Supplementary Materials

Immobilization of Aldoxime Dehydratases and Their Use as Biocatalysts in Aqueous Reaction Media

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Content

General experimental information

Chemicals used

Material for cell cultivation and aldoxime dehydratase (Oxd) expression was purchased by Carl Roth (Antibiotics, LB- and TB-premixed medium, D-glucose, D-lactose). Buffer salts (KH_2PO_4 and K_2HPO_4) were obtained from VWR chemicals. Immobilization carrier were purchased by Purolite®, Sodium alginate and TEOS were purchased by Sigma Aldrich. Solvents were purchased by VWR Chemicals in HPCL-grade and used without further purification.

Analytical methods

Conversions were determined by GC measurements (Shimadzu GC-2010 Plus) in comparison to a calibration curve. Measurements were conducted on a chiral SGE Analytik B6B-174 column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) with nitrogen as carrier gas. An injector temperature of 220 °C in a split injection mode was used and a sample amount of 1 µL was injected in this method. The following temperature gradient was used:): 140 °C starting temperature (hold 1 min), in 20 °C/min to 190 °C (hold 0.5 min) and in 50 °C/min to 200 °C. Retention times of the substances of interest are: t_R (octanaloxime) = 2.7 min; t_R (octanenitrile) = 2.4 min. Accuracy of the GC-analytical methods: the error of the determined conversions to the corresponding nitriles is in the range of up to 5% depending on the concentration of the aldoximes due to temperature depending degradation of the aldoximes on the GC-column.

Oxd sequences

Aldoxime dehydratase from *Bacillus* sp. OxB-1 (OxdB) (Accession number: GenBank: AP013294.1)

His-tagged variant in pET22a

ACAGGGAACGGGGGCGGAAATTCCCTCTAGAATAATTTTGGTTTAACTTTAAGGAAGGA
GGATATACATATGAAAAATATGCCGGAAAATCACAATCCACAAGCGAATGCCTGGACTG
CCGAATTTCCTCCTGAAATGAGCTATGTAGTATTTGCGCAGATTGGGATTCAAAGCAAGT
CTTTGGATCACGCAGCGGAACATTTGGGAATGATGAAAAAGAGTTTCGATTTGCGGACA
GGCCCCAAACATGTGGATCGAGCCTTGCATCAAGGAGCCGATGGATACCAAGATTCCA
TCTTTTTAGCCTACTGGGATGAGCCTGAAACATTTAAATCATGGGTTGCGGATCCTGAAG
TACAAAAGTGGTGGTCGGGTAAAAAAATCGATGAAAATAGTCCAATCGGGTATTGGAGT
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AATGGGGTTTCACACTTTGTACCGATCAAGCATACAGAAGTCCATGAATATTGGGGAGC
AATGCGCGACCGCATGCCGGTGTCTGCCAGTAGTGATTTGGAAAGCCCCCTTGGCCTT
CAATTACCGGAACCCATTGTCCGGGAGTCTTTCGGAAAACGGCTAAAAGTCACGGCGC
CGGATAATATTTGCTTGATTCGAACCGCTCAAAATTGGTCTAAATGTGGTAGCGGGGAA

AGGGAAACGTATATAGGACTAGTGGAACCGACCCTCATAAAAGCGAATACGTTTCTTCG
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CGGCGAAATAGTAGATAAATCATGTGTCATCGGATATTATCTCTCCATGGGGCATCTTGA
ACGCTGGACGCATGATCATCCAACACATAAAGCGATCTACGGAACCTTTTATGAGATGT
TGAAAAGGCATGATTTTAAGACCGAACTTGCTTTATGGCACGAGGTTTCGGTGCTTCAAT
CCAAAGATATCGAGCTTATCTATGTCAACTGCCATCCGAGTACTGGATTTCTTCCATTCT
TTGAAGTGACAGAAATTCAAGAGCCTTTACTGAAAAGCCCTAGCGTCAGGATCCAGCTC
GAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGAAGTTTTT
T

Without His-tag in pUC18

ATGAAAAATATGCCGGAAAATCACAATCCACAAGCGAATGCCTGGACTGCCGAATTTCC
TCCTGAAATGAGCTATGTAGTATTTGCGCAGATTGGGATTCAAAGCAAGTCTTTGGATCA
CGCAGCGGAACATTTGGGAATGATGAAAAAGAGTTTCGATTTGCGGACAGGCCCCAAA
CATGTGGATCGAGCCTTGCATCAAGGAGCCGATGGATACCAAGATTCCATCTTTTTAGC
CTACTGGGATGAGCCTGAAACATTTAAATCATGGGTTGCGGATCCTGAAGTACAAAAGT
GGTGGTCGGGTAAAAAAATCGATGAAAATAGTCCAATCGGGTATTGGAGTGAGGTAACG
ACCATTCCGATTGATCACTTTGAGACTCTTCATTCCGGAGAAAATTACGATAATGGGGTT
TCACACTTTGTACCGATCAAGCATACAGAAGTCCATGAATATTGGGGAGCAATGCGCGA
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GAACCCATTGTCCGGGAGTCTTTCGGAAAACGGCTAAAAGTCACGGCGCCGGATAATAT
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GTGAAACAGGCTGTATTAGTTCAAAATTAGTCTATGAACAGACCCATGACGGCGAAATA
GTAGATAAATCATGTGTCATCGGATATTATCTCTCCATGGGGCATCTTGAACGCTGGAC
GCATGATCATCCAACACATAAAGCGATCTACGGAACCTTTTATGAGATGTTGAAAAGGCA
TGATTTTAAGACCGAACTTGCTTTATGGCACGAGGTTTCGGTGCTTCAATCCAAAGATAT
CGAGCTTATCTATGTCAACTGCCATCCGAGTACTGGATTTCTTCCATTCTTTGAAGTGAC
AGAAATTCAAGAGCCTTTACTGAAAAGCCCTAGCGTCAGGATCCAGTGA

