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Publisher's version / Version de l'éditeur:
https://doi.org/10.1139/V11-086
Canadian Journal of Chemistry, 90, 1, pp. 39-45, 2011-10-19

Cyclohexylamine oxidase as a useful biocatalyst for the kinetic resolution and dereacemization of amines

Hannes Leisch, Stephan Grosse, Hiroaki Iwaki, Yoshie Hasegawa, and Peter C.K. Lau

Abstract: The biocatalytic performance of a cloned cyclohexylamine oxidase derived from *Brevibacterium oxydans* IH-35A towards structurally different amines was investigated. Cycloalkyl primary amines, alkyl aryl amines, and α-carbon-substituted aliphatic amines were identified as suitable substrates for the biocatalyst based on an activity assay. Kinetic resolutions of several amines by either recombinant whole cells or crude enzyme extracts prepared therefrom gave enantiomerically pure (*R*)-amines besides the corresponding ketones. When cyclohexylamine oxidase in combination with a borane–ammonia complex as reducing agent was applied to the deracemization of several substrates, excellent enantiomeric ratios (>99:1) and good isolated yields (62%–75%) of the corresponding (*R*)-amines were obtained.

Key words: biocatalysis, cyclohexylamine oxidase, chiral amines, kinetic resolution, deracemization.

Résumé : On a étudié la performance biocatalytique d'un clone de l'oxydase de la cyclohexylamine dérivé du *Brevibacterium oxydans* IH-35A vis-à-vis des amines de structures diverses. Sur la base d'un essai d'activité, il a été possible d'identifier les cycloalkylamines primaires, les alkylarylamines primaires et les amines aliphatiques portant un substituant sur le carbone en α comme des substrats appropriés pour le biocatalyseur. Les résolutions cinétiques de plusieurs amines par des cellules recombinantes complètes ou des extraits bruts d'enzyme préparés à partir de celles-ci conduisent à des amines (*R*) énantiomériquement pures aux côtés des cétones correspondantes. On a utilisé l'oxydase de la cyclohexylamine en combinaison avec un complexe de borane–ammoniac comme agent réducteur pour effectuer la déracémisation de plusieurs substrats, avec d'excellents rapports énantiomériques (>99/1) et de bons rendements en produits isolés (72 % à 75 %) des amines (*R*) correspondantes.

Mots-clés : biocatalyseur, oxydase de la cyclohexylamine, amines chirales, résolution cinétique, déracémisation.

[Traduit par la Rédaction]

Introduction

The importance of enantiomerically pure amines as starting materials for pharmaceuticals and agrochemicals, as chiral auxiliaries, and as resolving agents is beyond question.[1] Despite the wide applications of chiral amines in chemistry, medicine, and biology, their synthesis remains challenging. Chemical protocols include the resolution of racemic amines as their diasteriomeric salts, enantioselective enamide and imine reduction, reductive amination, olefin hydroamination, and enantioselective carbanion addition to imines.[2] Alternative to the chemical method biocatalytic routes have been developed as promising strategies.[3] Most notably are the applicatons of ω-transaminases, which can be either used for the kinetic resolution of racemic amines and amino acids or for the asymmetric synthesis of amines from ketones.[4]

In addition, the ability of flavoproteins to oxidize amines to imines has been exploited for the synthesis of chiral amines (Scheme 1).[5] Initial experiments by Hafner and Weller[6] using D-amino acid oxidase in combination with sodium borohydride led to the preparation of L-amino acids from the corresponding *R*-enantiomers. Applying the same concept, Soda and co-workers[7] demonstrated the deracemization of racemic amino acids. Synthetically useful protocols, which allowed for the synthesis of enantiopure amino acids

Received 11 April 2011. Accepted 26 June 2011. Published at www.nrcresearchpress.com/cjc on 19 October 2011.

