Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

One-pot one-step **deracemization of amines using ω-Transaminases**

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1. Materials

Glucose dehydrogenase from *Pseudomonas* sp., L-Lactic dehydrogenase from rabbit muscle, pyridoxal 5′-phosphate hydrate (PLP), and β-Nicotinamide adenine dinucleotide (NADH) were purchased from Sigma-Aldrich, Korea. All other chemicals used were of analytical or reagent grade.

2. Enzyme expression and purification

ω-TA from *Polaromonas* sp. JS666 ((*S*)-ω-TAPO) and ω-TA from *Vibrio fluviallis* JS17 ((*S*)-ω-TAVF) were expressed and purified as described elsewhere.[1,2] In the case of ω-TA from *Mycobacterium vanbaalenii* ((*R*)-ω-TAMV) and ω-TA from *Neosartorya fischeri* ((*R*)-ω-TANF)[3], both genes were chemically synthesized after codon optimization complying with the codon preferences of *E. coli* (Bioneer Inc., Taejeon, South Korea). Both synthetic genes were supplied by Bioneer Inc. after cloning in the pGEM-T easy vector (Promega**,** Madison, WI). The synthetic gene of (*R*)-ω-TAMV was digested with *Nde*I/*Not*I and the synthetic gene of (*R*)-ω-TANF was digested with *EcoR*I/*Xho*I, after which both genes were inserted into the IPTG-inducible expression vector pET24ma. The plasmid was then introduced into *E. coli* (BL21) and the transformants were grown at 37°C in 1L LB broth containing 100 μg mL^{-1} of kanamycin. When the OD_{600} reached 0.6, IPTG was added to a final concentration of 0.5 mM. The culture media was then immediately cooled to 20°C and incubated at the same temperature at 250 rpm. After overnight induction, the cells were harvested and washed twice with 50 mM phosphate buffer (pH 8.0). Following centrifugation, the cell pellet was resuspended in 2 volumes of 20 mM phosphate buffer (pH 7.0) containing 20 μM pyridoxal 5′-phosphate (PLP), 1 mM PMSF, 300 mM NaCl and 5 mM imidazole. The cells were then subjected to ultrasonic disruption for 15 min, after which the protein was purified at 4°C on a Ni-NTA agarose resin obtained from Qiagen (Hilden, Germany). The crude extract was passed directly into a column containing 3 mL of Ni-NTA agarose resin and then washed with 50 mL of phosphate buffer (pH 8.0) containing 20 mM imidazole, after which the protein was eluted with phosphate buffer (pH 8.0) containing 250 mM imidazole. The eluted solution containing purified protein was dialyzed against 100 mM potassium phosphate buffer (pH 8.0) containing 20 μM PLP, 0.2 mM EDTA, and 0.2% mercaptoethanol and then concentrated using an Amicon PM-10 ultrafiltration unit. Glycerol was

added to the purified enzyme solution (final 40% glycerol) and the samples were stored at –20°C until further analysis.

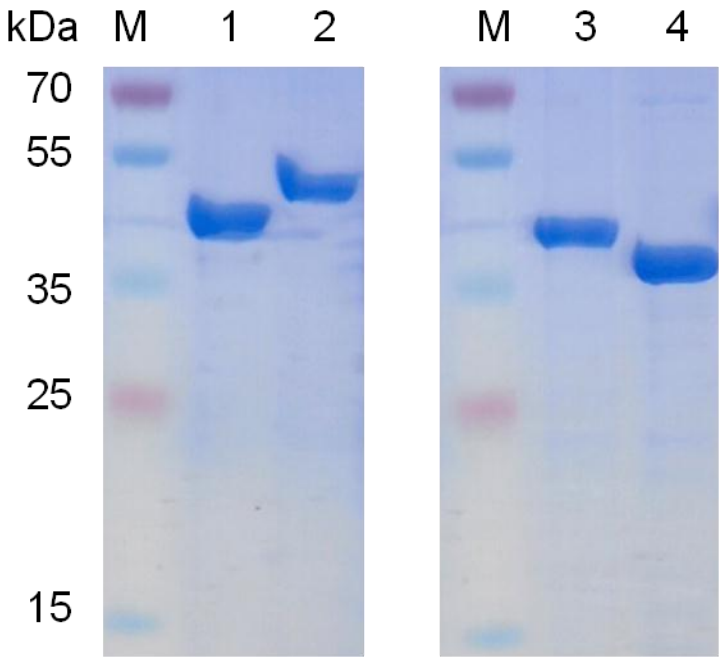

Figure S1. SDS-PAGE analysis of purified ω-TAs. Lane M; Molecular weight marker, lane 1; (*S*)-ω-TAPO (47kDa), lane 2; (*S*)-ω-TAVF (48kDa), lane 3;(*R*)-ω-TAMV(37.92kDa), lane 4; (*R*)-ω-TANF (37.1kDa).

