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# polyamine oxidase (propane-1,3-diamine-forming)

1.5.3.14

## 1 Nomenclature

### EC number

1.5.3.14

### Systematic name

spermidine:oxygen oxidoreductase (propane-1,3-diamine-forming)

### Recommended name

polyamine oxidase (propane-1,3-diamine-forming)

### Synonyms

MPAO <1,3> [2,3,4,7,11,12]

PAO <1,2> [7,10,13,14]

ZmPAO <1> [12,14]

flavin-containing polyamine oxidase <1,2> [14]

maize PAO <3> [2]

maize polyamine oxidase <1> [11]

## 2 Source Organism

<1> *Zea mays* [7,10,11,12,14]

<2> *Nicotiana tabacum* [13,14]

<3> *Zea mays* (UNIPROT accession number: O64411) [1,2,3,4,5,6,7,8,9]

## 3 Reaction and Specificity

### Catalyzed reaction

spermidine + O<sub>2</sub> + H<sub>2</sub>O = propane-1,3-diamine + 4-aminobutanal + H<sub>2</sub>O<sub>2</sub>

### Natural substrates and products

**S** spermidine + O<sub>2</sub> + H<sub>2</sub>O <1,2> (Reversibility: ?) [13,14]

**P** 1,3-diaminopropane + 4-aminopropanal + H<sub>2</sub>O<sub>2</sub>

**S** spermidine + O<sub>2</sub> + H<sub>2</sub>O <1> (Reversibility: ?) [7,11,12]

**P** propane-1,3-diamine + 4-aminobutanal + H<sub>2</sub>O<sub>2</sub>

**S** spermine + O<sub>2</sub> + H<sub>2</sub>O <2> (Reversibility: ?) [13,14]

**P** 1,3-diaminopropane + aminoaldehyde + H<sub>2</sub>O<sub>2</sub>

**S** Additional information <1,2> (<1> analysis of polymaine content in leaves under different salt conditions, overview [10]; <1> effects of increased H<sub>2</sub>O<sub>2</sub> production on the expression of enzymes involved in the

antioxidant machinery, overview [7]; <2>  $H_2O_2$  is the co-substrate for the peroxidase-driven reactions during cell-wall maturation and a key signalling molecule in defence mechanisms [14]; <1> involvement of polyamine oxidase in abscisic acid-induced cytosolic antioxidant defense in leaves of maize. MPAO contributes to abscisic acid-induced cytosolic antioxidant defense through  $H_2O_2$ , a spermidine catabolic product, overview [11]) (Reversibility: ?) [7,10,11,14]

P ?

### Substrates and products

S  $N^1$ -acetylspermidine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [1]

P ?

S  $N^1$ -acetylspermine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [1]

P 1,3-diaminopropane +  $H_2O_2$  + ?

S  $N^8$ -acetylspermine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [1]

P 1,3-diaminopropane +  $H_2O_2$  + ?

S spermidine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [1]

P 1,3-diaminopropane +  $H_2O_2$  + ?

S spermidine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [3,4,8]

P ?

S spermidine +  $O_2$  +  $H_2O$  <1> (<1> best substrate [11]; <1> enzyme activity is measured spectrophotometrically by following the formation of a pink adduct resulting from the  $H_2O_2$ -dependent oxidation of 4-aminoantipyrine catalyzed by horseradish peroxidase and the subsequent condensation of oxidized 4-aminoantipyrine with 3,5-dichloro-2-hydroxybenzenesulfonic acid [12]) (Reversibility: ?) [7,10,11,12]

P propane-1,3-diamine + 4-aminobutanal +  $H_2O_2$

S spermidine +  $O_2$  +  $H_2O$  <1,2> (<1,2> preferred substrate [14]) (Reversibility: ?) [13,14]

P 1,3-diaminopropane + 4-aminopropanal +  $H_2O_2$

S spermine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [1]

P 1,3-diaminopropane +  $H_2O_2$  + ?

