3,4-dihydroxyphenylalanine oxidative deaminase 1.13.12.15

1 Nomenclature

EC number
1.13.12.15

Systematic name
3,4-dihydroxy-L-phenylalanine:oxygen oxidoreductase (deaminating)

Recommended name
3,4-dihydroxyphenylalanine oxidative deaminase

Synonyms
3,4-dihydroxy-L-phenylalanine: oxidative deaminase
3,4-dihydroxyphenylalanine oxidative deaminase <1> [2]
DDC <1> [2]
DOPA decarboxylase <1> [2]
DOPA oxidative deaminase
DOPAODA
oxidative deaminase

2 Source Organism

<1> *Sus scrofa* [2]
<2> *Rhodobacter sphaeroides* [1]

3 Reaction and Specificity

Catalyzed reaction
2 3,4-dihydroxy-L-phenylalanine + O_2 = 2 3,4-dihydroxyphenylpyruvate + 2 NH_3

Reaction type
deamination

Natural substrates and products
S D-tryptophan methyl ester + ½ O_2 <1> (<1> production depending on the nature of the substrate, and ammonia with concomitant O_2 consumption in a 1:2 molar ratio with respect to the products [2]) (Reversibility: ?) [2]
P methyl 3-(1H-indol-3yl)-2-oxopropanoate + NH_3
S L-DOPA + O_2 <1> (Reversibility: ?) [2]

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_68,

P ?
S aromatic amine + ½ O_2 <1> (<1> oxidative deamination, unusual oxygen-consuming reaction catalyzed by the enzyme toward aromatic amines (serotonin, dopamine, and α-methyldopamine) and D-tryptophan methyl ester [2]) (Reversibility: ?) [2]
P aromatic aldehyde + NH_3 + H_2O (<1> production in equivalent amounts depending on the nature of the substrate, and ammonia with concomitant O_2 consumption in a 1:2 molar ratio with respect to the products. A ketimine accumulates during the linear phase of product formation. This species is reactive since it is converted back to pyridoxal 5'-phosphate when the substrate is consumed. Superoxide anion and hydrogen peroxide are both generated during the catalytic cycles. [2])
S Additional information <1> (<1> The novelty in DDC is the possibility of catalyzing a reaction involving dioxygen although the enzyme lacks any cofactor or metal related to O_2 chemistry. The external aldimine intermediate undergoes a decarboxylation or a deprotonation leading to a quinonoid species, that is protonated at C_4 producing the ketimine intermediate. Although it cannot be ruled out that this intermediate could be attacked by dioxygen, it seems much more likely, regarding enzymes proceeding through a carbanion chemistry on DDC, that the more electron dense quinonoid intermediate, in equilibrium with the ketimine, is reactive toward O_2. Aerobiosis shifts the quinonoid-ketimine equilibrium toward quinonoid, while anaerobiosis shifts the equilibrium toward ketimine. The reaction between dioxygen and the quinonoid give rise directly to a superoxide anion and semiquinone. Superoxide is deprotonated and its anionic form is thus able to couple with the semiquinone giving rise to a peroxide species that is further protonated, and thus forming a hydroperoxy-pyridoxal 5'-phosphate intermediate. This rearranges to produce aldehyde, ammonia and hydrogen peroxide. [2]) (Reversibility: ?) [2]
P ?

Substrates and products

S 3,4-dihydroxy-L-phenylalanine + O_2 <2> (Reversibility: ?) [1]
P 3,4-dihydroxyphenylpyruvate + NH_3
S D-tryptophan methyl ester + ½ O_2 <1> (<1> production depending on the nature of the substrate, and ammonia with concomitant O_2 consumption in a 1:2 molar ratio with respect to the products [2]) (Reversibility: ?) [2]
P methyl 3-(1H-indol-3yl)-2-oxopropanoate + NH_3
S L-DOPA + O_2 <1> (Reversibility: ?) [2]
P ?
S L-alanine + O_2 <2> (Reversibility: ?) [1]
P 2-oxo-propanoic acid + NH_3 (<2> 20% of the activity with 3,4-dihydroxy-L-phenylalanine [1])
S L-glutamate + O_2 <2> (Reversibility: ?) [1]
P 2-oxopentanedioic acid + NH_3 (<2> 60% of the activity with 3,4-dihydroxy-L-phenylalanine [1])