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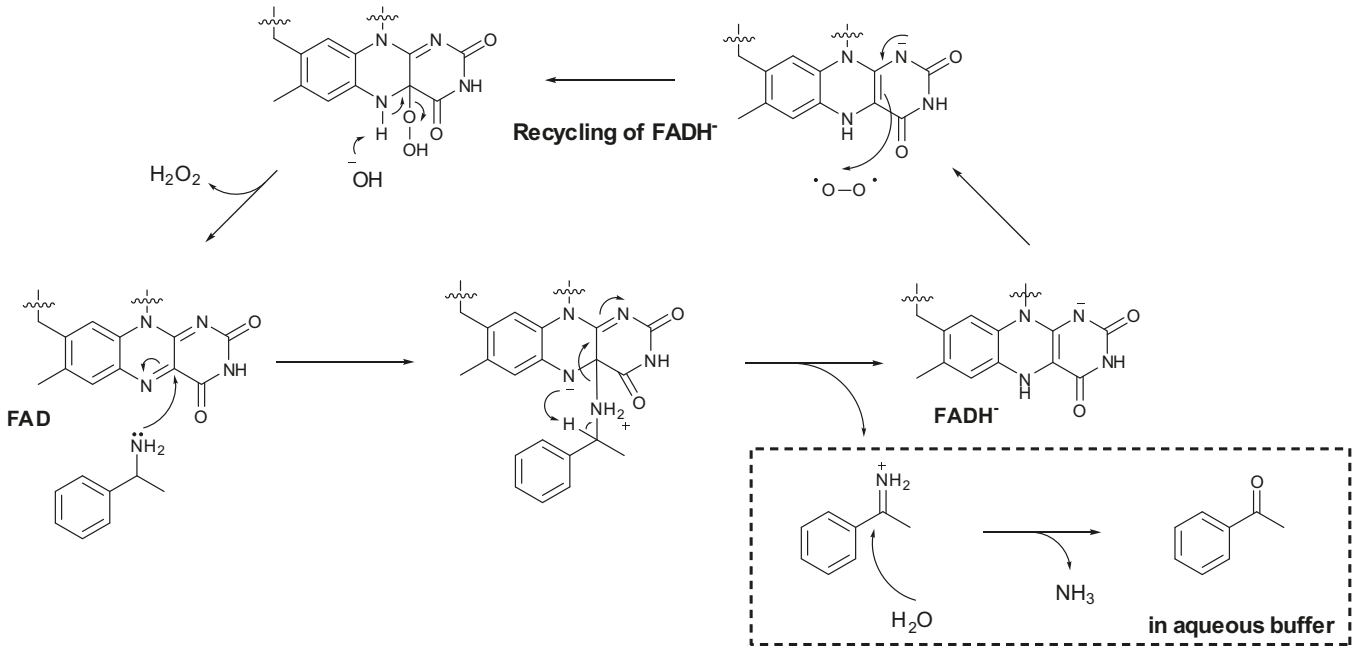
This article is part of a Special Issue dedicated to Professor Tak-Hang Chan in recognition and admiration of his long-standing contributions to green catalysis and synthesis.

Scheme 1. Proposed mechanism for the oxidation of amines with flavoproteins by nucleophilic attack of the heteroatam at C-4 of the flavoprotein.[5]



in excellent yields from racemic starting materials, were developed by Turner and co-workers[8] using porcine D-amino acid oxidase. Subsequently, the application of recombinant monoamine oxidase from *Aspergillus niger*[9] and mutants obtained by directed evolution led to the development of biocatalytic protocols for the deracemization of primary,[10] secondary,[11] and tertiary amines,[12] desymmetrization of substituted pyrrolidines,[13] enantioselective oxidation of hydroxylamines,[14] and the design of tandem biocatalysis multicomponent reactions.[15]

Prompted by the wide-ranging applications and excellent enantioselectivity of monoamine oxidase and its mutants from *A. niger*, we were interested in the exploitation of a bacterial amine oxidase as an alternative or complementary biocatalyst. As a result, cyclohexylamine oxidase (CHAO), a 50 kDa flavoprotein responsible for the oxidation of cyclohexylamine to cyclohexanone in *Brevibacterium oxydans* IH-35A,[16] was investigated and this new biocatalytic potential is reported herein.