S spermine +  $O_2$  +  $H_2O$  <1> (Reversibility: ?) [10]

P spermidine + propane-1,3-diamine +  $H_2O_2$

S spermine +  $O_2$  +  $H_2O$  <2> (Reversibility: ?) [13,14]

P 1,3-diaminopropane + aminoaldehyde +  $H_2O_2$

S spermine +  $O_2$  +  $H_2O$  <3> (Reversibility: ?) [4,8]

P ?

S Additional information <1,2,3> (<3> no activity with: acetylputrescine, acetylcadaverine [1]; <1> analysis of polyamine content in leaves under different salt conditions, overview [10]; <1> effects of increased  $H_2O_2$  production on the expression of enzymes involved in the antioxidant machinery, overview [7]; <2>  $H_2O_2$  is the co-substrate for the peroxidase-driven reactions during cell-wall maturation and a key signalling molecule in defence mechanisms [14]; <1> involvement of polyamine oxidase in abscisic acid-induced cytosolic antioxidant defense in leaves of maize. MPAO contributes to abscisic acid-induced cytosolic antioxidant defense

through H<sub>2</sub>O<sub>2</sub>, a spermidine catabolic product, overview [11]) (Reversibility: ?) [1,7,10,11,14]

**P** ?

### Inhibitors

- (N<sup>1</sup>-5-aminopentyl)-N<sup>3</sup>-(cyclohexylethyl)-N<sup>1</sup>,N<sup>2</sup>,N<sup>3</sup>-tris(tert-butoxycarbonyl)guanidine <1> [12]  
 1,10-diaminododecane <3> [3]  
 1,12-diaminododecane <3> [3]  
 1,19-bis(ethylamino)-5,10,15-triazanonadecane <2> (<2> i.e. SL-11061, 68% inhibition at 0.5 mM [13]) [13]  
 1,3-diaminopropane <3> [3]  
 1,4-diaminobutane <3> [3]  
 1,5-diaminopentane <3> [3]  
 1,6-diaminohexane <3> [3]  
 1,7-diaminoheptane <3> [3]  
 1,8-diaminooctane <1,3> [3,10,12]  
 1,9-diaminononane <3> [3]  
 1-(4-aminobutyl)-3-(4-fluorobenzyl)guanidine <1,3> (<1> competitive inhibition of spermidine oxidation [12]) [6,12]  
 1-(4-aminobutyl)-3-but-3-en-1-ylguanidine <1> (<1> competitive inhibition of spermidine oxidation [12]) [12]  
 1-(4-aminobutyl)-3-but-3-yn-1-ylguanidine <1> (<1> competitive inhibition of spermidine oxidation [12]) [12]  
 1-(4-aminobutyl)-3-prop-2-en-1-ylguanidine <3> [6]  
 1-(4-aminobutyl)-3-prop-2-yn-1-ylguanidine <3> [6]  
 1-(4-carbamimidamidobutyl)-3-(3-methylbut-2-en-1-yl)guanidine (non-preferred name) <1> [12]  
 1-(5-aminopentyl)-3-(2-cyclohexylethyl)guanidine <1> [12]  
 1-(5-aminopentyl)-3-(2-cyclopropylethyl)guanidine <1> [12]  
 1-(5-aminopentyl)-3-(3-methoxybenzyl)guanidine <1> [12]  
 1-(5-aminopentyl)-3-(3-methylbut-2-en-1-yl)guanidine <3> [6]  
 1-(5-aminopentyl)-3-(4-methylpent-3-en-1-yl)guanidine <1> (<1> competitive inhibition of spermidine oxidation [12]) [12]  
 1-(5-aminopentyl)-3-[(2E)-3-phenylprop-2-en-1-yl]guanidine <1> [12]  
 1-(6-aminohexyl)-3-(3-methylbut-2-en-1-yl)guanidine <3> [6]  
 1-(6-aminohexyl)-3-(4-methylpent-3-en-1-yl)guanidine <1> (<1> competitive inhibition of spermidine oxidation [12]) [12]  
 1-(guanidino)-17-(N<sup>1</sup>-(γ,γ-dimethylallyl)guanidino)-9-azaheptadecane tris(trifluoroacetate) <1> [12]  
 1-[3-[(3-aminopropyl)amino]propyl]-3-(3-methylbut-2-en-1-yl)guanidine <1> [12]  
 1-[7-[(9-carbamimidamidononyl)amino]heptyl]-3-(2-cyclopropylethyl)guanidine <1> [12]  
 1-[7-[(9-carbamimidamidononyl)amino]heptyl]-3-(2-phenylethyl)guanidine <1> [12]