S L-phenylalanine + O_2 <2> (Reversibility: ?) [1]
P 2-oxo-3-phenylpropanoic acid + NH_3 (<2> 60% of the activity with 3,4-dihydroxy-L-phenylalanine [1])
S L-tryptophan + O_2 <2> (Reversibility: ?) [1]
P 3-(1H-indol-3-yl)-2-oxopropanoic acid + NH_3 (<2> 50% of the activity with 3,4-dihydroxy-L-phenylalanine [1])
S L-tyrosine + O_2 <2> (Reversibility: ?) [1]
P 3-(4-hydroxyphenyl)-2-oxopropanoic acid + NH_3 (<2> 80% of the activity with 3,4-dihydroxy-L-phenylalanine [1])
S aromatic amine + ½ O_2 <1> (<1> oxidative deamination, unusual oxygen-consuming reaction catalyzed by the enzyme toward aromatic amines (serotonin, dopamine, and α-methyldopamine) and D-tryptophan methyl ester [2]) (Reversibility: ?) [2]
P aromatic aldehyde + NH_3 + H_2O (<1> production in equivalent amounts depending on the nature of the substrate, and ammonia with concomitant O_2 consumption in a 1:2 molar ratio with respect to the products. A ketimine accumulates during the linear phase of product formation. This species is reactive since it is converted back to pyridoxal 5'-phosphate when the substrate is consumed. Superoxide anion and hydrogen peroxide are both generated during the catalytic cycles. [2])
S glycine + O_2 <2> (Reversibility: ?) [1]
P oxoacetic acid + NH_3 (<2> 30% of the activity with 3,4-dihydroxy-L-phenylalanine [1])
S Additional information <1> (<1> The novelty in DDC is the possibility of catalyzing a reaction involving dioxygen although the enzyme lacks of any cofactor or metal related to O_2 chemistry. The external aldimine intermediate undergoes a decarboxylation or a deprotonation leading to a quinonoid species, that is protonated at C_4 producing the ketimine intermediate. Although it cannot be ruled out that this intermediate could be attacked by dioxygen, it seems much more likely, regarding enzymes proceeding through a carbanion chemistry on DDC, that the more electron dense quinonoid intermediate, in equilibrium with the ketimine, is reactive toward O_2. Aerobiosis shifts the quinonoid-ketimine equilibrium toward quinonoid, while anaerobiosis shifts the equilibrium toward ketimine. The reaction between dioxygen and the quinonoid give rise directly to a superoxide anion and semiquinone. Superoxide is deprotonated and its anionic form is thus able to couple with the semiquinone giving rise to a peroxide species that is further protonated, and thus forming a hydroperoxy-pyridoxal 5'-phosphate intermediate. This rearranges to produce aldehyde, ammonia and hydrogen peroxide. [2]) (Reversibility: ?) [2]
P ?

Inhibitors

2-oxoglutarate <2> [1]
3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methyl propionic acid <1> (<1> carbiDOPA, addition of 10 microM inhibitor to reaction mixtures (Y332F mutant

with L-dopa) in the presence or in the absence of catalase or superoxide dismutase, immediately stops the O_2 consumption. [2]) [2]
NADH <2> [1]

Cofactors/prosthetic groups
Additional information <2> (<2> no cofactor required [1]) [1]

Turnover number (s^{-1})
0.68 <2> (3,4-dihydroxy-L-phenylalanine, <2> pH 7.8, 30°C [1]) [1]
4.5 <1> (L-Dopa, <1> Y332F DDC mutant, reaction in 50 mM Hepes, pH 7.5, at 25°C causes the production of ammonia and 3,4-dihydroxyphenylacetaldehyde along with the consumption of molecular oxygen in a 1:2 molar ratio [2]) [2]

K_m-Value (mM)
0.01184 <2> (<2> K_m value for 3,4-dihydroxy-L-phenylalanine [1]) [1]

4 Enzyme Structure

Molecular weight
190000 <2> (<2> gel filtration [1]) [1]

Subunits
pentamer <2> (<2> five different subunits of 54000 Da, 43000 Da, 34000 Da, 25000 Da, and 22000 Da, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [2]
<2> [1]

Cloning
<1> (cloning and expression of wild-type 3,4-dihydroxyphenylalanine oxidative deaminase and Y332F and T246A mutants in SVS370 Escherichia coli cells.) [2]

Engineering
T246A <1> (<1> T246 act as an essential general base for the oxidative deamination reaction [2]) [2]
Y332F <1> (<1> wild-type enzyme and Y332F variant are able to perform the oxidation toward aromatic amines or aromatic L-amino acids, without the aid of any cofactor related to oxygen chemistry. [2]) [2]

References

[1] Ranjith, N.K.; Ramana, C.h.V.; Sasikala, C.H.: Purification and characterization of 3,4-dihydroxyphenylalanine oxidative deaminase from Rhodobacter sphaeroides OU5. Can. J. Microbiol., **54**, 829-834 (2008)
[2] Bertoldi, M.; Cellini, B.; Montioli, R.; Borri Voltattorni, C.: Insights into the mechanism of oxidative deamination catalyzed by DOPA decarboxylase. Biochemistry, **47**, 7187-7195 (2008)