aliphatic (R)-hydroxynitrile lyase 4.1.2.46

1 Nomenclature

EC number
4.1.2.46

Systematic name
(2R)-2-hydroxy-2-methylbutanenitrile butan-2-one lyase (cyanide forming)

Recommended name
aliphatic (R)-hydroxynitrile lyase

Synonyms
(R)-HNL <3> [7]
(R)-oxynitrilase <1,2> [2,3]
(R)-hydroxynitrile lyase <3> [7]
acetone cyanohydrin lyase <1,3> [1,6]
hydroxynitrile lyase <1,2,3> [5,6]
LuHNL <1,2,3> [4,5,6,9,10]

2 Source Organism

<1> *Linum usitatissimum* [1,3,4,5,10]
<2> *Linum usitatissimum* (UNIPROT accession number: O22574) [2,5]
<3> *Linum usitatissimum* (UNIPROT accession number: P93243) [6,7,8,9]

3 Reaction and Specificity

Catalyzed reaction
(2R)-2-hydroxy-2-methylbutanenitrile = cyanide + butan-2-one

Natural substrates and products
S 2-hydroxy-2-methylpropanenitrile <1,3> (<1> i.e. acetone cyanohydrin [1]; <3> the enzyme is involved in the catabolism of cyanogenic glycosides in young seedlings of Linum usitatissimum [6]) (Reversibility: ?) [1,6]
P cyanide + acetone
S cyanide + acetone <2> (<2> natural substrates for the (R)-oxynitrilase from Linum usitatissimum are acetone and butan-2-one, which are the building blocks of the cyanogenic glycosides in Linum, linamarin and lotaustralin, or linustatin and neolinustatin, respectively [2]) (Reversibility: ?) [2]

D. Schomburg and I. Schomburg (eds.), *Class 3.4–6 Hydrolases, Lyases, Isomerases, Ligases: EC 3.4–6*, Springer Handbook of Enzymes S10, DOI 10.1007/978-3-642-36260-6_36,

P 2-hydroxy-2-methylpropanenitrile
S cyanide + butan-2-one <2> (<2> natural substrates for the (R)-oxynitrilase from Linum usitatissimum are acetone and butan-2-one, which are the building blocks of the cyanogen glycosides in Linum, linamarin and lotaustralin, or linustatin and neolinustatin, respectively [2]) (Reversibility: ?) [2]
P (2R)-butan-2-one cyanohydrin

Substrates and products

S (2R)-2-hydroxy-2-methylbutanenitrile <1> (Reversibility: ?) [1]
P cyanide + 2-butanone
S (R)-2-butanone-cyanhydrin <1> (Reversibility: ?) [4]
P HCN + butanone
S 2-hydroxy-2-methylpropanenitrile <1,3> (<1> i.e. acetone cyanohydrin [1]; <3> the enzyme is involved in the catabolism of cyanogenic glycosides in young seedlings of Linum usitatissimum [6]) (Reversibility: ?) [1,6]
P cyanide + acetone
S HCN + 4-hydroxybutanal <1> (Reversibility: ?) [3]
P 2,5-dihydroxypentanenitrile <1> [3]
S HCN + benzaldehyde <1> (Reversibility: ?) [3]
P (R)-mandelonitrile <1> [3]
S HCN + butanone <2> (Reversibility: ?) [5]
P (R)-2-butanone cyanhydrin
S cyanide + 2-methylcyclopentanone <2> (Reversibility: ?) [2]
P ?
S cyanide + 2-pentanone <3> (<3> 93% enantiomeric excess [9]) (Reversibility: ?) [9]
P (2R)-2-hydroxy-2-methylpentanenitrile
S cyanide + acetone <2> (<2> natural substrates for the (R)-oxynitrilase from Linum usitatissimum are acetone and butan-2-one, which are the building blocks of the cyanogenic glycosides in Linum, linamarin and lotaustralin, or linustatin and neolinustatin, respectively [2]) (Reversibility: ?) [2]
P 2-hydroxy-2-methylpropanenitrile
S cyanide + acetylcyclopropane <2> (Reversibility: ?) [2]
P ?
S cyanide + acrolein <3> (<3> 74% enantiomeric excess [9]) (Reversibility: ?) [9]
P (2R)-2-hydroxybut-3-enenitrile
S cyanide + butan-2-one <2> (<2> natural substrates for the (R)-oxynitrilase from Linum usitatissimum are acetone and butan-2-one, which are the building blocks of the cyanogen glycosides in Linum, linamarin and lotaustralin, or linustatin and neolinustatin, respectively [2]) (Reversibility: ?) [2]
P (2R)-butan-2-one cyanohydrin