1-[7-[(9-carbamimidamidonyl)amino]heptyl]-3-(3-methylbut-3-en-1-yl)-guanidine <1> [12]  
 3-(4-methylpent-3-en-1-yl)-1-[9-[(7-[(4-methylpent-3-en-1-yl)carbamimidamido]heptyl)amino]nonyl]guanidine <1> [12]  
 3-[(2E)-but-2-en-1-yl]-1-[7-[(9-carbamimidamidonyl)amino]heptyl]guanidine <1> [12]  
 3-but-3-yn-1-yl-1-[7-[(9-carbamimidamidonyl)amino]heptyl]guanidine <1> [12]  
 $\Delta^1$ -pyrroline <3> (<3> competitive [8]) [8]  
 N,N''-butane-1,4-diylbis[3-(3-methylbut-2-en-1-yl)guanidine] <1,3> (<1> competitive inhibition of spermidine oxidation [12]) [6,12]  
 N,N'-bis(2,3-butadienyl)-1,4-butane-diamine <3> (<3> i.e. MDL72527 [9]) [9]  
 N,N'-diaminoguanidine <2> (<2> about 25% inhibition at 0.5 mM [13]) [13]  
 N-prenyl agmatine <1> [12]  
 N-prenylagmatine <1> (<1> i.e. G3, a specific and selective ZmPAO inhibitor. G3 strongly inhibits lignin and suberin polyphenolic domain deposition along the wound periderm in maize mesocoty [14]) [14]  
 N<sup>1</sup>,N<sup>2</sup>-bis(tert-butoxycarbonyl)-N<sup>1</sup>-(cyclohexylethyl)-S-methylisothiourea <1> [12]  
 N<sup>1</sup>,N<sup>2</sup>-bis(tert-butoxycarbonyl)-N<sup>1</sup>-( $\gamma,\gamma$ -dimethylallyl)-S-methylisothiourea <1> [12]  
 N<sup>1</sup>,N<sup>2</sup>-bis(tert-butoxycarbonyl)-N<sup>1</sup>-( $\gamma,\gamma$ -methylallyl)-S-methylisothiourea <1> [12]  
 N<sup>1</sup>-(3-methoxybenzyl)-N<sup>3</sup>-(5-aminopentyl)-N<sup>2</sup>,N<sup>3</sup>,N<sup>4</sup>-tris(tert-butoxycarbonyl)guanidine <1> [12]  
 N<sup>1</sup>-[(30-aminopropyl)-3-aminopropyl]-N<sub>3</sub>-( $\gamma,\gamma$ -dimethylallyl)-N<sup>2</sup>,N<sup>3</sup>-bis(tert-butoxycarbonyl)guanidine <1> [12]  
 N<sup>1</sup>-acetyl-3-aminopropyl-4-aminobutanal <3> (<3> competitive [1]) [1]  
 N<sup>1</sup>-acetylspermine <3> (<3> non-competitive [1]; <3> poor competitive inhibitor [8]) [1,8]  
 N<sup>1</sup>-benzylamine-N<sup>3</sup>-( $\gamma,\gamma$ -dimethylallyl)-N<sup>2</sup>,N<sup>3</sup>,N<sup>4</sup>-tris(tert-butoxycarbonyl)-guanidine <1> [12]  
 N<sup>1</sup>-benzylamine-N<sup>3</sup>-( $\gamma,\gamma$ -dimethylallyl)guanidine bis-(trifluoroacetate) <1> [12]  
 SL-11061 <1> (<1> i.e. 1,19-bis-(ethylamine)-5,10,15 triazanonadecane [10]) [10]  
 agmatine <1> (<1> competitive inhibition of spermidine oxidation [12]) [12]  
 agmatinec <3> [5]  
 diazabicyclononane <3> (<3> competitive [8]) [8]  
 diphenylene iodonium <1> (<1> slight inhibition of PAO [10]) [10]  
 guazatine <2,3> (<2> 75% inhibition at 0.5 mM [13]) [5,13]  
 iminooctadine <1> [12]  
 prenylagmatine <3> [5]  
 tert-butyl (2E)-but-2-en-1-yl[(E)-[(tert-butoxycarbonyl)imino](methylsulfonyl)methyl]carbamate <1> [12]  
 tert-butyl (4-[(tert-butoxycarbonyl)[(E)-[(tert-butoxycarbonyl)imino](methylsulfonyl)methyl]amino]butyl)methylcarbamate <1> [12]

