6-phospho-3-hexuloisomerase 5.3.1.27

1 Nomenclature

EC number
5.3.1.27

Systematic name
D-arabino-hex-3-ulose-6-phosphate isomerase

Recommended name
6-phospho-3-hexuloisomerase

Synonyms
3-hexulose-6-phosphate isomerase
6-phospho-3-hexuloisomerase <1,2,3,4,5,6,7,9,10,11,12,20,21> [10]
PHI <1,2,3,4,5,6,7,9,10,11,12,20,21> [10]
YckF <15> [1]
[acyl-carrier protein]:acetate ligase
phospho-3-hexuloisomerase

2 Source Organism

<1> *Pyrococcus sp.* [10]
<2> *Brevibacillus brevis* [10]
<3> *Bacillus subtilis* [6,10]
<4> *Methylococcus capsulatus* [4,10]
<5> *Bacillus methanolicus* [10]
<6> *Thermococcus sp.* [10]
<7> *Pyrococcus horikoshii* [10]
<8> *uncultured bacterium* [2]
<9> *Thermococcus kodakarensis* [8,10]
<10> *Methanosarcina sp.* [10]
<11> *Methylobacillus flagellatus KT* [10]
<12> *Methylomonas aminofaciens* [10]
<13> no activity in *Hansenula polymorpha* [2]
<14> *Methanocaldococcus jannaschii* (UNIPROT accession number: Q58644) [9]
<15> *Bacillus subtilis* (UNIPROT accession number: P42404) [1]
<16> no activity in *Kloeckera sp.* [2]
<17> *Mycobacterium gastri* (UNIPROT accession number: Q9LBW5) [3]
<18> *Methylomonas aminofaciens* (UNIPROT accession number: Q950X3) [5]
<19> *Pyrococcus horikoshii* (UNIPROT accession number: O59601) [7]

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_78,

<20> *Mycobacterium gastri* [10]
<21> *Aminomonas aminovorus* [10]

3 Reaction and Specificity

Catalyzed reaction
D-arabino-hex-3-ulose 6-phosphate = D-fructose 6-phosphate

Reaction type
isomerization

Natural substrates and products
S D-arabino-hex-3-ulose 6-phosphate <1,2,3,4,5,6,7,9,10,11,12,20,21> (Reversibility: ?) [10]
P D-fructose 6-phosphate
S Additional information <9> (<9> bifunctional 3-hexulose-6-phosphate synthase/6-phospho-3-hexuloisomerase is essential for the biosynthesis of ribulose 5-phosphate. The ribulose monophosphate pathway substitutes for the classical pentose phosphate pathway in Thermococcus kodakarensis [8]) (Reversibility: ?) [8]
P ?

Substrates and products
S D-arabino-3-hexulose 6-phosphate <4,17> (Reversibility: r) [3,4]
P D-fructose 6-phosphate
S D-arabino-hex-3-ulose 6-phosphate <1,2,3,4,5,6,7,8,9,10,11,12,15,20,21> (Reversibility: ?) [1,2,10]
P D-fructose 6-phosphate
S D-fructose 6-phosphate <8> (Reversibility: r) [2]
P D-arabino-hex-3-ulose 6-phosphate
S D-fructose 6-phosphate <4> (Reversibility: r) [4]
P D-arabino-3-hexulose 6-phosphate
S Additional information <4,9> (<9> bifunctional 3-hexulose-6-phosphate synthase/6-phospho-3-hexuloisomerase is essential for the biosynthesis of ribulose 5-phosphate. The ribulose monophosphate pathway substitutes for the classical pentose phosphate pathway in Thermococcus kodakarensis [8]; <4> enzyme is specific for substrates D-arabino-3-hexulose 6-phosphate and D-fructose 6-phosphate [4]; <4> no substrate: D-ribulose 5-phosphate, D-xylulose 5-phosphate or D-allulose 6-phosphate [4]) (Reversibility: ?) [4,8]
P ?

