Alanine racemase 5.1.1.1

1 Nomenclature

EC number
5.1.1.1

Systematic name
Alanine racemase

Recommended name
Alanine racemase

Synonyms
l-Alanine racemase
l-Alanine:D-alanine racemase
Racemase, alanine

CAS registry number
9024-06-0

2 Source Organism

<1> Streptococcus faecalis (O-carbamoyl-d-Ser-resistant mutant [6]) [1,6,24]
<2> Bacillus subtilis [2,3,10,24]
<3> Salmonella typhimurium (overproducing strain [7]; encoded by the dadB and alr gene [11]; encoded by alr and dadB gene [14]) [4,5,7,10,11,14,15,24,31]
<4> Bacillus stearothermophilus [8-12,14-18,20,21,23-25,30]
<5> Pseudomonas aeruginosa (strain A237) [13]
<6> Staphylococcus sp. [14]
<7> E. coli (strain B [28]) [14,22,28,29]
<8> Pseudomonas fluorescens [19,25]
<9> Bacillus sp. (strain YM-1) [21]
<10> Pseudomonas putida [24]
<11> Penaeus monodon [26]
<12> Tolypocladium niveum [27]
<13> Serratia marcescens [29]
3 Reaction and Specificity

Catalyzed reaction

$\text{l-Ala} = \text{d-Ala}$ (<2>, the active site of the alanine racemase reacts asymmetrically with the enantiomers of the substrate and has a conformation which greatly favors the d-enantiomer [3]; <4>, the alanine racemase builds two different bases in the active site. The base for d-Ala may be closer to the enzyme surface, and that for l-Ala inside [18])

Reaction type

Racemization

Natural substrates

l-Ala <3,4,7,9> (<3,4,9>, enzyme provides d-Ala as an essential building block for biosynthesis of the peptidoglycan layer of the cell wall) [11,15,21,28] Additional information <3,12> (<3>, two nonhomologous alanine racemase genes, one of which is associated with the catabolic function and the other of which presumably represents the biosynthetic function [4]; <3>, alr racemase is constitutive and serves an anabolic function, dadB encoded enzyme is inducible and required for cell growth on l-Ala [14,24]; <12>, key enzyme in cyclosporin A biosynthesis [27]) [4,14,24,27]

Substrates and products

$S$ L-Ala <1-13> (<1,4>, specific for Ala [1,8]) [1-31]

$P$ D-Ala <1-13> [1-31]

S Additional information <2> (<2>, exchange of the $\alpha$-hydrogen of d-Ala and l-Ala with D$_2$O) [2]

P ?

Inhibitors

(1-Aminoethyl)boronic acid <4> [16]
(1-Aminoethyl)phosphonate <1,4> (<1>, d- and L-(1-aminoethyl)phosphonate [6]) [6,12]
2-Amino-3-chlorobut-3-enoic acid <7> (<7>, i.e. 3-chlorovinylglycine, irreversible [22]) [22,28]
2-Amino-3-fluorobut-3-enoic acid <7> (<7>, i.e. 3-fluorovinylglycine, irreversible [22]) [22,28]
$\text{d-Chloroalanine} <2,3> <$2>, Ki: 0.005 mM, competitive [3]) [3,7]
$d$-O-Acetylseryline <3> [7]
$d$-$\beta$-Fluoroalanine <3> [7]
FAD <11> (slight activation at low concentrations, inhibition at high concentrations) [26]
$L$-Chloroalanine <2,3> (<2>, Ki: 1.71 mM, noncompetitive [3]) [3,7]
$L$-$\beta$-Chloroalanine <3> [7]
NaCl <11> (<11>, slight inhibition above 600 mM) [26]
O-Carbamoyl-d-Ser <1> (<1>, inhibition of wild type enzyme but not of the O-carbamoyl-d-Ser mutant) [6]
Pyridoxal 5'-phosphate <11> (<11>, slight activation at low concentrations, inhibition at high concentrations) [26]
Pyruvate <11> [26]
Vinylglycine <5> [13]
β,β,β-Trifluoroalanine <3,4> (<3,4>, nucleophilic attack of Lys38 on the electrophilic β-difluoro-α,β-unsaturated imine) [15]