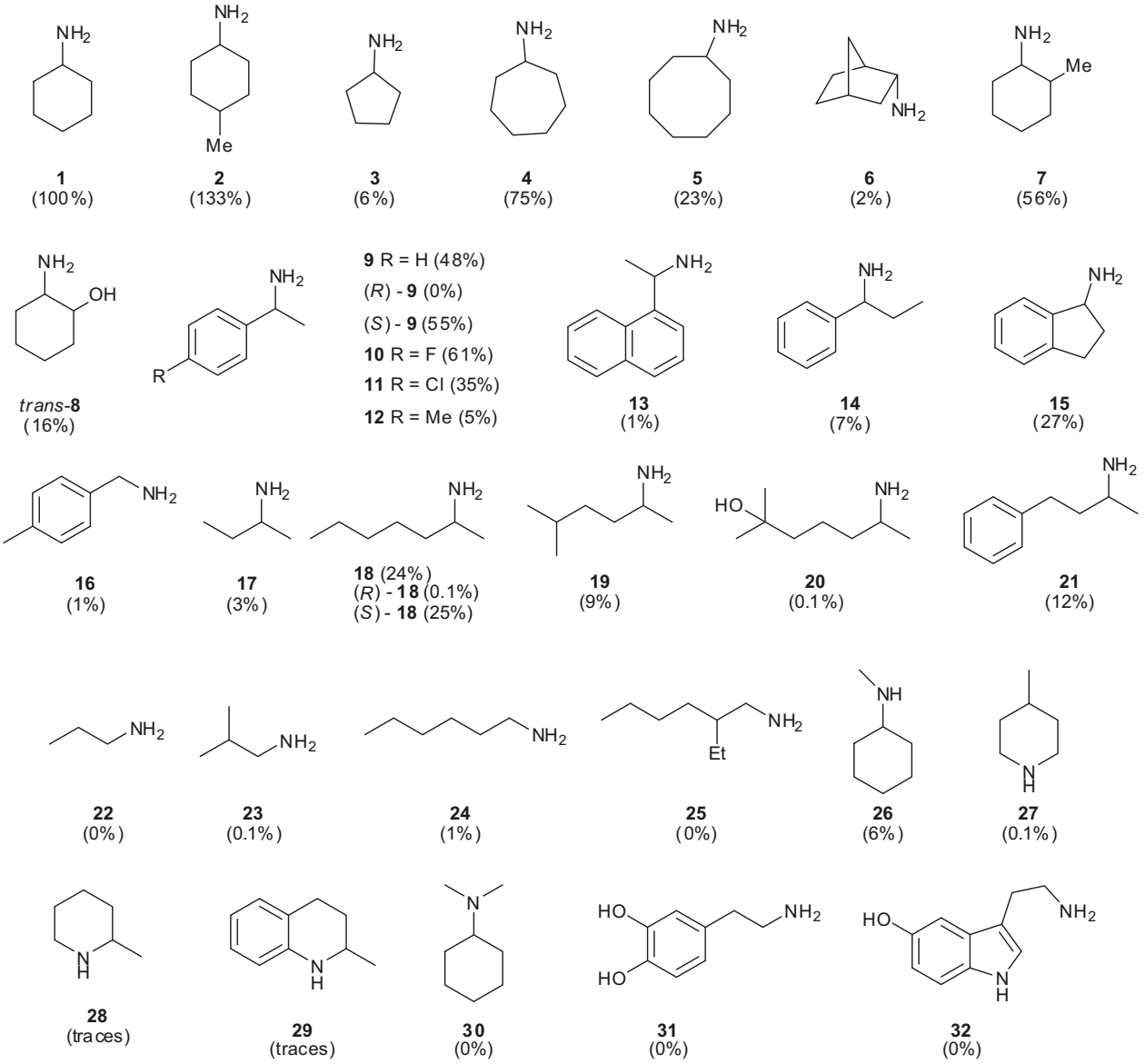
Results and discussion

To gain access to sufficient amounts of CHAO, the CHAO-encoding gene was cloned in a conventional isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible expression vector (pSD80), and the recombinant plasmid was produced in *Escherichia coli* strain BL 21.[17] Crude cell extracts, prepared from a Luria Bertani medium grown and with IPTG-induced cells, were assayed for their rate of oxidation of structurally diverse amines (Fig. 1). CHAO was found to exhibit activity towards a wide range of amines with cycloalkyl primary amine derivatives **1**–**8** showing the highest activity. The specific activity for cyclohexylamine was determined to be 10.7 U per mg crude enzyme preparation. Given the fact that a nonoptimized 1 L Terrific Broth medium fermentation experiment produced 650 mg of crude CHAO enzyme preparation (6955 U/L), this amount of biocatalyst produced would be capable of transforming 40 g of cyclohexylamine per h. Interestingly, 4-methyl cyclohexylamine (**2**) was oxidized 1.3 times faster than cyclohexylamine (**1**). Additionally, α-alkyl aryl derivatives **9**–**15** and α-carbon-substituted aliphatic amines **17**–**21** were acceptable substrates, whereas compounds of the structure R–CH_2–NH_2 (**22**–**25**) and straight-chain and cyclic secondary and tertiary amines showed very limited activity. Amino acids and monoamine neurotransmitters such as dopamine (**31**) or serotonin (**32**) were not oxidized by CHAO. With respect to enantioselectivity of CHAO we tested the oxidation rates of enantiomerically pure (*R*)- and (*S*)-α-methyl benzylamine (**9**) and 2-heptylamine (**18**). In both cases, CHAO displayed virtually no detectable activity for the (*S*)-enantiomers, indicating a highly enantioselective biocatalyst.

