quinolinate synthase 2.5.1.72

1 Nomenclature

EC number
2.5.1.72

Systematic name
glycerone phosphate:iminosuccinate alkyltransferase (cyclizing)

Recommended name
quinolinate synthase

Synonyms
NadA <2> [3]
Old5 <3> [11]
SufE3 <3> (<3> bifunctional enzyme, quinolinate synthase activity and stimulation of CpNifS cysteine desulfurase activity [10]) [10]
quinolinate synthetase <2> [13]

CAS registry number
39434-08-7

2 Source Organism

<1> *Bacillus subtilis* [5]
<2> *Escherichia coli* [2,3,4,6,7,8,13]
<3> *Arabidopsis thaliana* [1,10,11]
<4> *Mycobacterium tuberculosis* [3]
<5> *Pyrococcus horikoshii* [3]
<6> *Pyrococcus horikoshii* (UNIPROT accession number: O57767) [9]
<7> *Cyanophora paradoxa* (UNIPROT accession number: P31179) [12]

3 Reaction and Specificity

Catalyzed reaction
glycerone phosphate + iminosuccinate = pyridine-2,3-dicarboxylate + 2 H_2O + phosphate (<6> model of the catalytic state. Elimination of phosphate from dihydroxyacetone phosphate may precede the condensation reaction [9])

Substrates and products
S dihydroxyacetone phosphate + iminoaspartate <1,2,4,5> (Reversibility: ?) [3,5]

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*, Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_60,

P ? + H_2O + phosphate
S Additional information <2> (<2> residues C291 and C294 of the C291XXC294XXC297 motif undergo reversible disulfide formation, which regulates the activity of the enzyme [4]) (Reversibility: ?) [4]
P ?

Inhibitors

1,10-phenanthroline <2> (<2> inhibits reactivation of O_2-inactivated enzyme [2]) [2]
2,2'-dipyridyl <2> (<2> inhibits reactivation of O_2-inactivated enzyme [2]) [2]
H_2O_2 <2> (<2> 1 mM, inactivation [2]) [2]
O_2 <1,2> (<1> complete loss of activity upon purification of enzyme in aerobic conditions or exposure to oxygen overnight [5]; <2> the activity of the enzyme within Escherichia coli is diminished by exposure of the cells to 4.2 atm O_2, while the activity in extracts is rapidly decreased by 0.2 atm O_2. Inactivation in extracts can be gradually reversed during anaerobic incubation, but is blocked by α, α-dipyridyl or by 1,10-phenanthroline [2]) [2,5]
paraquat <2> (<2> inactivation [2]) [2]

Cofactors/prosthetic groups

iron-sulfur centre <1,2,3,4,5> (<3> enzyme carries a higly oxygen-sensiteve [4Fe-4S] cluster at the NadA domain [10]; <1> enzyme contains a [4Fe-4S]-cluster, coordinated by residues C110, C230, C320. 3.8 mol iron per mol of enzyme, 3.3 mol sulfur per mol of enzyme [5]; <2> protein contains 3-3.5 mol iron and 3-3.5 mol sulfur per mol. Majority of the iron is in the form of a $[4Fe\text{-}4S]^{2+}$ cluster. The cluster is absolutely required for activity [6]; <2> protein contains 4 mol of iron and sulfur per mol [7]; <2,4,5> protein contains a [4Fe-4S] cluster absolutely required for activity [3]; <2> protein contains a $[4Fe\text{-}4S]^{2+}$ cluster plus a small amount of a $[3Fe\text{-}4S]^{+}$ cluster species. Protein contains 5 mol of iron and 2.8 mol of sulfur per mol [8]; <2> sequence contains a Cys-W-X-Cys-Y-Z-Cys sequence characteristic for $(Fe\text{-}S)_4$-containing proteins. Enzyme is inhibited by Fe(II)-chelating agents [2]) [2,3,5,6,7,8,10]