**Cofactors / prosthetic groups / activating substances**

FAD <11> (<11>, not required as cofactor, slight activation at low concentrations, inhibition at high concentrations) [26]
Glutathione <1> (<1>, required for maximal activity) [1]
Pyridoxal 5'-phosphate <1,3,4,8,12> (<1-4>, pyridoxal 5'-phosphate dependent enzyme [11,24]; <1,12>, required as coenzyme [1,27]; <3>, 1 mol of pyridoxal 5'-phosphate is bound per subunit [5]; <1>, 1 pyridoxal 5'-phosphate per 42000 MW subunit [6]; <3>, contains one mol of pyridoxal 5'-phosphate per mol of enzyme [7]; <3>, the sequence of 10 amino acid residues around the Lys residue, to which pyridoxal 5'-phosphate is bound, is identical with that of the dadB racemase [7]; <3>, Kₘ: 0.000033 mM [7]; <4, 8>, 2 mol of pyridoxal 5'-phosphate bound per mol of enzyme dimer [8,19]; <4>, the monomeric inactive enzyme appears to bind the cofactor pyridoxal 5'-phosphate by a non-covalent linkage, although the native dimeric enzyme binds the cofactor through an aldimine Schiff base linkage [17]; <1-4>, pyridoxal 5'-phosphate binds to Lys of the enzyme protein and forms an aldimine Schiff base. The α-proton of the substrate is then abstracted, and the pyridoxal 5'-phosphate carbanion is generated [24]; <11>, not required as cofactor, slight activation at low concentrations, inhibition at high concentrations [26]; <4>, Arg219 forms a hydrogen bond with the pyridine nitrogen of the cofactor, Arg136 donates a hydrogen bond to the phenolic oxygen of pyridoxal 5'-phosphate and may be involved in the binding of substrate as well as stabilization of intermediates [30]) [1,5-8,11,17,19,24,26,27,30]

**Turnover number (min⁻¹)**
66 <4> (l-Ala, <4>) [11]
96 <3> (d-Ala, dadB encoded enzyme, <3>) [11]
156 <3> (d-Ala, alr encoded enzyme, <3>) [11]
420 <4> (d-Ala, <4>) [11]
438 <3> (l-Ala, dadB encoded enzyme, <3>) [11]
582 <3> (l-Ala, alr encoded enzyme, <3>) [11]

**Specific activity (U/mg)**
22 <3> [7]
133 <7> [28]
143 <12> [27]
567 <11> [26]
587 <8> [19]
910 <3> [5]
2920 <1> [6]

Additional information <1> [1]
**Kₘ-Value (mM)**

- 0.5 <3> (d-Ala, alr gene encoded, <3>) [11]
- 1.7 <3> (l-Ala, alr gene encoded, <3>) [11]
- 2 <3,12> (d-Ala, <3,12>) [7,27]
- 2.1 <3> (d-Ala, <3>) [5]
- 2.2 <1,3> (d-Ala, <1> [6]; d-Ala, dadB encoded enzyme, <3> [11]) [6,11]
- 2.7 <3> (l-Ala, <3> [7]; d-Ala, <4> [8,11]) [7,8,11]
- 4.25 <4> (l-Ala, <4>) [8]
- 4.4 <4> (l-Ala, <4>) [11]
- 7.8 <1> (l-Ala, <1>) [6]
- 8.2 <3> (l-Ala, <3>) [5]
- 8.5 <1> (Ala, <1>) [1]
- 11 <3> (l-Ala, dadB gene encoded, <3>) [11]
- 12.8 <8> (d-Ala, <8>) [19]
- 18.9 <8> (l-Ala, <8>) [19]
- 119 <11> (d-Ala, <11>) [26]

Additional information <4,8><4,8>, Kₘ values of l-Ala in the presence of urea at various concentrations) [25]

**pH-Optimum**

- 8.3 <8> [19]
- 8.5 <1,3,11> [1,7,26]
- 8.8 <12> (<12>, l-Ala) [27]
- 9 <1> [6]
- 9.5 <12> (<12>, d-Ala) [27]

**pH-Range**

7–9.5 <11> (<11>, 7.0: about 45% of maximal activity, 9.5: about 95% of maximal activity) [26]

**Temperature optimum (°C)**

- 30 <8> [19]
- 35–40 <11> [26]
- 42 <12> (<12>, l-Ala) [27]

**Temperature range (°C)**

0–40 <8> (<8>, 0 C: about 25% of maximal activity, 40 C: about 45% of maximal activity) [19]
- 20–50 <11> (<11>, 20 C: about 60% of maximal activity, 50 C, about 35% of maximal activity) [26]

### 4 Enzyme Structure

**Molecular weight**

- 43000 <3> (<3>, gel filtration) [7]
- 50000 <3> (<3>, gel filtration) [5]
- 67000 <1> (<1>, gel filtration) [6]
5.1.1.1 Alanine racemase

76000 <8> (<8>, gel filtration in presence or absence of 2-mercaptoethanol) [19]
78000 <4> (<4>, equilibrium sedimentation method) [8]
85000 <11> (<11>, HPLC gel filtration) [26]
120000–150000 <12> (<12>, gel filtration) [27]

