. The enantiomers of the racemic α -hydroxy acid are interconverted by mandelate racemase, with concurrent oxidation of D-mandelic acid by a D-selective mandelate dehydrogenase (ADH) to give the corresponding α -oxo acid. The latter was transformed into the corresponding α -amino acid via an asymmetric reductive amination using an L-selective amino acid dehydrogenase (AADH) (Scheme 11). Because the formal hydrogen abstracted in the oxidation was used in the reduction, both reactions (the enantioselective oxidation and the stereoselective reduction) can occur simultaneously.



2.4.1.2 Amine Dehydrogenases

Recently, an amine dehydrogenase (AmDH) was developed via protein engineering of amino acid dehydrogenases (AADHs). Amine dehydrogenases catalyze the reduction of prochiral ketones using ammonia, to afford chiral amines (Scheme 12).

cpgp.BommariusAu.210cck.3d・2014-10-22・Rev: 1
Ziegler + Müller, Kirchentellinsfurt・APEX Version: 1.0.1-26

16 (16 of 24)



Amine dehydrogenase catalyzed reductive amination of ketones with ammonia offers an alternative to the enzymatic route involving transaminases. Transaminations of ketones with amine donors such as alanine or isopropylamine often suffer from equilibrium limitations and require a sacrificial amine donor instead of just ammonia.^[118] The insertion of two residue mutations in the active site of an amino acid dehydrogenase scaffold created the respective amine dehydrogenase. The current family of AmDHs includes leucine dehydrogenase (LeuDH) derived amine dehydrogenase (L-AmDH; LeuDH from *Bacillus stearothermophilus*; Lys67Ser/Asn260Leu),^[119] phenylalanine dehydrogenase (PheDH) derived amine dehydrogenase of L- and F-AmDH (termed cFL1-AmDH; Lys77Ser/Asn270Leu) (Scheme 13).^[121]

Scheme 13 Typical Reactions of a Leucine Amine Dehydrogenase (L-AmDH), Phenylalanine Amine Dehydrogenase (F-AmDH), and Chimeric Amine Dehydrogenase (cFL1-AmDH)^[119–121]



The substrate targets of amine dehydrogenases are specialty chemicals and intermediates that contain chiral amines, as well as active pharmaceutical ingredients (APIs). Substrate specificity is listed in Table 9.^[119-121]

Table 9	Activity	of Amine	Dehydrogenases	s toward V	/arious S	Substrates	119,120,121
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Process	Substrate	Substra	Substrate Specificity ^a (mU/mg)			
		L-AmDH	F-AmDH	cFL1-AmDH		
reductive amination	4-methylpentan-2-one	690	77	n.d.	[119–121]	
	3,3-dimethylbutan-2-one	n.d.	0	133	[119–121]	

for references see p 20

cpgp.BommariusAu.210cck.3d · 2014-10-22 · Rev: 1
Ziegler + Müller, Kirchentellinsfurt · APEX Version: 1.0.1-26

17 (17 of 24)

Process	Substrate	Substra	te Specifici	tyª (mU/mg)	Ref
		L-AmDH	F-AmDH	cFL1-AmDH	
	1-(adamantan-1-yl)ethanone	n.d.	0	69	[119–121]
	1-(4-fluorophenyl)propan-2-one	0	4000	1725	[119–121]
	1-phenoxypropan-2-one	0	540.8	n.d.	[119–121]
	3,4-dihydronaphthalen- 1(2H)-one	n.d.	0	107	[119–121]
oxidative de- amination	(R)-4-methylpentan-2-amine	586	166.3	n.d.	[119–121]
	rac-1-phenylethan-1-amine	484	0.5	21	[119–121]
	rac-1-methoxypropan-2-amine	130	0	40	[119–121]

Biocatalysis 2.4 Transamination and Reductive Amination

^a Substrate concentration: 20 mM in 5 M NH₄Cl buffer (pH 9.6); L-AmDH and F-AmDH measured at 25 °C, cFL1-AmDH measured at 60 °C; n.d. = not determined.

2.4.1.2.1 Synthesis with a Leucine Dehydrogenase Derived Amine Dehydrogenase

The first amine dehydrogenase successfully developed was the leucine dehydrogenase (LeuDH) derived amine dehydrogenase (L-AmDH).^[119,122] L-AmDH was developed by the addition of mutations to leucine dehydrogenase from *Bacillus stearothermophilus* (see Section 2.4.1.2). L-AmDH converts 4-methylpentan-2-one into 4-methylpentan-2-amine. A high-throughput absorbance-based assay based on NAD⁺ increase at 340 nm and cell density measurement at 600 nm was employed to screen variants of the two-site library 67DDK/ 260DDK. Eleven rounds of protein engineering resulted in a successful variant Lys67Ser/Asn260Leu with a k_{cat} value of 0.46 s⁻¹. Lab-scale (30 mg) reaction with L-AmDH and cofactor regeneration with formate dehydrogenase (FDH) resulted in 92.5% conversion and 99.8% ee.