Metals, ions

Iron <1,2,4,5> (<1> enzyme contains a [4Fe-4S]-cluster, coordinated by residues C110, C230, C320. 3.8 mol iron per mol of enzyme [5]; <2> protein contains 3-3.5 mol iron and 3-3.5 mol sulfur per mol [6]; <2> protein contains 4 mol of iron and sulfur per mol [7]; <4,5> protein contains a [4Fe-4S] cluster absolutely required for activity [3]; <2> protein contains a [4Fe-4S] cluster absolutely required for activity. 3 iron ions per polypeptide [3]; <2> protein contains a $[4Fe\text{-}4S]^{2+}$ cluster plus a small amount of a $[3Fe\text{-}4S]^{+}$ cluster species. Protein contains 5 mol of iron and 2.8 mol of sulfur per mol [8]; <2> sequence contains a Cys-W-X-Cys-Y-Z-Cys sequence characteristic for $(Fe\text{-}S)_4$-containing proteins. Enzyme is inhibited by Fe(II)-chelating agents [2]) [2,3,5,6,7,8]

Specific activity (U/mg)

0.027 <1> (<1> 25°C, presence of oxygen to reoxidize NadB in the coupled assay [5]) [5]
0.05 <1> (<1> 25°C, presence of fumarate as electron acceptor for NadB in the coupled assay [5]) [5]
0.6 <2> (<2> pH 7.0, 25°C [13]) [13]
Additional information <1,2> (<2> continuous assay method [13]; <1> discontinuous enzymatic assay that couples the production of iminoaspartate by NadB with the condensation between DHAP and iminoaspartate to form quinolinic acid catalyzed by NadA. The assay is linear up to 0.25 mg of NadA, 10 microg of NadB is the lowest amount suitable to measure NadA activity, and under anaerobic conditions, NadA activity becomes independent of fumarate concentration, starting from 1 mM fumarate, but decreases at concentrations higher than 2 mM fumarate, due to inhibition of NadB by fumarate [5]) [5,13]

K_m-Value (mM)

0.36 <1> (dihydroxyacetone phosphate, <1> 25°C [5]) [5]

4 Enzyme Structure

Molecular weight

40000 <2> (<2> gel filtration [7]; <2> gel filtration, minor amount of protein [6]) [6,7]
80000 <2> (<2> gel filtration, major amount of protein [6]) [6]
124000 <1> (<1> gel filtration [5]) [5]

Subunits

? <2,7> (<7> x * 36760, calculated [12]; <2> x * 38246, mass spectrometry, x * 38240, calculated [13]) [12,13]
dimer <2> (<2> plus minor amount of monomer. 2 * 40000, SDS-PAGE, 2 * 39300, calculated [6]) [6]
monomer <2> (<2> 1 * 40000, SDS-PAGE, 1 * 39300, calculated [7]) [7]
trimer <1> (<1> 3 * 41000, calculated [5]) [5]

Posttranslational modification

Additional information <2> (<2> residues C291 and C294 of the C291XXC294XXC297 motif undergo reversible disulfide formation, which regulates the activity of the enzyme. Disulfide-bond formation and reduction are effected by oxidized and reduced forms of thioredoxin, with a midpoint potential of -264 mV for the redox couple [4]) [4]

5 Isolation/Preparation/Mutation/Application

Localization

plastid <3> [1,10]

Purification

<1> (recombinant protein, complete loss of activity upon purification of enzyme in aerobic conditions or exposure to oxygen overnight) [5]
<2> [3]
<2> (recombinant enzyme, purification from inclusion bodies) [13]
<2> (recombinant protein) [7,8]
<4> [3]
<5> [3]

Renaturation

<2> (O_2-dependent inactivation inactivation in extracts can be gradually reversed during anaerobic incubation, but is blocked by α,α'-dipyridyl or by 1,10-phenanthroline) [2]

Crystallization

<6> (in presence of substrate analogue malate. Diffraction to 2.0 A resolution. Triangular architecture composed of a 3fold repeat of three-layer $\alpha\beta\alpha$ sandwich folding. The active site is located at the interface of the three domains and is centered on the pseudo-3fold axis. The malate molecule is tightly held near the bottom of the active site cavity) [9]