To demonstrate the practicality of CHAO in the syntheses of chiral amines, we tested either whole cells overexpressing CHAO or crude enzyme extracts for the kinetic resolution of racemic amines (Table 1). α-Alkyl aryl derivatives **9**–**12**, **14**, and **15** were resolved in excellent enantioselectivities and CHAO selectively oxidized the (*S*)-enantiomer of the aryl amine derivative to the corresponding ketone, whrereas the (*R*)-enantiomer remained unreacted even after complete consumption of the (*S*)-enantiomer. Preparative scale experiments led to the isolation of enantiopure (*R*)-α-methyl benzylamine (**9**) with recombinant whole cells in 38% and with crude enzyme extracts in 42% isolated yield after simple acid–base extraction. In contrast, the kinetic resolution of 2-heptylamine (**18**) had to be stopped after approximately 50% conversion, since after complete consumption of the (*S*)-enantiomer the less reactive (*R*)-enantiomer (Fig. 1) was oxidized by CHAO at a considerable rate. Interestingly, CHAO oxidized both enantiomers of 2-phenylbutylamine (**21**) at the same rate and only racemic **21** was detected by GC analysis.

Since the kinetic resolution of a racemate only produced a maximum yield of 50% of enantiopure amine, we examined

Fig. 1. Relative rates of the oxidation of amines using crude enzyme extracts of cyclohexylamine oxidase (CHAO). Activities are relative to the oxidation of cyclohexylamine (**1**).



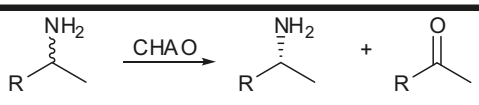
CHAO for the deracemization of amines. Based on the existing deracemization protocols developed by Turner and co-workers,[8,10–12] we investigated the deracemization of α-methyl benzylamine (**9**) under varying reaction conditions (Table 2). Initial experiments using 20 equiv of borane–ammonia complex as the reducing reagent led to the formation of (*R*)-**9** in 79% as determined by GC, confirming the concept of deracemization.

In addition, we observed the formation of 21% phenylethanol, most likely derived from hydrolyzed imine, which was further reduced. The use of sodium cyanoborohydride or sodium borohydride as alternative and more reactive reducing agents led to either decreased yields of amines or insufficient reaction rates. The use of increased equivalents of borane–ammonia complex led to the formation of higher amounts of amine and (*R*)-**9** was isolated in 69% yield after workup. The use of whole cells gave a slightly lower yield. In addition, CHAO in combination with borane–ammonia complex was applied successfully to the deracemization of phenylpropan-1-amine (**14**) and 1-indanamine (**15**).

The great potential of CHAO as a biocatalyst for the deracemization of amines is reflected by the fact that both phenylpropan-1-amine (**14**) and 1-indanamine (**15**) were isolated in higher yield than α-methylbenzylamine (**9**), although the relative activities toward both compounds were much lower than that of compound **9**. This underscores the fact that even compounds with relatively low activity can be used for this powerful biocatalytic application.

Although relative oxidation rates of secondary amines were very low as determined by colorimetric assay, we applied the same protocol to the deracemization of 2-methylpiperidine (**28**) and tetrahydroquinaldine (**29**). As expected, we observed only a slight enrichment of one enantiomer of tetrahydroquinaldine, and 2-methylpiperidine was recovered in racemic form.

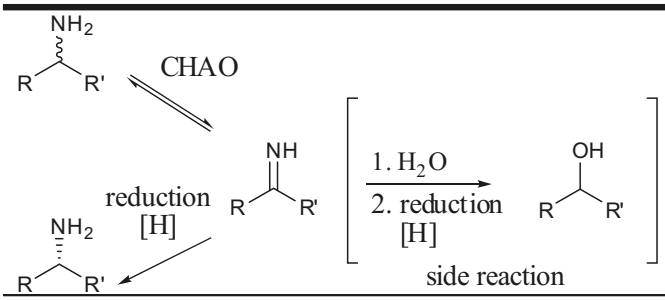
Table 1. Kinetic resolution of amines with cyclohexylamine oxidase (CHAO).