3. Codon optimized nucleotide sequence of (*R*)-ω-TAMV

5′-

CATATGGGCATCGACACTGGCACCTCGAACCTGGTGGCGGTGGAGCCGGGGGCG
ATTCGCGAGGACACACCGGCAGGCAGCGTCATCCAGTATTCCGATTACGAGATTG
ATTACAGCTCGCCGTTCGCGGGCGGCGTGGCGTGGATTGAGGGTGAGTACCTGC
CGGCCGAAGATGCGAAAATTAGCATCTTCGACACCGGTTTCGGCCACTCGGACCT
GACCTACACGGTGGCGCACGTTTGGCACGGCAACATTTTCCGCTTGGGCGATCAC
CTGGACCGCCTGCTCGACGGCGCACGGAAGCTCCGCCTGGACAGCGGCTACACC
AAAGACGAGCTCGCCGACATCACCAAGAAGTGCGTGAGCCTGAGCCAGCTGCGT
GAAAGCTTCGTGAACCTGACCATCACCCGCGGGTACGGCAAGCGCAAGGGGGAA
AAGGACCTGAGCAAGCTGACCCACCAAGTGTACATTTACGCCATTCCGTATCTGTG
GGCGTTCCCCCCGGCGGAGCAGATCTTCGGCACCACCGCGGTGGTGCCGCGCCA
CGTGCGCCGCGCCGGCCGCAATACCGTGGACCCGACCATCAAGAACTACCAGTG
GGGCGACCTGACCGCGGCCAGCTTCGAGGCTAAGGATCGGGGCGCGCGCACCG
CCATTCTGATGGACGCGGACAACTGCGTCGCGGAGGGCCCGGGATTCAACGTGT
GCATTGTGAAGGACGGCAAGCTGGCAAGCCCGTCCCGCAACGCGCTGCCGGGCA
TCACCCGCAAGACCGTGTTCGAGATTGCGGGAGCCATGGGAATTGAAGCGGCGC
TCCGCGACGTGACCTCCCATGAGCTGTACGACGCCGACGAGATTATGGCGGTGAC
CACCGCGGGCGGCGTGACCCCGATTAACACCCTGGACGGCGTGCCGATCGGCGA
CGGCGAGCCGGGTCCGGTGACCGTGGCGATTCGCGACCGCTTCTGGGCGCTGAT
GGACGAGCCGGGGCCACTGATTGAAGCGATTCAGTACGCGGCCGC-3′

4. Codon optimized nucleotide sequence of (*R*)-ω-TANF

5′-

GAATTCATGGCCTCTATGGACAAAGTCTTTTCGGGATATCATGCGCGCCAGAAGCT
GCTTGAACGGAGCGACAATCCTTTCTCTAAGGGCATTGCCTATGTGGAAGGAAAG
CTCGTCTTACCCAGCGACGCCAGAATACCGCTACTTGACGAAGGCTTCATGCACG
GTGACCTAACTTATGATGTTACAACGGTTTGGGATGGACGCTTCTTTCGATTGGAT
GATCATATGCAACGGATCCTGGAAAGCTGCGATAAAATGCGGCTCAAGTTCCCACT
TGCACCGAGCACGGTGAAAAATATCCTGGCTGAGATGGTCGCCAAGAGTGGTATT
CGGGATGCGTTTGTGGAAGTTATCGTGACACGTGGTCTGACAGGTGTACGTGGTT
CGAAGCCCGAGGATCTGTATAATAACAACATATACCTGCTTGTTCTCCCATACGTTT
GGGTTATGGCGCCTGAGAACCAGCTCCTTGGTGGCAGTGCTATCATTACAAGGAC
AGTGCGACGAACACCCCCGGGTGCATTTGATCCTACGATCAAAAATCTACAGTGG
GGTGACTTAACAAAGGGACTTTTTGAGGCAATGGACCGTGGCGCAACGTACCCAT
TTCTCACTGACGGAGACACCAACCTTACCGAAGGATCTGGATTTAACATTGTCTTG
GTGAAGAACGGTATTATCTATACCCCTGATCGAGGTGTCTTGCGAGGGATCACAC
GTAAAAGTGTGATTGACGTTGCCCGAGCCAACAACATCGACATCCGCCTTGAGGT
CGTACCAGTGGAGCAGGTTTATCACTCCGATGAAATCTTCATGTGCACAACAGCCG
GTGGCATTATGCCTATAACGTTGCTTGATGGTCAACCAGTTAATGACGGCCAGGTT
GGCCCGATCACAAAGAAGATATGGGATGGTTACTGGGAGATGCACTACAATCCGG
CGTATAGTTTTCCGGTCGACTATGGCAGTGGCCTCGAG-3′