tert-butyl (6-aminohexyl)[(tert-butoxycarbonyl)(cyclopropylmethyl)carbami-  
midoyl]carbamate <1> [12]

tert-butyl (6-aminohexyl)[(tert-butoxycarbonyl)[(3E)-4-phenylbut-3-en-1-yl]-  
carbamiimidoyl]carbamate <1> [12]

tert-butyl [(1E)-[(tert-butoxycarbonyl)(cyclopropylmethyl)amino](methylsul-  
fanyl)methylidene]carbamate <1> [12]

tert-butyl [(E)-[(tert-butoxycarbonyl)imino](methylsulfanyl)methyl][(2E)-3-  
phenylprop-2-en-1-yl]carbamate <1> [12]

tert-butyl [(E)-[(tert-butoxycarbonyl)imino](methylsulfanyl)methyl]prop-2-  
yn-1-ylcarbamate <1> [12]

tert-butyl benzyl[(E)-[(tert-butoxycarbonyl)imino](methylsulfanyl)methyl]car-  
bamate <1> [12]

Additional information <1,3> (<3> not diaminopropane, H<sub>2</sub>O<sub>2</sub> or in combina-  
tion [1]; <3> docking simulations carried out with the charged and uncharged  
forms of MPAO inhibitors indicate that the stereoelectronic properties of the  
MPAO active site are consistent with the binding of inhibitors in the protonated  
form, a feature which can shed light on the still obscure MPAO catalytic mech-  
anism [3]; <3> no inhibition by 1,3-diaminopropane (1 mM) and H<sub>2</sub>O<sub>2</sub>  
(1 mM) [8]; <1> computational structure-function analysis of inhibitors, over-  
view [12]; <1> no inhibition by 1,3-diaminopropane [10]) [1,3,8,10,12]

### Cofactors/prosthetic groups

FAD <1,2,3> (<3> oxidized FAD is the prominent form during steady-state  
turnover, consistent with the reductive half-reaction being rate-limiting [4];  
<3> the FAD prosthetic group is non-covalently bound to the protein and is  
deeply embedded within the structure. All FAD atoms are solvent-inaccessi-  
ble, with the exception of the flavin C5a, N5 and C4a atoms that line the  
active centre [9]) [4,7,9,14]

### Metals, ions

NaCl <1> (<1> salt increases PAO activity [10]) [10]

### Turnover number (s<sup>-1</sup>)

0.004 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme K300M [4]) [4]

0.0053 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme K300M [4]) [4]

2.8 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme E62Q [4]) [4]

4.6 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme E170Q [4]) [4]