Amine	Biocatalyst	Conversion (%)[a]	R:S[b]
9	Whole cells[c]	50 (38)	>99:1 (*R*)
9	Crude enzyme[d]	50 (42)	>99:1 (*R*)
10	Whole cells	50	>99:1 (*R*)
11	Whole cells	50	>99:1 (*R*)
12	Whole cells	50	>99:1 (*R*)
14	Whole cells	50	>99:1 (*R*)
15	Whole cells	50	>99:1 (*R*)
18	Whole cells[e]	65	>99:1 (*R*)
18	Crude enzyme[e]	47	94:6 (*R*)
21	Whole cells[e]	85	1:1
21	Crude enzyme[e]	60	1:1

[a]Determined by GC; isolated yields are in parentheses.
[b]Determined by GC after acetamide formation.
[c]Resuspended cells OD_{600} 5.
[d]CHAO 1.2 U/mL.
[e]Reaction was stopped after 2 h.

Table 2. Deracemization of amines with CHAO.



Starting material	Biocatalyst	Reducing agent[a]	Recovered amine (%)[b]	R:S[c]	Alcohol (%)[b]
9	Crude enzyme[d]	$NH_3{\cdot}BH_3$	79	>99:1 (*R*)	21
9	Crude enzyme	$NaBH_4$	96	67:33 (*R*)	4
9	Crude enzyme	$NaCNBH_4$	56	>99:1 (*R*)	44
9	Crude enzyme	$NH_3{\cdot}BH_3$ (30 equiv)	86 (69)	>99:1 (*R*)	14
9	Whole cells[e]	$NH_3{\cdot}BH_3$	75 (62)	>99:1 (*R*)	25
14	Crude enzyme	$NH_3{\cdot}BH_3$	84 (75)	>99:1 (*R*)	16
15	Crude enzyme	$NH_3{\cdot}BH_3$	80 (69)	>99:1 (*R*)	20
28	Crude enzyme	$NH_3{\cdot}BH_3$	100	50:50	—
29	Crude enzyme	$NH_3{\cdot}BH_3$	100	57:43	—
29	Whole cells	$NH_3{\cdot}BH_3$	100	52:48	—

[a]Twenty equivalents of reducing agent were used unless otherwise stated.
[b]Determined by GC; isolated yield in parentheses.
[c]Enantiomeric ratio determined by GC after acetamide formation.
[d]CHAO 1.2 U/mL.
[e]Resuspended cells OD_{600} 5.

Conclusion

We demonstrated for the first time the application of a bacterial amine oxidase to the synthesis of chiral amines. Recombinant CHAO from *B. oxydans* IH-35A displays high activity towards a wide range of structurally different amines, a substrate spectrum that is different from that of the fungal monoamine oxidase.[13] In addition, we demonstrated that CHAO can be applied to the synthesis of primary (*R*)-amines by either kinetic resolution or deracemization protocols. Currently, we are working on the preparation of CHAO mutants with increased activities towards secondary and tertiary amines and their application to biocatalysis.

Experimental

All chemicals and reagents, including amines **1**–**30**, chiral amines (*R*)-**9**–**12**, (*R*)-**14**, (*R*)-**15**, and (*R*)-**18**, (*S*)-**9**, (*S*)-**18**, and the corresponding ketones derived from **9**–**12**, **14**, **15**, and **18** were purchased from Sigma-Aldrich (Mississauga, Ontario), Alfar Aesar (USA), TCI America (USA), and Thermo Fisher Scientific (USA) and were used without further purification.

For the determination of the conversion of amines by GC, appropriate calibration curves and response factors for product/substrate mixtures were prepared.[18] The enantiomeric ratio of α-methylbenzylamine (**9**) was determined after reaction with propionyl chloride (4 equiv) in the presence of triethylamine (8 equiv) in DCM; for all other amines (**10**–**12**, **14**, **15**, **18**, and **28**) acetamides were prepared using acetylchloride (4 equiv) and triethylamine (8 equiv) in DCM. Enantiomers of **29** were separated by chiral GC without derivatization. Co-injection with samples of acetamides prepared from commercial (chiral) standards allowed for the determination of the absolute configuration. The structure of acetamides were confirmed by GC–MS analysis, and the structures of isolated amines were confirmed by ^{1}H nuclear magnetic resonance (NMR) analysis performed on a Bruker (500 MHz) spectrometer.

