(+)-pulegone reductase 1.3.1.81

1 Nomenclature

EC number
1.3.1.81

Systematic name
(-)-menthone:$NADP^+$ oxidoreductase

Recommended name
(+)-pulegone reductase

Synonyms
pulegone reductase <1> [1,3,4,5]
Additional information <1,2> (<1> (+)-pulegone reductase is a member of the medium-chain dehydrogenase/reductase superfamily [3]; <1,2> the enzyme belongs to the distinct medium chain dehydrogenase/reductase superfamily [2]; <1> the enzyme is a member of the medium-chain dehydrogenase/reductase superfamily [1]) [1,2,3]

CAS registry number
138066-95-2

2 Source Organism

<1> *Mentha x piperita* [1,2,3,4,5]
<2> *Mentha spicata* [2]
<3> *Mentha x piperita* (UNIPROT accession number: Q6WAU0) [6,7]

3 Reaction and Specificity

Catalyzed reaction
(+)-isomenthone + $NADP^+$ = (+)-pulegone + NADPH + H^+
(-)-menthone + $NADP^+$ = (+)-pulegone + NADPH + H^+

Natural substrates and products
S (+)-pulegone + NADPH + H^+ <3> (Reversibility: r) [6]
P (-)-menthone + $NADP^+$
S (+)-pulegone + NADPH + H^+ <1,2,3> (<1> branchpoint enzyme in essential oil biosynthesis [5]; <1,2> pathways of monoterpene biosynthesis, overview [2,3]; <1> regulation of pulegone metabolism involves menthofuran, the flux of (+)-pulegone through pulegone reductase correlates ne-

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gatively with the essential oil content of menthofuran, such that menthofuran, and pulegone increase, or decrease, in concert, overview [4]; <1> the enzyme is required for catalyzation of the reduction of the $\Delta^{4,8}$ double bond to produce menthone on route to menthol, monoterpene biosynthetic pathways in peppermint, overview [1]) (Reversibility: ?) [1,2,3,4,5,7]

P (-)-menthone + (+)-isomenthone + $NADP^+$ (<3> ratio of 90% (-)-menthone and 10% (+)-isomenthone [7])

S Additional information <1> (<1> kinetic modeling and simulations of the developmental patterns of monoterpenoid essential oil accumulation in peppermint, overview [5]; <1> pathways for monoterpene biosynthesis in peppermint, overview [4]) (Reversibility: ?) [4,5]

P ?

Substrates and products

S (+)-pulegone + NADPH + H^+ <3> (Reversibility: r) [6]

P (-)-menthone + $NADP^+$

S (+)-pulegone + NADPH + H^+ <1,2,3> (<1> branchpoint enzyme in essential oil biosynthesis [5]; <1,2> pathways of monoterpene biosynthesis, overview [2,3]; <1> regulation of pulegone metabolism involves menthofuran, the flux of (+)-pulegone through pulegone reductase correlates negatively with the essential oil content of menthofuran, such that menthofuran, and pulegone increase, or decrease, in concert, overview [4]; <1> the enzyme is required for catalyzation of the reduction of the Δ^4,8 double bond to produce menthone on route to menthol, monoterpene biosynthetic pathways in peppermint, overview [1]; <1> the NADPH-dependent reductase reduces the conjugated double bond to yield both (-)-menthone and (+)-isomenthone [2]; <1> the reductase catalyzes the reduction of the 4(8)-double bond of (+)-pulegone to produce both (-)-menthone and (+)-isomenthone in a 70:30 ratio [1]; <1> the reductase catalyzes the reduction of the 4(8)-double bond of (+)-pulegone to produce both (-)-menthone and (+)-isomenthone in a 70:30 ratio, product analysis by GC-MS, stereospecific reductase [3]) (Reversibility: ?) [1,2,3,4,5,7]

P (-)-menthone + (+)-isomenthone + $NADP^+$ (<3> ratio of 90% (-)-menthone and 10% (+)-isomenthone [7])

S Additional information <1> (<1> kinetic modeling and simulations of the developmental patterns of monoterpenoid essential oil accumulation in peppermint, overview [5]; <1> pathways for monoterpene biosynthesis in peppermint, overview [4]) (Reversibility: ?) [4,5]

P ?

