benzoyl-CoA-dihydrodiol lyase 4.1.2.44

1 Nomenclature

EC number
4.1.2.44

Systematic name
2,3-dihydro-2,3-dihydroxybenzoyl-CoA 3,4-didehydroadipyl-CoA semialdehyde-lyase (formate-forming)

Recommended name
benzoyl-CoA-dihydrodiol lyase

Synonyms
BoxC <1> [2]
benzoyl-CoA oxidation component C <1> [2]
dihydrodiol transforming enzyme <1> [2]

2 Source Organism

<1> *Azoarcus evansii* (UNIPROT accession number: Q84HH6) [1,2]

3 Reaction and Specificity

Catalyzed reaction
2,3-dihydro-2,3-dihydroxybenzoyl-CoA + H_2O = 3,4-didehydroadipyl-CoA semialdehyde + formate

Natural substrates and products
S 2,3-dihydro-2,3-dihydroxybenzoyl-CoA + H_2O <1> (<1> the enzyme is involved in the aerobic benzoyl-CoA catabolic pathway. Benzoyl-CoA is oxidized to 2,3-dihydro-2,3-dihydroxybenzoyl-CoA (benzoyl-CoA dihydrodiol) by benzoyl-CoA oxygenase/reductase BoxBA in the presence of molecular oxygen. The next, ring cleaving step is catalysed by BoxC [2]) (Reversibility: ?) [2]
P 3,4-dehydroadipyl-CoA semialdehyde + formate

Substrates and products
S 2,3-dihydro-2,3-dihydroxybenzoyl-CoA + H_2O <1> (<1> the enzyme is involved in the aerobic benzoyl-CoA catabolic pathway. Benzoyl-CoA is oxidized to 2,3-dihydro-2,3-dihydroxybenzoyl-CoA (benzoyl-CoA dihydrodiol) by benzoyl-CoA oxygenase/reductase BoxBA in the presence of

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molecular oxygen. The next, ring cleaving step is catalysed by BoxC [2]; <1> NADPH and semicarbazide are analysed directly by NMR spectroscopy and mass spectrometry. The purified protein does not require molecular oxygen for activity [2]) (Reversibility: ?) [2]

P 3,4-dehydroadipyl-CoA semialdehyde + formate
S Additional information <1> (<1> no activity with crotonyl-CoA [2]) [2]
P ?

Inhibitors

Additional information <1> (<1> acetoacetyl-CoA (0.2 mM), a potential inhibitor of enoyl-CoA hydratase, has no impact on enzyme activity of BoxC-mal. Crotonyl-CoA (0.2 mM), a potential substrate of enoyl-CoA hydratase (crotonase), is neither converted to 3-hydroxybutyryl-CoA nor does it inhibit the standard assay [2]) [2]

Cofactors/prosthetic groups

Additional information <1> (<1> the purified protein does not require divalent metals or any cosubstrates or coenzymes for activity [2]) [2]

Activating compounds

Additional information <1> (<1> addition of 1 mM thiamine diphosphate to the standard assay causes only a minimal stimulation (8%) [2]) [2]

Metals, ions

Additional information <1> (<1> the purified protein does not require divalent metals or any cosubstrates or coenzymes for activity [2]) [2]

Turnover number (s^{-1})

20 <1> (2,3-dihydro-2,3-dihydroxybenzoyl-CoA) [2]

Specific activity (U/mg)

4.9 <1> [2]

K_m-Value (mM)

0.017 <1> (2,3-dihydro-2,3-dihydroxybenzoyl-CoA) [2]

pH-Optimum

9 <1> [2]

pH-Range

7-11 <1> (<1> half maximal activity at pH 7 and pH 11 [2]) [2]

pi-Value

5.44 <1> (<1> calculated from sequence [1]) [1]
5.6 <1> [2]

4 Enzyme Structure

Molecular weight

120000 <1> (<1> gel filtration [2]) [2]

Subunits
? <1> (<1> x * 61000, calculated from sequence [1]) [1]
homodimer <1> (<1> 2 * 60000, SDS-PAGE [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
culture condition:benzoate-grown cell <1> [2]

Localization
cytosol <1> [1]

Purification
<1> (wild type and recombinant proteins) [2]

Cloning
<1> [1]
<1> (the boxC gene is expressed in a recombinant Escherichia coli strain as a fusion protein with maltose binding protein (BoxCmal)) [2]

6 Stability

Storage stability
<1>, -20°C, the protein can be stored without appreciable loss of activity for months in the presence of 10% (v/v) glycerol [2]

References

[1] Gescher, J.; Zaar, A.; Mohamed, M.; Schägger, H.; Fuchs, G.: Genes coding for a new pathway of aerobic benzoate metabolism in Azoarcus evansii. J. Bacteriol., **184**, 6301-6315 (2002)
[2] Gescher, J.; Eisenreich, W.; Wörth, J.; Bacher, A.; Fuchs, G.: Aerobic benzoyl-CoA catabolic pathway in Azoarcus evansii: studies on the non-oxygenolytic ring cleavage enzyme. Mol. Microbiol., **56**, 1586-1600 (2005)