7.7 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme Y298F [4]) [4]

8.3 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme E62Q [4]) [4]

16.5 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme Y298F [4]) [4]

17.3 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme E170Q [4]) [4]

32.9 <3> (spermine, <3> pH 6.0, 25°C, wild-type enzyme [4]) [4]

39.3 <3> (spermine, <3> pH 6.0, 25°C, native enzyme [4]) [4]

50.2 <3> (spermidine, <3> pH 6.0, 25°C, wild-type enzyme [4]) [4]

55.1 <3> (spermidine, <3> pH 6.0, 25°C, native enzyme [4]) [4]

### Specific activity (U/mg)

Additional information <1> (<1> PAO activity in unsalinized and salt-trea-  
ted plants, overview [10]) [10]

**K<sub>m</sub>-Value (mM)**

- 0.0007 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme Y298F [4]) [4]  
 0.0012 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme Y298F [4]) [4]  
 0.0016 <3> (spermine, <3> pH 6.0, 25°C, wild-type enzyme [4]) [4]  
 0.0017 <3> (spermidine, <3> pH 6.0, 25°C, native enzyme [4]) [4]  
 0.0017 <3> (spermine, <3> pH 6.0, 25°C, native enzyme [4]) [4]  
 0.0021 <3> (spermidine, <3> pH 6.0, 25°C, wild-type enzyme [4]) [4]  
 0.0125 <3> (spermine, <3> pH 6.0, 25°C, mutant enzyme E170Q [4]) [4]  
 0.0147 <3> (Spermine, <3> pH 6.0, 25°C, mutant enzyme E62Q [4]) [4]  
 0.0167 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme E170Q [4]) [4]  
 0.0177 <1> (spermine, <1> pH 6.5, 30°C [10]) [10]  
 0.0246 <3> (spermidine, <3> pH 6.0, 25°C, mutant enzyme E62Q [4]) [4]  
 0.038 <3> (spermine) [1]  
 0.04 <3> (spermidine) [1]  
 0.062 <3> (N<sup>1</sup>-acetylspermine) [1]  
 0.274 <3> (N<sup>1</sup>-acetylspermidine) [1]  
 1.13 <3> (N<sup>8</sup>-acetylspermine) [1]

**K<sub>i</sub>-Value (mM)**

- 0.00000008 <1> (1-[7-[(9-carbamimidamidononyl)amino]heptyl]-3-(2-cyclopropylethyl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00000005 <1> (1-[7-[(9-carbamimidamidononyl)amino]heptyl]-3-(3-methylbut-3-en-1-yl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00000007 <1> (3-but-3-yn-1-yl-1-[7-[(9-carbamimidamidononyl)amino]heptyl]guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.0000001 <1> (1-[7-[(9-carbamimidamidononyl)amino]heptyl]-3-(2-phenylethyl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00000011 <1> (3-[(2E)-but-2-en-1-yl]-1-[7-[(9-carbamimidamidononyl)amino]heptyl]guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00000017 <1> (3-(4-methylpent-3-en-1-yl)-1-[9-[(7-[(4-methylpent-3-en-1-yl)-carbamimidamido]heptyl]amino)nonyl]guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.0000003 <1> (1-(guanidino)-17-(N<sup>1</sup>-(γ,γ-dimethylallyl)guanidino)-9-azaheptadecane tris(trifluoroacetate), <1> pH 6.5, 25°C [12]) [12]  
 0.00000075 <3> (guazatine) [5]  
 0.00000075 <1> (iminooctadine, <1> pH 6.5, 25°C [12]) [12]  
 0.00001 <3> (1-(5-aminopentyl)-3-(3-methylbut-2-en-1-yl)guanidine) [6]  
 0.00001 <1> (1-(5-aminopentyl)-3-(4-methylpent-3-en-1-yl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.000015 <1> (N-prenylagmatine, <1> pH 6.5, 25°C [12]) [12]  
 0.000015 <3> (prenylagmatine) [5]  
 0.000022 <1> (1-(6-aminohexyl)-3-(4-methylpent-3-en-1-yl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00013 <1> (1-(4-aminobutyl)-3-but-3-en-1-ylguanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00013 <3> (1-(4-aminobutyl)-3-prop-2-en-1-ylguanidine) [6]  
 0.00017 <3> (1,12-diaminododecane, <3> pH 6.0, 25°C [3]) [3]  
 0.00022 <3> (1-(6-aminohexyl)-3-(3-methylbut-2-en-1-yl)guanidine) [6]