S cyanide + butan-2-one <1,2,3> (<3> 95% enantiomeric excess [9]; <1> reaction with an immobilized form of the hydroxynitrile lyase as cross-linked enzyme aggregate with high specific activity and recovery on a preparative scale [5]) (Reversibility: ?) [2,5,9]
P (2R)-2-hydroxy-2-methylbutanenitrile (<2> 77.2% enantiomeric excess [2])
S cyanide + butyraldehyde <2,3> (<3> 98% enantiomeric excess [9]) (Reversibility: ?) [2,9]
P (2R)-2-hydroxypentanenitrile
S cyanide + chloroacetone <2> (Reversibility: ?) [2]
P (2R)-3-chloro-2-hydroxy-2-methylpropionitrile
S cyanide + crotonaldehyde <2,3> (<3> 99% enantiomeric excess [9]) (Reversibility: ?) [2,9]
P (2R)-2-hydroxy-3-pentenenitrile
S cyanide + hexan-2-one <2> (Reversibility: ?) [2]
P (2R)-2-hydroxy-2-methylhexanenitrile
S cyanide + hydroxyacetone <2> (Reversibility: ?) [2]
P (2R)-1,2-dihydroxy-2-methyl-propane-3-nitrile
S cyanide + hydroxypivaldehyde <3> (<3> 73% enantiomeric excess [9]) (Reversibility: ?) [9]
P (2R)-2,4-dihydroxy-3,3-dimethylbutanenitrile
S cyanide + isobutyraldehyde <3> (<3> 93% enantiomeric excess [9]) (Reversibility: ?) [9]
P (2R)-2-hydroxy-4-methylpentanenitrile
S cyanide + methacrolein <3> (<3> 98% enantiomeric excess [9]) (Reversibility: ?) [9]
P (2R)-2-hydroxy-3-methylbut-3-enenitrile
S cyanide + methyl vinyl ketone <2> (Reversibility: ?) [2]
P (2R)-2-hydroxy-2-methyl-3-butenenitrile
S cyanide + methyl vinyl ketone <3> (Reversibility: ?) [9]
P (2R)-2-hydroxy-2-methylbut-3-enenitrile (<3> despite a short reaction time of 0.8 h, the conversion of methyl vinyl ketone results in a poor (38%) enantiomeric excess value. As in the same time there is almost no conversion without enzyme. This compound is one of the rare examples, where the enzyme exerts only a partial stereoselectivity for a defined substrate [9])
S cyanide + pentan-2,4-dione <2> (Reversibility: ?) [2]
P ?
S cyanide + pentan-2-one <2> (Reversibility: ?) [2]
P (2R)-2-hydroxy-2-methylpentanenitrile
S cyanide + pinacolone <2> (Reversibility: ?) [2]
P (2R)-2-hydroxy-2,3,3-trimethylbutyronitrile
S cyanide + pivalaldehyde <2> (Reversibility: ?) [2]
P (2R)-3,3-dimethyl-2-hydroxybutyronitrile
S cyanide + propionaldehyde <2,3> (<3> 97% enantiomeric excess [9]) (Reversibility: ?) [2,9]
P (2R)-2-hydroxybutyronitrile

S cyanide + pyruvic acid ethyl ester <2> (Reversibility: ?) [2]
P ?
S Additional information <1,3> (<1> no activity with aromatic substrates [4]; <1> no activity towards mandelonitrile and *p*-hydroxymandelonitrile [1]; <1> synthesis of aromatic (S)-cyanohydrins. Most active towards derivatives of phenylacetone, converting 30-65% of the starting material to (S)-cyanohydrin with 55-95% enantiomeric excess in less than 1 day [10]; <3> the enzyme catalyzes the stereoselective synthesis of aliphatic (R)-cyanohydrins. Conversion of aromatic aldehydes (3-phenylpropionaldehyde or cinnamic aldehyde) and the aliphatic ketones is incomplete and gives poor enantiomeric excess-values, caused by the long reaction time [9]) (Reversibility: ?) [1,4,9,10]
P ?