GC analysis of products and reaction mixtures

GC analysis of reaction mixtures, acetamides, and racemic and chiral standards was carried out on a Hewlett-Packard 6890 Series gas chromatograph with a Hewlett-Packard 6890 Series autosampler equipped with a flame ionization detector. One microlitre was injected under pulse split (1:5) conditions on a Varian Chirasil-Dex CB column (25 m × 0.25 mm × 0.25 μm) with 8 mL/min helium flow. The following temperature program was used: 75 °C for 5 min; 2.5 °C/min to 175 °C, 10 min at 175 °C, 10 °C/min to 200 °C, 2.5 min at 200 °C. Table 3 illustrates retention times for the ketones and acetamides.

GC–MS analysis of acetamides

Analysis of acetamides derived from compounds **10**, **11**, **12**, **14**, and **15** was performed on an Agilent Technologies 7890A GC coupled to a 5975C quadrupole MS and a CTC Analytics Combipal autosampler. One microlitre was injected under pulse split (1:10) conditions on a 30 m × 0.25 mm × 0.25 μm HP-5MS capillary column (Agilent). The temperature program was as follows: 70 °C for 1.5 min; 65 °C/min to 135 °C, 5 °C/min to 280 °C, and 15 min at 280 °C. Helium was used as the carrier gas. The injector and the detector were adjusted to 250 and 280 °C, respectively. The MSD detector was run in the EI (70 eV) scan mode between 45 and 500 amu.

Analysis of propionate amide derived from **9** and acetamide from **18** was performed on a Hewlett-Packard 6890 GC coupled to a 5973 quadrupole MS and a 7683 autosampler. One microlitre was injected under pulse splitless conditions on a 50 m × 0.2 mm × 0.25 μm HP-5MS capillary column (Agilent). The temperature program was as follows: 70 °C for 1.5 min; 65 °C/min to 135 °C, 5 °C/min to 280 °C, and 15 min at 280 °C. Helium was used as the carrier gas. The injector and the detector were adjusted to 250 and 280 °C, respectively. The MSD detector was run in the EI (70 eV) scan mode between 45 and 500 amu.

Preparation of bacterial cells and the crude enzyme extract of CHAO

Escherichia coli BL21 harboring the CHAO-containing plasmid was maintained on LB medium containing glycerol (50%, v/v) at –80 °C. For biotransformation experiments, a fresh LB agar plate (1.5% agar) containing ampicillin (100 μg/mL) was prepared from the stock culture, and one colony was transferred to a preculture (10 mL) containing LB medium supplemented with ampicillin (100 μg/mL) and grown at 30 °C at 200 rpm on an orbital shaker overnight. In a 2 L DASGIP bioreactor system (Germany) an aliquot of the suspension (10 mL) was used to inoculate 1 L of TB medium. The temperature was set to 30 °C and the pH was controlled at 7.0 by the addition of concentrated ammonium hydroxide. The cell broth was aerated at 1.0 vvm and stirred at 400–1000 rpm to maintain 30% dissolved oxygen. If necessary, the airflow was supplemented with oxygen. At an OD_{600} of 10 (~7 h), protein expression was induced by the addition of IPTG (final concentration 1 mmol/L) and glucose (33% w/w aqueous solution) was fed at an initial rate of 3 mL/h and gradually increased to 7 mL/h. The cells were harvested by centrifugation (6300*g*, 4 °C, 20 min) 16 h after induction and washed with 20 mmol/L sodium phosphate buffer (pH 7), followed by centrifugation. Whole cells (could be stored at 4 °C up to 3 days without loss of activity) were resuspended in the appropriate amount of 100 mmol/L phosphate buffer (pH 7) to give a final OD_{600} of 5 and used for the biotransformations.

Alternatively, the centrifuged and washed cells were resuspended in 20 mmol/L sodium phosphate buffer (OD_{600} ~ 70) and broken using a French press cell operating at 20 000 psi (1 psi = 6.894757 kPa). The crude extract was obtained by centrifugation (20 000*g*, 45 min), followed by sterile filtration using a 0.22 μm membrane. Protein concentration was determined by Bradford assay[19] and the activity was determined as described below. A 1 L fermentation yielded ~650 mg of crude CHAO.