Inhibitors

(+)-menthofuran <1> (<1> weak competitive inhibition, in peppermint plants grown under low-light conditions, (+)-menthofuran is selectively retained in secretory cells and accumulated to very high levels of up to 20 mM, whereas under regular growth conditions, (+)-menthofuran levels remain very low, below 0.4 mM [5]) [5]

Additional information <1> (<1> although (+)-menthofuran does not inhibit (+)-pulegone reductase activity, stem feeding with menthofuran selectively decreases enzyme transcript levels in immature leaves, thereby accounting for decreased reductase activity and increased pulegone content, overview, the flux of (+)-pulegone through pulegone reductase correlates negatively with the essential oil content of menthofuran, such that menthofuran, and pulegone increase, or decrease, in concert, overview [4]) [4]

Cofactors/prosthetic groups

$NADP^+$ <3> [6,7]
NADPH <1,2,3> (<1> dependent on [1,3]) [1,2,3,4,5,6,7]

Turnover number (s^{-1})

1.8 <1> ((+)-pulegone, <1> pH 5.0, recombinant enzyme [3]) [3]
1.8 <1> (NADPH, <1> pH 5.0, recombinant enzyme [3]) [3]

Specific activity (U/mg)

Additional information <1> (<1> essential oil composition of wild-type peppermint [4]) [4]

K_m-Value (mM)

0.0023 <1> ((+)-pulegone, <1> pH 5.0, recombinant enzyme [3]) [3]
0.0069 <1> (NADPH, <1> pH 5.0, recombinant enzyme [3]) [3]
0.04 <1> ((+)-pulegone, <1> pH 6.6 [5]) [5]
Additional information <1> (<1> kinetic data analysis, overview [5]) [5]

K_i-Value (mM)

0.3 <1> ((+)-menthofuran, <1> pH 6.6 [5]) [5]

pH-Optimum

5 <1> [3]
6.6 <1> (<1> assay at [5]) [5]

pH-Range

4.5-6.5 <1> (<1> 70% of maximal activity within this range [3]) [3]

pi-Value

5.2 <1> (<1> about, isoelectric focusing [3]) [3]

4 Enzyme Structure

Molecular weight

45000 <1> (<1> gel filtration [3]) [3]

Subunits

? <1> (<1> x * 38000 [1]) [1]
monomer <1> (<1> 1 x * 43000, SDS-PAGE, 1 * 37914, sequence calculation [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

epidermis <1> [3]
leaf <1,3> [3,4,5,6,7]
secretory cell <1,3> (<3> essential oil biosynthetic enzyme expression patterns, overview [7]) [3,5,7]
secretory trichome <1> (<1> secretory cells of peltate glandular trichomes with abundant labeling corresponding to the secretory phase of gland development [1]) [1]
trichome <3> (<3> glandular [7]) [7]

Localization

soluble <1> [1,4]

Purification

<1> (native enzyme partially by isolation of secretory cells from leaves) [5]
<1> (recombinant enzyme from Escherichia coli by anion exchange and hydrophobic interaction chromatography followed by SDS-PAGE and gel excision) [1]
<1> (recombinant enzyme from Escherichia coli strain BL21(DE3), native enzyme by oil gland secretory cell isolation procedure, two steps of anion exchange chromatography, hydroxyapatite chromatography, and β-NADPH affinity chromatography, the activity is rapidly lost during purification on each matrix, 10fold purification accompanied by loss of over 98% of starting activity) [3]

Cloning

<1> (DNA and amino acid sequence determination and analysis, functional expression in Escherichia coli strain BL21(DE3)) [3]
<1> (expression in Escherichia coli strain BL21(DE3)) [1]
<3> (expression analysis with and without UV-B irradiation) [6]

6 Stability

General stability information

<1>, neither protease inhibitors nor flavins improve the activity or stability of the purified native enzyme, and the addition of glycerol to the buffers leads to rapid loss of activity upon storage [3]

References

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