Subunits
? <1,4,12> (<1>, x * 42000, SDS-PAGE [6]; <4>, x * 43341, calculation from nucleotide sequence [10,20]; <12>, 3 or 4 * 37000, SDS-PAGE [27]) [6,10,20,27]
Dimer <4,8> (<4>, 2 * 39000, SDS-PAGE [8]; <4> [14,24]; <8>, 2 * 38000, SDS-PAGE [19]) [8,14,19,24]
Monomer <1,3> (<3>, 1 * 39000, SDS-PAGE [5]; <3>, 1 * 39044, calculation from nucleotide sequence [5]; <3>, 1 * 42000, SDS-PAGE [7]; <3> [14]; <3>, dadB enzyme and alr enzyme [24]; <1> [24]) [5,7,14,24]

5 Isolation/Preparation/Mutation/Application

Source/tissue
Muscle <11> [26]

Purification
<1> [1,6]
<3> (enzyme encoded by the dadB gene [5]; alr gene encoded [7]) [5,7]
<4> (enzyme overproduced in E. coli W3110 lacIq [9]) [8,9,20]
<7> [28]
<8> [19]
<11> (partial) [26]
<12> [27]

Renaturation
<4> (enzyme denatured in 6 M guanidine hydrochloride is renatured either by dialysis or dilution to reduce the guanidine hydrochloride concentration) [17]

Crystallization
<4> [9,30]

Cloning
<3> (two genes: dadA and dadB [4]; dadB [5]) [4,5]
<4> (expression in E. coli C600 [8]; 31-54% sequence homologies with Bacillus subtilis and Salmonella typhimurium dadB and alr enzymes [10]; expression in E. coli [20,21]) [8,10,20,21]

Engineering
Additional information <3,4> (<3>, double mutant for the alr encoded enzyme and the dadB encoded enzyme display a phenotype of requirement for exogenous \( \delta \)-Ala for growth [14,24]; <4>, mutant gene which tandemly encodes the two polypeptides of the enzyme subunit, fragment 1 and fragment
2, cleaved at the position corresponding to the predicted hinge region. The mutant fragmentary alanine racemase is active at about 40% of the activity of the wild type enzyme [21,23,24]) [14,21-24]

6 Stability

pH-Stability
7.2 <3> (<3>, 80 C, stable) [24]
8.3–10.5 <4,8> (<4,8>, 1 h, 0 C, stable) [25]

Temperature stability
20 <8> (<8>, 1 h, stable) [19]
30 <8> (<8>, over 30 C, 1 h, quick loss of activity [19,25]; <8>, 5 min, about 50% loss of activity after incubation with 0.08% SDS, 1 M guanidine hydrochloride, 4 M urea, 45% ethanol or 50% dimethyl sulfoxide [25]; <4>, 5 min, resistant to incubation with 0.08% SDS, 1 M guanidine hydrochloride, 4 M urea, 45% ethanol or 50% dimethyl sulfoxide [25]) [19,25]
40–50 <11> (<11>, pH 8.0, 5 min, sharp decrease of activity between 40 C and 50 C) [26]
60 <11> (<11>, pH 8.0, 5 min, almost complete inactivation) [26]
70 <3> (<3>, 80 min, pH 7.2, stable) [24]
75 <4> (<4>, 1 h, inactivation over 75 C) [25]

General stability information
<3>, NaB3H4-reduced DadB holoenzyme is resistant to α-chymotrypsin and trypsin and is labile only towards subtilisin [31]
<3>, stable in 30% ammonium sulfate. Irreversibly diminished activity by exposure to ammonium sulfate concentrations near or above 40%, where precipitation occurs [5]
<4>, 5 min, resistant to incubation with 0.08% SDS, 1 M guanidine hydrochloride, 4 M urea, 45% ethanol or 50% dimethyl sulfoxide [25]
<4>, denatured by 3.5 M urea in one transition phase [25]
<4>, in 0.6 M to 1.5 M guanidine hydrochloride the dimeric enzyme is dissociated into a monomeric form, which is catalytically inactive [17,21]
<8>, 5 min, about 50% loss of activity after incubation with 0.08% SDS, 1 M guanidine hydrochloride, 4 M urea, 45% ethanol or 50% dimethyl sulfoxide [25]
<8>, denatured by urea in two transition phases, dissociation of pyridoxal 5'-phosphate with 4.0 M urea, unfolding with 5.5 M urea [25]

Storage stability
<3>, -70°C, 10% glycerol, more than 90% of the activity is retained after 2 years [5]
<8>, -20°C, stable for 1 month [19]
<8>, 4°C, stable for 1 week [19]
<11>, enzyme loses about 20% of activity during storage for 20 days at -20°C, 0°C and 5°C [26]
References

Springer Handbook of Enzymes
Schomburg, D.; Schomburg, I. (Eds.)