Activity assay

Cyclohexylamine oxidase activity was determined by a modified procedure of Braun et al.[20]

The formation of a dye (ε = 29.4 mmol/L^{-1} cm^{-1}) produced by the action of horseradish peroxidase with liberated hydrogen peroxide from the reaction of the amine (5 mmol/L) and CHAO, 4-aminoantipyrine, and 2,4,6-tribromo-3-hydroxybenzoic acid was monitored at 510 nm using a Beckman spectrophotometer (model DU 640). The standard assay mixture (1 mL total volume) contained: 965 μL of 50 mmol/L phosphate buffer (pH 7), 10 μL of a 2,4,6-tribromo-3-hydroxybenzoic acid stock solution (20 mg/ mL in DMSO), 0.15 mg of 4-aminoantipyrine, 5 μL of an amine stock solution (1 mol/L in DMSO), and 10 μL of a horse radish peroxidase stock solution (5 mg/mL). The reaction (1 mL) was started by the addition of 10 μL of an appropriate amount of enzyme at room temperature. Enzymatic assays were performed in triplicate with the appropriate control experiments. One enzyme unit (U) was defined as the amount of enzyme that produced 1 μmol of hydrogen peroxide from

Table 3. Retention times of ketones and alcohols and response factors for amines and ketones.

Compound	Retention time (min) (*R*)-Enantiomer acetamide	(*S*)-Enantiomer acetamide	Ketone	Alcohol[a]	Amine:ketone response factor
9[b]	38.97	38.66	18.69	25.59 and 26.32	1:1.48
10	38.22	37.54	18.59	na	1:1.51
11	45.41	45.95	27.54	na	1:1.63
12	40.69	40.13	23.50	na	1.02:1[c]
14	38.84	38.29	22.47	29.56 and 29.94	1:1.68
15	45.14	44.52	29.06	32.18 and 32.39	1:1.81
18	44.27	43.88	26.83	na	1:1.25
21	28.70	27.65	8.76	na	1:2.57
28	29.66	29.26	na	na	na
29[e]	32.34	32.73	na	na	na

Note: na, not applicable.

[a]Two enantiomers.

[b]Propionate amides were prepared.

[d]Response factor based on acetamide and ketone.

[e]Absolute configuration was not determined; (*R*)- and (*S*)- based on enrichment by CHAO.

cyclohexylamine (**1**) per min. Relative activities (%) were calculated by dividing the activity of the amine tested by the activity of cyclohexylamine (**1**).

Kinetic resolution of racemic amines using either whole cells or crude enzyme extract

Screening scale

The amine (0.2 mmol) dissolved in DMSO (0.2 mL) was added to either a solution of CHAO (final concentration 1.2 U/mL) in 100 mmol/L phosphate buffer (pH 7, 20 mL) or a suspension of whole cells overexpressing CHAO (OD_{600} 5) resuspended in 100 mmol/L phosphate buffer (pH 7, 20 mL). The mixture was shaken at 200 rpm at 30 °C on an orbital shaker for 18 h, before the pH of the reaction mixture was adjusted to 2 with 2 mol/L HCl. The suspension was centrifuged (6300*g*, 20 min) and the supernatant was basified to pH 9 with 5 mol/L NaOH. The aqueous layer was extracted 3 times with DCM and the organic layers were combined, washed with brine, dried over anhydrous sodium sulphate, and filtered. An aliquot (1 mL) was analyzed for the conversion of amine to ketone. For determination of the enantiomeric ratio the organic solvent was concentrated to 1 mL, cooled to 0 °C, and triethylamine (81 mg, 0.8 mmol) was added followed by the dropwise addition of acetylchloride (31 mg, 0.4 mmol). Then the reaction mixture was allowed to react at room temperature for 1 h. An aliquot of the reaction mixture (20 µL) was added to a mixture of DCM–MeOH, 8:2 (1 mL) and analyzed by chiral GC and GC–MS.