Inhibitors
Co^{2+} <4> (<4> 1 mM, 35% residual activity [4]) [4]
Cu^{2+} <4> (<4> 1 mM, 8% residual activity [4]) [4]
Mg^{2+} <4> (<4> 5 mM, 30% loss of activity [4]) [4]
Ni^{2+} <4> (<4> 1 mM, 14% residual activity [4]) [4]
Zn^{2+} <4> (<4> 1 mM, 40% residual activity [4]) [4]

Additional information <4> (<4> at 1 mM sugar phosphate concentration, D-allulose 6-phosphate, D-fructose 6-phosphate, 6-phospho-D-gluconate, D-ribulose 5-phosphate, D-xylulose 5-phosphate, D-erythrose 4-phosphate and glyceraldehyde 3-phosphate do not affect the isomerase activity [4]) [4]

Activating compounds

formaldehyde <3> (<3> concomitant induction of 3-hexulose 6-phosphate synthase and 6-phospho-3-hexuloisomerase by formaldehyde [6]) [6]
Additional information <3> (<3> no induction by by methanol, formate, or methylamine [6]) [6]

Metals, ions

Additional information <4> (<4> no requirement for divalent cations [4]) [4]

Specific activity (U/mg)

20 <8> (<8> pH 7.0, 30°C [2]) [2]
154 <17> (<17> 3-hexulose-6-phosphate synthase/6-phospho-3-hexuloisomerasefusion enzyme, 30°C [3]) [3]
563 <17> (<17> 6-phospho-3-hexuloisomerase, 30°C [3]) [3]
1560 <4> (<4> 30°C, pH 7.0 [4]) [4]

K_m-Value (mM)

0.029 <8> (D-arabino-hex-3-ulose 6-phosphate, <8> pH 7.0, 30°C [2]) [2]
0.1 <4> (D-arabino-3-hexulose 6-phosphate, <4> 30°C, pH 7.0 [4]) [4]
0.67 <8> (D-fructose 6-phosphate, <8> pH 7.0, 30°C [2]) [2]
1.1 <4> (D-fructose 6-phosphate, <4> 30°C, pH 7.0 [4]) [4]

pH-Optimum

7.5 <8> [2]
8.3 <4> [4]

Temperature optimum (°C)

30 <8,17> (<17> both recombinant 6-phospho-3-hexuloisomerase and 3-hexulose-6-phosphate synthase /6-phospho-3-hexuloisomerase fusion enzyme [3]) [2,3]
60 <19> (<19> separately expressed 6-phospho-3-hexuloisomerase domain [7]) [7]
80-85 <19> (<19> bifunctional enzyme [7]) [7]

4 Enzyme Structure

Molecular weight

67000 <4> (<4> gel filtration [4]) [4]
75000 <19> (<19> separately expressed 6-phospho-3-hexuloisomerase domain [7]) [7]
94000 <17> (<17> gel filtration, recombinant 6-phospho-3-hexuloisomerase [3]) [3]
162000 <19> (<19> gel filtration, bifunctional enzyme [7]) [7]

Subunits

? <17> (<17> x * 42000, recombinant 3-hexulose-6-phosphate synthase /6-phospho-3-hexuloisomerasefusion enzyme [3]) [3]
tetramer <17,19> (<17> 4 * 21000, SDS-PAGE, recombinant 6-phospho-3-hexuloisomerase [3]; <19> 4 * 47000, SDS-PAGE, bifunctional enzyme, 4 * 21000, SDS-PAGE, and 4 * 21475, calculated, separately expressed 6-phospho-3-hexuloisomerase domain [7]) [3,7]

5 Isolation/Preparation/Mutation/Application

Purification

<4> [4]

Crystallization

<14> (diffraction to 2.0 A resolution. MJ1247 is an α/β structure consisting of a five-stranded parallel β-sheet flanked on both sides by α-helices, forming a three-layered α-β-α sandwich. The fold represents the nucleotide binding motif of a flavodoxin type. Protein forms a tetramer in the crystal an in solution and each monomer has a folding similar to the isomerase domain of glucosamine 6-phosphate synthase) [9]
<15> (diffraction to 1.7 A, space group P6522 or P6122) [1]

Cloning

<9> [8]
<14> (expression in Escherichia coli) [9]
<15> (expression in Escherichia coli) [1]
<19> (expression in Escherichia coli) [7]

Engineering

Additional information <3,9,17,19> (<9> deletion of bifunctional 3-hexulose-6-phosphate synthase /6-phospho-3-hexuloisomerase results in loss of growth in minimal medium, which can be recovered by addition of nucleosides to the medium [8]; <19> expression of the full-length gene encoding the hybrid enzyme 3-hexulose 6-phosphate synthase/6-phospho-3-hexuloisomerase, the sequence corresponding to the 3-hexulose 6-phosphate synthase region, and the sequence corresponding to the 6-phospho-3-hexuloisomerase region produces active enzymes in Escherichia coli. The bifunctional enzyme catalyzes the sequential reaction much more efficiently than a mixture of the isolated domains [7]; <17> fusion gene construct of 3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase. The gene product of 3-hexulose-6-phosphate synthase /6-phospho-3-hexuloisomerase exhibits both activities at room temperature and catalyzes the sequential reactions more efficiently than a simple mixture of the individual enzymes. The gene product of 6-phospho-3-hexuloisomerase /3-hexulose-6-phosphate synthase fails to display any enzyme activity. Escherichia coli strains harboring the 3-hexulose-6-phosphate synthase /6-phospho-3-hexuloisomerase gene consume formaldehyde more efficiently and exhibited better growth in

a formaldehyde containing medium than the host strain [3]; <3> gene disruption causes moderate sensitivity to formaldehyde [6]) [3,6,7,8]

