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

\[ \text{L-Ala} = \text{D-Ala} \]  
(\text{<2>, the active site of the alanine racemase reacts asymmetri-

ically with the enantiomers of the substrate and has a confor-

mation 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

\text{L-Ala} <3,4,7,9> (\text{<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> (\text{<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

\text{S} \text{L-Ala } <1-13> (\text{<1,4>, specific for Ala [1,8]) [1-31]}
\text{P} \text{D-Ala } <1-13> [1-31]
\text{S} \text{Additional information } <2> (\text{<2>, exchange of the } \alpha \text{-hydrogen of D-Ala
and L-Ala with D}_2\text{O}) [2]
\text{P} ?

Inhibitors

(1-Aminoethyl)boronic acid <4> [16]
(1-Aminoethyl)phosphonate <1,4> (\text{<1>, D- and L-(1-aminoethyl)phospho-

nate [6]) [6,12]
2-Amino-3-chlorobut-3-enoic acid <7> (\text{<7>, i.e. 3-chlorovinylglycine, irre-

versible [22]) [22,28]
2-Amino-3-fluorobut-3-enoic acid <7> (\text{<7>, i.e. 3-fluorovinylglycine, irre-

versible [22]) [22,28]
D-Chloroalanine <2,3> (\text{<2>, Ki: 0.005 mM, competitive [3]} [3,7]
D-O-Acetylserine <3> [7]
D-\(\beta\)-Fluoroalanine <3> [7]
FAD <11> (slight activation at low concentrations, inhibition at high concen-

trations) [26]
L-Chloroalanine <2,3> (\text{<2>, Ki: 1.71 mM, noncompetitive [3]} [3,7]
L-\(\beta\)-Chloroalanine <3> [7]
NaCl <11> (\text{<11>, slight inhibition above 600 mM}) [26]
O-Carbamoyl-D-Ser <1> (\text{<1>, inhibition of wild type enzyme but not of the
O-carbamoyl-D-Ser mutant}) [6]
Cofactors / prosthetic groups / activating substances

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]

Turnover number (min⁻¹)

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Specific activity (U/mg)

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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]
Alanine racemase

5.1.1.1

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 Bacil-
lus subtilis and Salmonella typhimurium dadB and alr enzymes [10]; expres-
sion in E. coli [20,21]) [8,10,20,21]

Engineering
Additional information <3,4> (<3>, double mutant for the alr encoded en-
zyme and the dad B encoded enzyme display a phenotype of requirement for
exogenous D-Ala for growth [14,24]; <4>, mutant gene which tandemly en-
codes 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.)