5. Enzyme assay

One unit of activity was defined as the amount of enzyme that produced 1μmol acetophenone per minute. Reaction conditions; incubation of 1 mL of reaction volume, 100 mM phosphate buffer (pH 7.0), 10 mM pyruvate, 10 mM (*S*)-α-MBA (or (*R*)-α-MBA) and the enzyme (1–20 μg mL^{-1}) for 30 min at 37°C . Different concentrations of enzymes were used to measure the exact specific activity. When 2–10% acetophenone had formed, the specific activity was determined.

6. Analytical methods

6.1 Analysis of amines (α-MBA and d1–d8)

The conversion and *ee* analysis of amines was performed accomplished by HPLC using a Crownpak CR (Daicel Co., Japan) column at 210 nm with an elution of pH 1.5 perchloric acid solution (0.6 mL min^{-1}). Each enantiomer was separated by these analytical conditions except ***d4***. Quantitative chiral analysis of **d4** was performed using a C_{18} symmetry column (Waters, MA) with a Waters HPLC system at 254 nm after derivatization of the sample with GITC.[2] Separation of each enantiomer of ***d4*** was achieved through an isocratic elution with a mixture of 50% methanol and 50% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1}.

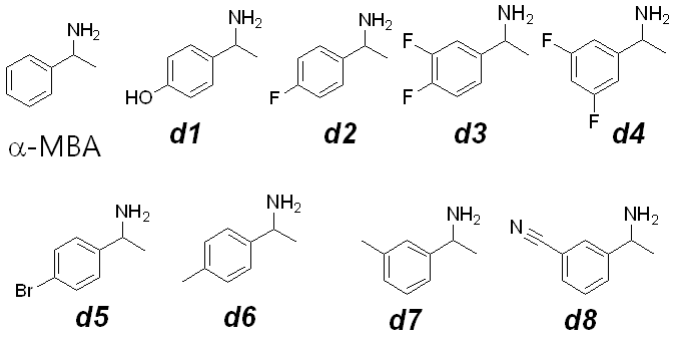

Figure S2. Amine structures.

Table S1. Amine retention times

Substrate	Retention time (min)[a]	
	(S)	(*R*)
α-MBA	15.9	22.3
d1	17.5	30
d2	38.2	56
d3	49.3	58.7
d4 [b]	19.9	17.8
d5	81.3	102.7
d6	39.4	49.7
d7	101	112.5
d8	24.5	28.2

[a]sample was analyzed using a Crownpak CR(+) column.

[b]sample was analyzed using a C_{18} symmetry column after GITC derivatization

6.2 Analysis of pyruvate and acetophenone

Pyruvate was analyzed using an Aminex HPX-87H HPLC column (Bio-Rad, CA) with an elution of 5 mM sulfuric acid solution at UV 210 nm. Acetophenone was analyzed using a C_{18} Symmetry column (Waters, MA) with an elution mixture of 50% methanol and 50% water (0.1% TFA) at a flow rate of 1.0 mL min^{-1}.[2]

References

1. H. Yun, B. Y. Hwang, J. H. Lee, B. G. Kim, *Appl. Environ.Microbiol*. 2005, **71**, 4220-4224.

2. H. S. Bea, H. J. Park, S. H. Lee, H. Yun, *Chem. Commun*. 2011, **47**, 5894-5896.

3. M. Hohne, S. Schatzle, H. Jochens, K, Robins, U. T. Bornscheuer, *Nat. Chem. Biol*. 2010, **6**, 807-813.