2.4.1.2.2 Synthesis with a Phenylalanine Dehydrogenase Derived Amine Dehydrogenase

An amine dehydrogenase derived from phenylalanine dehydrogenase (F-AmDH; see Section 2.4.1.2) was the second amine dehydrogenase developed, and resulted from the same two active-site mutations to the Bacillus badius phenylalanine dehydrogenase (PheDH) scaffold.^[120,122] F-AmDH converts 1-(4-fluorophenyl)propan-2-one into (R)-1-(4-fluorophenyl)propan-2-amine (2). 1-Phenylpropan-2-one (phenylacetone) was not used in this study because it is regulated as a schedule II controlled substance by the United States Department of Justice, Drug Enforcement Administration. A high-throughput library was screened for F-AmDH activity, and, interestingly, the best F-AmDH variant consisted of the same two mutations as those present in the previously reported amine dehydrogenase derived from leucine dehydrogenase (L-AmDH; Section 2.4.1.2.1). Use of a restricted codon DDK at each of the two mutation sites was implemented for more efficient screening. A total of 21 unique F-AmDH variants were identified with k_{cat} values greater than 1 s⁻¹. The top variant (Lys77Ser/Asn275Leu) achieved a k_{cat} of 6.85 s⁻¹, at pH 9.5 and 25 °C, in the presence of all saturated substrates. Successful lab-scale reaction with a cofactor regeneration system was achieved with 93% conversion and >99.8% ee (Scheme 14). In addition, a biphasic organic solvent reaction system has been developed to allow for conversion of hydrophobic substrates that are nearly insoluble in aqueous solution, and thus would not register any activity in purely aqueous medium.^[123]

cpgp.BommariusAu.210cck.3d・2014-10-22・Rev: 1
Ziegler + Müller, Kirchentellinsfurt・APEX Version: 1.0.1-26

18 (18 of 24)



(R)-1-(4-Fluorophenyl)propan-2-amine (2); Typical Procedure:^[120]

F-AmDH was expressed in pET 28a, BL21(DE3) system at 37 °C (120 rpm) in MagicMedia for 24 h. Purification of the enzyme was achieved by His-tag using Ni-NTA affinity chromatography. The reaction was performed in 225 mM NH₄Cl/NH₄OH buffer (pH 9.6; 150 mL). 1-(4-Fluorophenyl)propan-2-one (20 mM), NAD⁺ (2 mM), F-AmDH (17.5 mg), and glucose dehydrogenase (GDH) from *B. megaterium* or *B. licheniformis* (200 U) were added. The mixture was reacted at 25 °C for 48 h. After completion of the reaction, the mixture was adjusted to pH 13 with 10 M NaOH (5 mL). The amine was extracted with *t*-BuOMe (3 × 75 mL) and the organic phase was dried (anhyd MgSO₄). The solvent was removed under reduced pressure using a rotary evaporator and (*R*)-1-(4-fluorophenyl)propan-2-amine (**2**) was isolated; yield: 73.9%; >99.8% ee [determined by chiral GC (Shimadzu GC-2010 unit equipped with Restec Rt-BDEXcst column)].

2.4.1.2.3 Synthesis with a Chimeric Amine Dehydrogenase

The chimeric amine dehydrogenase (cFL1-AmDH; see Section 2.4.1.2) was created by combining the ketone-binding domain (residues 1–151 of *B. badius* PheDH) from the phenylalanine dehydrogenase derived amine dehydrogenase (F-AmDH) and the cofactor-binding domain (residues 141–368 of *B. stearothermophilus* LeuDH) from the leucine dehydrogenase derived amine dehydrogenase (L-AmDH).^[121] The amine dehydrogenase cFL1-AmDH possesses unique properties, as it accepts benzylic ketones as substrates in addition to the substrates of F-AmDH. The model reaction for cFL1-AmDH is the conversion of acetophenone into (*R*)-1-phenylethanamine. In addition, cFL1-AmDH has a higher temperature optimum (60 °C) than that of the F-AmDH (50 °C). Thus, the development of cFL1-AmDH has further expanded the amine dehydrogenase family.



cpgp.BommariusAu.210cck.3d・2014-10-22・Rev: 1
Ziegler + Müller, Kirchentellinsfurt・APEX Version: 1.0.1-26

19 (19 of 24)

19

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