Application

synthesis <18> (<18> production of D-[1-^{13}C]fructose 6-phosphate from ^{13}C-enriched formaldehyde and D-ribose 5-phosphate using 3-hexulose 6-phosphate synthase and 6-phospho-3-hexuloisomerase from Methylomonas aminofaciens and spinach ribose 5-phosphate isomerase [5]) [5]

6 Stability

Temperature stability

60 <4> (<4> 10 min, complete loss of activity [4]) [4]

90 <19> (<19> half-life above 90 min, bifunctional enzyme. Half-life below 5 min, separately expressed 6-phospho-3-hexuloisomerase domain [7]) [7]

Storage stability

<4>, -15°C, l0 mM-Tris-HCI, 1 mM-EDTA buffer, pH 7.5, stable for 4 months [4]

<4>, 0-4°C, l0 mM-Tris-HCI, 1 mM-EDTA buffer, pH 7.5, stable for 4 months [4]

References

[1] Taylor, E.J.; Charnock, S.J.; Colby, J.; Davies, G.J.; Black, G.W.: Cloning, purification and characterization of the 6-phospho-3-hexulose isomerase YckF from Bacillus subtilis. Acta Crystallogr. Sect. D, **D57**, 1138-1140 (2001)

[2] Kato, N.; Ohashi, H.; Hori, T.; Tani, Y.; Ogata, K.: Properties of 3-hexulose phosphate synthase and phospho-3-hexuloisomerase of a methanol-utilizing bacterium, 77a. Agric. Biol. Chem., **41**, 1133-1140 (1977)

[3] Orita, I.; Sakamoto, N.; Kato, N.; Yurimoto, H.; Sakai, Y.: Bifunctional enzyme fusion of 3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase. Appl. Microbiol. Biotechnol., **76**, 439-445 (2007)

[4] Ferenci, T.; Stroem, T.; Quayle, J.R.: Purification and properties of 3-hexulose phosphate synthase and phospho-3-hexuloisomerase from Methylococcus capsulatus. Biochem. J., **144**, 477-486 (1974)

[5] Yanase, H.; Koike, Y.; Matsuzaki, K.; Kita, K.; Sato, Y.; Kato, N.: Production of D-[1-^{13}C]fructose 6-phosphate by a formaldehyde-fixing system in Methylomonas aminofaciens 77a. Biosci. Biotechnol. Biochem., **56**, 541-542 (1992)

[6] Yasueda, H.; Kawahara, Y.; Sugimoto, S.: Bacillus subtilis yckG and yckF encode two key enzymes of the ribulose monophosphate pathway used by methylotrophs, and yckH is required for their expression. J. Bacteriol., **181**, 7154-7160 (1999)

[7] Orita, I.; Yurimoto, H.; Hirai, R.; Kawarabayasi, Y.; Sakai, Y.; Kato, N.: The archaeon Pyrococcus horikoshii possesses a bifunctional enzyme for for-

maldehyde fixation via the ribulose monophosphate pathway. J. Bacteriol., **187**, 3636-3642 (2005)
[8] Orita, I.; Sato, T.; Yurimoto, H.; Kato, N.; Atomi, H.; Imanaka, T.; Sakai, Y.: The ribulose monophosphate pathway substitutes for the missing pentose phosphate pathway in the archaeon Thermococcus kodakaraensis. J. Bacteriol., **188**, 4698-4704 (2006)
[9] Martinez-Cruz, L.A.; Dreyer, M.K.; Boisvert, D.C.; Yokota, H.; Martinez-Chantar, M.L.; Kim, R.; Kim, S.: Crystal structure of MJ1247 protein from M. jannaschii at 2.0 A resolution infers a molecular function of 3-hexulose-6-phosphate isomerase. Structure, **10**, 195-204 (2002)
[10] Yurimoto, H.; Kato, N.; Sakai, Y.: Genomic organization and biochemistry of the ribulose monophosphate pathway and its application in biotechnology. Appl. Microbiol. Biotechnol., **84**, 407-416 (2009)