Cloning

<1> (expression with C-terminal His-tag) [5]
<2> [3,6,13]
<2> (expression in Escherichia coli, N-terminal His-tag) [7]
<2> (expression with N-terminal His-tag) [8]
<4> [3]
<5> [3]
<6> (expression in Escherichia coli) [9]

Engineering

C110S <1> (<1> 0.4 mol iron per mol of protein, no enzymic activity [5]) [5]
C113A <2> (<2> 0.8 iron ions per polypeptide, no catalytic activity [3]; <2> 1 mol of iron and sulfur per mol of protein, oligomer formation [7]) [3,7]
C113S <2> (<2> 1.3 iron ions per polypeptide, no catalytic activity [3]) [3]
C119A <2> (<2> 2.9 mol of iron and sulfur per mol of protein [7]) [7]
C119S <2> (<2> 1.0 iron ions per polypeptide [3]) [3]
C128S <2> (<2> 2.7 iron ions per polypeptide [3]) [3]
C195S <2> (<2> 1.5 iron ions per polypeptide [3]) [3]
C200A <2> (<2> 0.9 mol of iron and sulfur per mol of protein, oligomer formation [7]; <2> 1.5 iron ions per polypeptide, no catalytic activity [3]) [3,7]
C200S <2> (<2> 1.0 iron ions per polypeptide, no catalytic activity [3]) [3]
C230S <1> (<1> 0.6 mol iron per mol of protein, no enzymic activity [5]) [5]
C259S <1> (<1> 4.5 mol iron per mol of protein, 80% of wild-type activity [5]) [5]
C291A <2> (<2> 3.9 mol of iron and sulfur per mol of protein [7]) [7]
C291A/C294A <2> (<2> 3.7 mol of iron and sulfur per mol of protein [7]) [7]

C291A/C294A/C297A <2> (<2> 0.5 mol of iron and sulfur per mol of protein [7]) [7]
C291S <2> (<2> 0.8 iron ions per polypeptide [3]) [3]
C294A <2> (<2> 3.2 mol of iron and sulfur per mol of protein [7]) [7]
C294A/C297A <2> (<2> 0.6 mol of iron and sulfur per mol of protein [7]) [7]
C294S <2> (<2> 2.1 iron ions per polypeptide [3]) [3]
C297A <2> (<2> 0.3 iron ions per polypeptide, no catalytic activity [3]; <2> 0.4 mol of iron and sulfur per mol of protein, oligomer formation [7]) [3,7]
C297S <2> (<2> 0.3 iron ions per polypeptide, no catalytic activity [3]) [3]
C318S <1> (<1> 3.3 mol iron per mol of protein, 75% of wild-type activity [5]) [5]
C318S/C320S <1> (<1> 0.3 mol iron per mol of protein, no enzymic activity [5]) [5]
C320S <1> (<1> 1.5 mol iron per mol of protein, no enzymic activity [5]) [5]
C64S <2> (<2> 1.4 iron ions per polypeptide [3]) [3]
C82S <1> (<1> 4.3 mol iron per mol of protein, activity similar to wild-type [5]) [5]
Additional information <3> (<3> functional complementation of a corresponding Escherichia coli mutant. Gene disruption in Arabidospis thaliana is embryo-lethal [1]; <3> knock-out of SufE3 is embryo-lethal [10]; <3> the old5 lesion does not affect quinolinate synthase activity but decreases the proteins cysteine desulfurase activity resulting in increased NAD steady state levels concomitant with increased activity of enzymes in the NAD salvage pathway [11]) [1,10,11]

Application

synthesis <2> (<2> production of quinolinic acid from L-aspartate, dihydroxyacetone phosphate, and O_2 by use of enzymes NadA and NadB [13]) [13]