- 0.00025 <1> (1-(4-aminobutyl)-3-but-3-yn-1-ylguanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00025 <3> (1-(4-aminobutyl)-3-prop-2-yn-1-ylguanidine) [6]  
 0.0003 <1,3> (1,8-diaminooctane, <1> pH 6.5, 25°C [12]; <3> pH 6.0, 25°C [3]) [3,12]  
 0.0004 <3> (1,7-diaminoheptane, <3> pH 6.0, 25°C [3]) [3]  
 0.00055 <3> (N,N'-bis(2,3-butadienyl)-1,4-butane-diamine, <3> pH 6.5, 25°C [9]) [9]  
 0.0006 <3> (1,10-diaminodecane, <3> pH 6.0, 25°C [3]) [3]  
 0.00063 <1,3> (1-(4-aminobutyl)-3-(4-fluorobenzyl)guanidine, <1> pH 6.5, 25°C [12]) [6,12]  
 0.0007 <1> (1-(4-carbamimidamidobutyl)-3-(3-methylbut-2-en-1-yl)guanidine (non-preferred name), <1> pH 6.5, 25°C [12]) [12]  
 0.00115 <1> (1-(5-aminopentyl)-3-(3-methoxybenzyl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.0012 <3> (1,9-diaminononane, <3> pH 6.0, 25°C [3]) [3]  
 0.00121 <1> (1-[3-[(3-aminopropyl)amino]propyl]-3-(3-methylbut-2-en-1-yl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.0015 <1,3> (N,N''-butane-1,4-diylbis[3-(3-methylbut-2-en-1-yl)guanidine], <1> pH 6.5, 25°C [12]) [6,12]  
 0.00153 <1> (1-(5-aminopentyl)-3-[(2E)-3-phenylprop-2-en-1-yl]guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00172 <1> (1-(5-aminopentyl)-3-(2-cyclohexylethyl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.00258 <1> (1-(5-aminopentyl)-3-(2-cyclopropylethyl)guanidine, <1> pH 6.5, 25°C [12]) [12]  
 0.003 <3> (1,6-diaminohexane, <3> pH 6.0, 25°C [3]) [3]  
 0.003 <1,3> (agmatine, <1> pH 6.5, 25°C [12]) [5,12]  
 0.018 <1> (N<sup>1</sup>-benzylamine-N<sup>3</sup>-( $\gamma,\gamma$ -dimethylallyl)guanidine bis-(trifluoroacetate), <1> pH 6.5, 25°C [12]) [12]  
 0.038 <3> (1,5-diaminopentane, <3> pH 6.0, 25°C [3]) [3]  
 0.1 <3> (1,3-diaminopropane, <3> pH 6.0, 25°C [3]) [3]  
 0.1 <3> (diazabicyclononane) [8]  
 0.13 <3> (1,4-diaminobutane, <3> pH 6.0, 25°C [3]) [3]  
 0.4 <3> ( $\Delta^1$ -pyrroline) [8]

**pH-Optimum**

- 5 <3> [8]  
 6.5 <1,3> (<1> assay at [12]) [1,10,12]  
 7.8 <1> (<1> assay at [7]) [7]

**Temperature optimum (°C)**

- 25 <1> (<1> assay at [12]) [12]  
 30 <1> (<1> assay at [10]) [10]