Inhibitors

benzaldehyde <2> (<2> leads to a complete and irreversible deactivation of the enzyme within 2 h incubation [2]) [2]
diisopropyl fluorophosphate <3> (<3> 10 mM, 25% inhibition [6]) [6]
Additional information <1> (<1> no inhibition by 10 mM 2-mercaptoethanol, 1 mM iodoacetamide or iodoacetic acid, 10 mM isobutyronitrile or isopropanol [1]) [1]

Cofactors/prosthetic groups

Additional information <1> (<1> not a flavoprotein [1]) [1]

Activating compounds

Additional information <1> (<1> molecular imprinting using 2-butanone as additive in the immobilization process improves the synthetic activity of the biocatalyst [5]) [5]

Metals, ions

Zn^{2+} <3> (<3> LuHNL has significant homologies to members of the Zn^{2+}-containing alcohol dehydrogenases. In particular, residues responsible for coordination of Zn^{2+} ions or fulfilling structural or functional tasks in Zn^{2+}-alcohol dehydrogenases are conserved. Contains about 2-4 mol zinc per mol of recombinant enzyme. Hydroxynitrile lyase from Linum usitatissimum and Zn^{2+}-alcohol dehydrogenases have similar structural requirements with respect to maintaining a catalytically active structure. Residues essentially involved in catalysis of Zn^{2+}-ADHs are also of functional importance in hydroxynitrile lyase from Linum usitatissimum [9]) [9]

Specific activity (U/mg)

34.1 <1> [1]
52.9 <2> [2]
Additional information <1,2> (<2> development of an immobilized form of the hydroxynitrile lyase as crosslinked enzyme aggregate (CLEA) with high specific activity (303.5 U/g) and recovery (33%), 180.5 U/g LuCLEA (crosslinked enzyme aggregate) (using sat. $(NH_4)_2SO_4$), 9.9 U/g LuEA (Enzyme aggregate of LuHNL) (using sat. $(NH_4)_2SO_4$), 110.9 U/g LuCLEA (using tert-

butanol), 221.9 U/g LuEA (using tert-butanol) [5]; <1> development of an immobilized form of the hydroxynitrile lyase as crosslinked enzyme aggregate with high specific activity (303.5 U/g) and recovery [5]) [5]

K_m-Value (mM)
1.25 <1> ((2R)-2-hydroxy-2-methylbutanenitrile, <1> pH 5.5, 25°C [1]) [1]
2.5 <1> (2-hydroxy-2-methylpropanenitrile, <1> pH 5.5, 25°C [1]) [1]

pH-Optimum
5.5 <1,2> [1,2]

pH-Range
4.1-5.5 <2> (<2> pH 4.1: about 70% of maximal activity, pH 5.5: maximal activity [2]) [2]

pi-Value
4.5-4.8 <1> (<1> chromatofocusing [1]) [1]

Temperature optimum (°C)
25 <2> (<2> assay at [2]) [2]

4 Enzyme Structure

Molecular weight
80000 <3> (<3> gel filtration [9]) [9]
82000 <1> (<1> gel filtration [1]) [1]
87000 <2> (<2> gel filtration [2]) [2]

Subunits
? <3> (<3> x * 45780, calculated from sequence [6]) [6]
dimer <1,2,3> (<3> 2 * 40000, SDS-PAGE [9]; <1> 2 * 42000, SDS-PAGE [1]; <2> 2 * 43000, SDS-PAGE [2]) [1,2,9]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cotyledon <3> [8]
seedling <2> [2]
shoot <1> [1]

Localization
cytoplasm <3> (<3> highest detection level in cytoplasm, with lower levels in organelles, not detected in cell wall or vacuole [8]) [8]

Purification
<1> [1,3]
<2> [2]
<3> [7]

Cloning

<1> (cloned into Pichia pastoris, expressed in Escherichia coli as an N-terminal hexa-histidine fusion protein) [4]
<3> (cloning of a myc-His-tagged LuHNL-cDNA under control of the methanol-inducible AOX1 (alcohol oxidase) promotor of Pichia pastoris and introduction in the SMD1168 strain. Recombinant LuHNL is kinetically indistinguishable from the authentic flax enzyme) [9]
<3> (expressed in Escherichia coli as N-terminal hexa-histidine fusion protein) [7]
<3> (expression in Escherichia coli) [6]

Engineering

G104A <3> (<3> 5-10% of wild-type activity [9]) [9]
G95A <3> (<3> complete destruction of enzymatic activity [9]) [9]

Application

synthesis <1> (<1> optically active aliphatic ω-hydroxycyanohydrins are valued materials in organic synthesis [3]) [3]

6 Stability

pH-Stability

4 <2> (<2> half-life: 1 h [2]) [2]
5 <2> (<2> immobilization on Eupergit (carrier consisting of macroporous beads) improves the stability considerably in the pH range below pH 5 [2]) [2]
6-11 <2> (<2> stable [2]) [2]

General stability information

<2>, immobilization on Eupergit (carrier consisting of macroporous beads) improves the stability considerably in the pH range below pH 5 [2]

Storage stability

<1>, 4°C, stable for at least 45 d [1]

References

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