Aldoxime dehydratase from *Rhodococcus erythropolis* N-771 (OxdRE) (Accession number: GenBank: AB094201.1)

His-tagged variant in pET28a

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC
CATATGGAAAGCGCAATTGGTGAACATCTGCAGTGTCCGCGTACCCTGACCCGTCGTGT
TCCGGATACCTATACCCCTCCGTTTCCGATGTGGGTTGGTCGTGCAGATGATGCACTGC
AGCAGGTTGTTATGGGTTATCTGGGTGTTCAGTTTCGTGATGAAGATCAGCGTCCGGCA
GCACTGCAGGCAATGCGTGATATTGTTGCAGGTTTTGATCTGCCGGATGGTCCGGCAC
ATCATGATCTGACCCATCATATTGATAATCAGGGCTATGAAAACCTGATTGTGGTGGGTT
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CGCGTGCAGAACAGTTTGAAACCCTGTATGCATTTCAAGAAGATCTGCCTGGCGTTGGT
GCAGTTATGGATGGTATTAGCGGTGAAATTAACGAACATGGTTATTGGGGTAGCATGCG
TGAACGTTTTCCGATTAGCCAGACCGATTGGATGCAGGCAAGCGGTGAACTGCGTGTTA
TTGCCGGTGATCCGGCAGTTGGTGGTCGTGTTGTTGTTCGTGGTCATGATAACATTGCA
CTGATTCGTAGCGGTCAGGATTGGGCAGATGCCGAAGCAGATGAACGTAGCCTGTATC
TGGATGAAATTCTGCCGACCCTGCAGAGCGGTATGGATTTTCTGCGTGATAATGGTCCT
GCAGTTGGTTGTTATAGCAATCGTTTTGTGCGCAACATTGATATCGATGGCAATTTTCTG
GATCTGAGCTATAACATTGGTCATTGGGCAAGCCTGGATCAGCTGGAACGTTGGAGCG
AAAGCCATCCGACCCATCTGCGTATTTTTACCACCTTTTTTCGCGTTGCAGCCGGTCTGA
GCAAACTGCGTCTGTATCATGAAGTTAGCGTTTTTGATGCAGCAGATCAGCTGTATGAAT
ACATTAATTGTCATCCGGGTACAGGTATGCTGCGTGATGCAGTTACCATTGCAGAACATT
AA

SDS-PAGE

(a) (b)

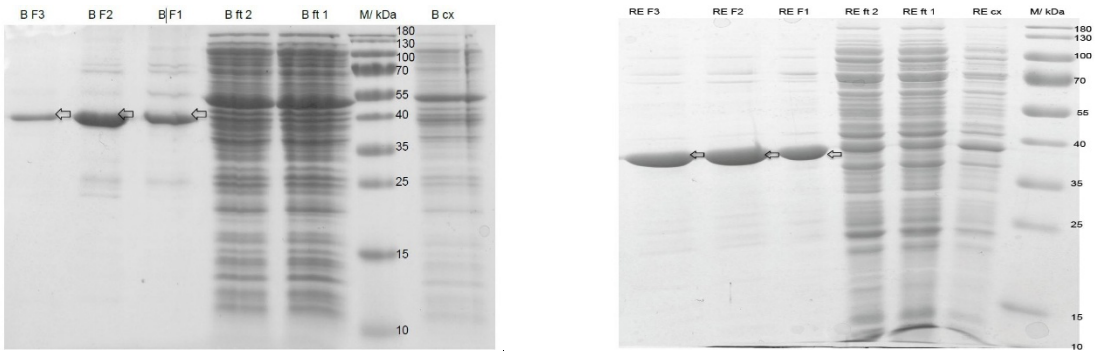

Figure 1. SDS-PAGEs of OxdB (a)- and OxdRE (b)-crude extracts (cx) and fractions of protein purification (flowthrough (ft), fractions of elution (F)).

Immobilization carrier and buffers used for immobilization

Table 1. Immobilization carrier used for the immobilization of OxdB or OxdRE purified enzymes.