References

[1] Katoh, A.; Uenohara, K.; Akita, M.; Hashimoto, T.: Early steps in the biosynthesis of NAD in Arabidopsis start with aspartate and occur in the plastid. Plant Physiol., **141**, 851-857 (2006)
[2] Gardner, P.R.; Fridovich, I.: Quinolinate synthetase: the oxygen-sensitive site of de novo $NAD(P)^+$ biosynthesis. Arch. Biochem. Biophys., **284**, 106-111 (1991)
[3] Saunders, A.H.; Griffiths, A.E.; Lee, K.H.; Cicchillo, R.M.; Tu, L.; Stromberg, J.A.; Krebs, C.; Booker, S.J.: Characterization of quinolinate synthases from Escherichia coli, Mycobacterium tuberculosis, and Pyrococcus horikoshii indicates that [4Fe-4S] clusters are common cofactors throughout this class of enzymes. Biochemistry, **47**, 10999-11012 (2008)
[4] Saunders, A.H.; Booker, S.J.: Regulation of the activity of Escherichia coli quinolinate synthase by reversible disulfide-bond formation. Biochemistry, **47**, 8467-8469 (2008)

[5] Marinoni, I.; Nonnis, S.; Monteferrante, C.; Heathcote, P.; Haertig, E.; Boettger, L.H.; Trautwein, A.X.; Negri, A.; Albertini, A.M.; Tedeschi, G.: Characterization of L-aspartate oxidase and quinolinate synthase from Bacillus subtilis. FEBS J., **275**, 5090-5107 (2008)
[6] Ollagnier-de Choudens, S.; Loiseau, L.; Sanakis, Y.; Barras, F.; Fontecave, M.: Quinolinate synthetase, an iron-sulfur enzyme in NAD biosynthesis. FEBS Lett., **579**, 3737-3743 (2005)
[7] Rousset, C.; Fontecave, M.; Ollagnier de Choudens, S.: The [4Fe-4S] cluster of quinolinate synthase from Escherichia coli: investigation of cluster ligands. FEBS Lett., **582**, 2937-2944 (2008)
[8] Cicchillo, R.M.; Tu, L.; Stromberg, J.A.; Hoffart, L.M.; Krebs, C.; Booker, S.J.: Escherichia coli quinolinate synthetase does indeed harbor a [4Fe-4S] cluster. J. Am. Chem. Soc., **127**, 7310-7311 (2005)
[9] Sakuraba, H.; Tsuge, H.; Yoneda, K.; Katunuma, N.; Ohshima, T.: Crystal structure of the NAD biosynthetic enzyme quinolinate synthase. J. Biol. Chem., **280**, 26645-26648 (2005)
[10] Narayana Murthy, U.M.; Ollagnier-de-Choudens, S.; Sanakis, Y.; Abdel-Ghany, S.E.; Rousset, C.; Ye, H.; Fontecave, M.; Pilon-Smits, E.A.; Pilon, M.: Characterization of Arabidopsis thaliana SufE2 and SufE3: functions in chloroplast iron-sulfur cluster assembly and Nad synthesis. J. Biol. Chem., **282**, 18254-18264 (2007)
[11] Schippers, J.H.; Nunes-Nesi, A.; Apetrei, R.; Hille, J.; Fernie, A.R.; Dijkwel, P.P.: The Arabidopsis onset of leaf death5 mutation of quinolinate synthase affects nicotinamide adenine dinucleotide biosynthesis and causes early ageing. Plant Cell, **20**, 2909-2925 (2008)
[12] Michalowski, C.B.; Flachmann, R.; Loeffelhardt, W.; Bohnert, H.J.: Gene nadA, encoding quinolinate synthetase, is located on the cyanelle DNA from Cyanophora paradoxa. Plant Physiol., **95**, 329-330 (1991)
[13] Ceciliani, F.; Caramori, T.; Ronchi, S.; Tedeschi, G.; Mortarino, M.; Galizzi, A.: Cloning, overexpression, and purification of Escherichia coli quinolinate synthetase. Protein Expr. Purif., **18**, 64-70 (2000)