## 4 Enzyme Structure

### Posttranslational modification

flavoprotein <1,2> [7,14]

## 5 Isolation/Preparation/Mutation/Application

### Source/tissue

leaf <1,2> (<1> apoplast [10,11]) [7,10,11,12,13,14]

mesocotyl <1> [14]

seedling <3> (<3> etiolated [2]) [2,4]

shoot <1,3> (<1> apical meristem [7]) [7,8]

### Localization

apoplast <1,2> [10,11,13,14]

cell wall <2,3> [1,14]

extracellular <1> [10,11]

### Purification

<1> (native enzyme from apoplastic fluids of leaf blade segments) [10]

<3> [1,4,8]

### Crystallization

<3> (crystallised by the hanging drop vapour-diffusion method, with the protein solution consisting of 5 mg enzyme/ml in 300 mM NaCl and 50 mM sodium phosphate buffer, pH 6.0. Crystal structure of polyamine oxidase is determined to a resolution of 1.9 Å. The enzyme contains two domains, which define a remarkable 30 Å long U-shaped catalytic tunnel at their interface. The structure of PAO in complex with the inhibitor MDL72527 reveals the residues forming the catalytic machinery and unusual enzyme-inhibitor CH-OH bonds. A ring of glutamate and aspartate residues surrounding one of the two tunnel openings contributes to the steering of the substrate towards the inside of the tunnel) [9]

### Cloning

<1> (ZmPAO overexpression in tobacco cell wall greatly accelerates the phenomenon in wounded tobacco stem, that enzyme inhibition inhibits lignin and suberin polyphenolic domain deposition along the wound periderm in maize mesocoty) [14]

<1> (overexpression of PAO in transgenic *Nicotiana tabacum* plants leading to dramatically increased expression levels of Mpao and high 1,3-diaminopropane content in the tobacco plant leaves, stems, and roots) [7]

<3> [2]

<3> (expression in *Pichia pastoris*, wild-type and mutant enzymes) [4]

<3> (transgenic tobacco plants overexpressing polyamine oxidase from *Zea mays* exhibit high 1,3-diaminopropane content) [7]



## Engineering

E170Q <3> (<3> mutation results in moderate change of enzyme activity and apparent  $K_m$ -values [4]) [4]

E62Q <3> (<3> mutation results in moderate change of enzyme activity and apparent  $K_m$ -values [4]) [4]

K300M <3> (<3> mutation results in a 1400fold decrease in the rate of flavin reduction and a 160fold decrease in the equilibrium dissociation constant for the K300M-spermidine complex, consistent with a major role for this residue in the mechanism of substrate oxidation [4]) [4]

Y298F <3> (<3> specific activity or  $K_M$ -values are not substantially altered [4]) [4]

Additional information <1> (<1> transgenic Mpao overexpressing tobacco plants show highly increased enzyme activity and 1,3-diaminopropane levels, also specific isoforms of the antioxidant machinery, i.e. peroxidase, superoxide dismutase and catalase, are induced in the transgenics but not in the wild-type, along with increase in activities of additional enzymes contributing to redox homeostasis. Nevertheless, further increase in the intracellular reactive oxygen species by exogenous  $H_2O_2$ , or addition of methylviologen or menadione to transgenic leaf discs, results in oxidative stress as evidenced by the lower quantum yield of PSII, the higher ion leakage, lipid peroxidation and induction of programmed cell death, overview [7]) [7]

## 6 Stability

### pH-Stability

5 <3> (<3> single two-state transition at pH 6 with  $T_m$  49.8°C. At pH 5 the thermal stability is increased by more than 14°C [8]) [8]

### Temperature stability

50 <3> (<3> single two-state transition at pH 6 with  $T_m$  49.8°C. At pH 5 the thermal stability is increased by more than 14°C.  $\Delta^1$ -pyrroline and diazabicyclononane improve the thermal stability of the enzyme [8]) [8]

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