#	Carrier material	Abbreviation	Pore size /Å	Purolite no.[1]
1	Octadecyl methacrylate	h1	500-700	Lifetech ECR8806
2	Macroporous styrene	h2	900-1100	Lifetech ECR1090
3	Divinylbenzene/Methyacrylate	h3	200-300	Lifetech ECR1030M$^{\neq}$
4	Amino C2 methacrylate	a1	600-1200	Lifetech ECR8309F
5	Epoxy methacrylate	e1	300-600	Lifetech ECR8204
6	Epoxy/butyl methacrylate	e2	400-600	Lifetech ECR8285

Table 2. Used buffer for washing of the carrier material, the enzyme solution and washing after immobilization.

Carrier material	PPB for washing of the carrier	PPB of the enzyme solution	PPB for washing the immobilizates	Incubation time /h
Hydrophobic	50 mM	50 mM	50 mM	21-23
Amino	50 mM	50 mM	10 mM, afterwards 0.5 mM NaCl in 50 mM PPB	18
Epoxy	0.5 mM	0.5 mM	3x 10 mM, afterwards 1x 0.5 mM in 50 mM PPB	20, then further 25

Determination of water content in immobilizates

To determine the solvent content, approx. 5 mg of each immobilizate of OxdB and OxdRE are balanced and lyophilized after freezing for approx. 24 h. The immobilizates were then balanced again. The determination was performed in triplicates and results shown in Table 3.

Table 3. Mass of immobilizates before and after drying.

Enzyme	Immobilizate	Mass before drying /mg	Mass after drying /mg
OxdB	h1	5.11	2.96
	h2	5.33	1.97
	h3	6.37	3.26
	a	4.32	1.80
	e1	4.59	2.14
	e2	5.31	1.87
OxdRE	h1	4.20	3.15
	h2	5.24	2.74
	h3	5.62	3.33
	a	4.24	2.46
	e1	5.59	3.33
	e2	5.68	2.14

Activity assay for purified or immobilized enzymes

Octanaloxime was prepared as described in previous publication.[2] To the enzyme (purified enzyme or immobilizates, 50 µL, 5 mg/mL final concentration) PPB (437.5 µL, 50 mM, pH=7.0) was added. After an incubation time of 5 min at 30 °C and 1400 rpm octanaloxime (12.5 µL, final concentration 5 mM) in acetonitrile (final concentration 2.5% (*v*/*v*)), was added. The conversion to octanenitrile was determined after 5 min at 30 °C and 1400 rpm by extraction with ethyl acetate (500 µL) and analyzation of the organic phase by gas chromatography. The data were compared to standard curves of octanaloxime and octanenitrile, giving conversions from which activities in $U/mg_{protein}$ were determined.

Table 4. Activities of OxdB and OxdRE purified enzymes ans immobilizates.

Enyzme	Formulation	Activity /$U \cdot mg^{-1}$
OxdB	purified	1.51
	h1	0.19
	h2	0.04
	h3	0.02
	a	0.11
	e1	0.04
	e2	0.03
OxdRE	purified	0.65
	h1	0.11
	h2	0.14
	h3	0.11
	a	0.13
	e1	0.03
	e2	0.03

Storage stability of enzymes and immobilizates were performed by comparison of the activity for octanaloxime after storage of the enzymes or immobilizates for 6 or 32 days at 4 °C.

Buffer screening using OxdB whole cells

The measurements of the activities of the selected buffers were recorded by GC analytic and performed with OxdB in whole cells . For the buffer activity, 50 µl of the cell-suspension (333 mg/ml in PPB) were placed into Eppendorf tubes. The suspension was centrifuged (1 min, 17000 g), the PPB removed and resuspend in the selected buffer to a final concentration of 333 mg/mL. The selected buffers were HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), TRIS-HCl (2-Amino-2-(hydroxymethyl)propane-1,3-diol), MOPS (3-Morpholinopropane-1-sulfonic acid) and ammonium acetate all in a concentration of 50 mM and pH of 7.0. 400 µL of the selected buffer was placed into an Eppendorf tube and 50 µL of the cell suspension in the resepective buffer was added (final concentration of cells 33 mg/mL). After incubation (30 °C, 1000 rpm, 5 min), 50 µL of an octanaloxime solution in ethanol solution (1 M, 100 mM final concentartion) was added. The samples were incubate for 15 min at 1000 rpm and 30 °C. Afterwards ethyl acetate (500 µl) was added, the mixture was vortexed and centrifuged. The organic phase was analyzed by GC. The conversions are shown in Table 5.

Table 5. Conversion of octanaloxime in different buffers using OxdB in whole cells.

Buffer	**Conversion /%**
PPB	80
HEPES	90
MOPS	91
TRIS-HCl	91
Ammium acetate	89

References

[1] Purolite, *User Man. Immobil. Carr.* **2015**.

[2] A. Hinzmann, S. Glinski, M. Worm, H. Gröger, *J. Org. Chem.* **2019**, *84*, 4867–4872.