Preparative scale

α-Methylbenzylamine (**9**) (121 mg, 1 mmol) dissolved in DMSO (0.5 mL) was added to either a solution of CHAO (final concentration 1.2 U/mL) in 100 mmol/L phosphate buffer (pH 7, 100 mL) or a suspension of whole cells overexpressing CHAO (OD_{600} 5) resuspended in 100 mmol/L phosphate buffer (pH 7, 100 mL). The mixture was shaken at 200 rpm at 30 °C on an orbital shaker for 18 h, before the pH of the reaction mixture was adjusted to 2 with 2 mol/L HCl. The suspension was centrifuged (6300*g*, 20 min) and the supernatant was basified to pH 9 with 5 mol/L NaOH. For the crude enzyme extracts the centrifugation step could be omitted. The aqueous layer was extracted 3 times with DCM and the organic layers were combined. An aliquot (1 mL) was analyzed for the conversion by GC analysis. The organic solvent was concentrated (25 mL) and extracted 3 times with 1 mol/L HCl. The aqueous layers were combined, basified to pH 9 with 5 mol/L NaOH, and extracted 3 times with DCM. The organic layers were combined, washed with brine, dried over anhydrous sodium sulphate, and filtered. Evaporation of the solvent gave enantiopure (*R*)-α-methylbenzylamine (**9**) in 38% yield (46 mg) with whole cell transformations and in 42% yield (51 mg) with the crude enzyme extract experiment. The enantiomeric ratio of the obtained products was determined as described before.

Deracemization of racemic amines using either whole cells or crude enzyme extract

Screening scale

The amine (0.2 mmol) dissolved in DMSO (0.2 mL) was either added to a solution of CHAO (final concentration 1.2 U/mL) and borane–ammonia complex (123 mg, 4.0 mmol) in 100 mmol/L phosphate buffer (pH 7, 20 mL) or to a suspension of whole cells overexpressing CHAO (OD_{600} 5) resuspended in 100 mmol/L phosphate buffer (pH 7, 20 mL) and borane–ammonia complex (123 mg, 4.0 mmol). The mixture was shaken at 200 rpm at 30 °C on an orbital shaker for 18 h, before the pH of the reaction mixture was carefully adjusted to 2 with 2 mol/L HCl. The suspension was centrifuged (6300*g*, 20 min) and the supernatant was basified to pH 9 with 5 mol/L NaOH. The aqueous layer was extracted 3 times with DCM and the organic layers were combined, washed with brine, dried over anhydrous sodium sulphate, and filtered. An aliquot (1 mL) was analyzed for the content of amine, the corresponding ketone, and alcohol derivatives. For determination of the enantiomeric ratio the organic solvent was concentrated to 1 mL, treated with acetylchloride, and analyzed by chiral GC as described before.

Preparative scale

The amine (1 mmol) dissolved in DMSO (0.5 mL) was either added to a solution of CHAO (final concentration 1.2 U/mL) and borane–ammonia complex (617 mg, 20.0 mmol; for the deracemization of **9**, 926 mg, 30 mmol) in 100 mmol/L phosphate buffer (pH 7, 20 mL) or to a suspension of whole cells overexpressing CHAO (OD_{600} 5) resuspended in 100 mmol/L phosphate buffer (pH 7, 20 mL) and borane–ammonia complex (617 mg, 20.0 mmol). The mixture was shaken at 200 rpm at 30 °C on an orbital shaker for 18 h, before the pH of the reaction mixture was carefully adjusted to 2 with 2 mol/L HCl. The suspension was centrifuged (6300*g*, 20 min) and the supernatant was basified to pH 9 with 5 mol/L NaOH. The aqueous layer was extracted 3 times with DCM, the organic layers were combined, and an aliquot (1 mL) was analyzed for the content of amine and the corresponding ketone and alcohol. The organic solvent was concentrated (25 mL) and extracted 3 times with 1 mol/L HCl. The aqueous layers were combined, basified to pH 9 with 5 mol/L NaOH, and extracted 3 times with DCM. The organic layers were combined, washed with brine, dried over anhydrous sodium sulphate, and filtered.

Evaporation of the solvent gave 83 mg (69% yield) of (*R*)-**9** using crude enzyme extracts, 75 mg (62% yield) of (*R*)-**9** using whole cells, 101 mg (75% yield) of (*R*)-**14** using crude enzyme extracts, and 92 mg (69% yield) of (*R*)-**14** using crude enzyme extracts. The enantiomeric ratio of the obtained products was determined after derivatization of an aliquot of the product as described before.

Acknowledgements

We thank A. Corriveau, S. Deschamps, and C. Beaulieu for their expert help in various analytical analyses, and K. Morley for suggestions and comments on the manuscript.

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