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-3-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

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

Reaction type

Racemization

Natural substrates

\[ \text{l-Ala} <3,4,7,9> \quad (\text{<3, 4, 9>}, \text{enzyme provides d-Ala as an essential building block for biosynthesis of the peptidoglycan layer of the cell wall}) \quad [11,15,21,28] \]

Additional information \[ \text{<3,12> (\text{<3>, two nonhomologous alanine racemase genes, one of which is associated with the catabolic function and the other of which presumably represents the biosynthetic function} \quad [4]; \quad \text{<3>, alr racemase is constitutive and serves an anabolic function, dadB encoded enzyme is inducible and required for cell growth on l-Ala \quad [14,24]; \quad \text{<12>, key enzyme in cyclosporin A biosynthesis} \quad [27])} \quad [4,14,24,27] \]

Substrates and products

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

Inhibitors

\[ \begin{align*}
\text{(1-Aminoethyl)boronic acid} & \quad \text{<4>} \quad [16] \\
\text{(1-Aminoethyl)phosphonate} & \quad \text{<1,4>, \text{d- and l-(1-aminoethyl)phosphonate} \quad [6]} \quad [6,12] \\
\text{2-Amino-3-chlorobut-3-enolic acid} & \quad \text{<7>, i.e. 3-chlorovinylglycine, irreversible} \quad [22] \quad [22,28] \\
\text{2-Amino-3-fluorobut-3-enolic acid} & \quad \text{<7>, i.e. 3-fluorovinylglycine, irreversible} \quad [22] \quad [22,28] \\
\text{d-Chloroalanine} & \quad \text{<2>, \text{Ki: 0.005 mM, competitive} \quad [3]} \quad [3,7] \\
\text{d-O-Acetylseryline} & \quad \text{<3>} \quad [7] \\
\text{d-β-Fluoroalanine} & \quad \text{<3>} \quad [7] \\
\text{FAD} & \quad \text{<11>, (slight activation at low concentrations, inhibition at high concentrations)} \quad [26] \\
\text{l-Chloroalanine} & \quad \text{<2,3>, \text{Ki: 1.71 mM, noncompetitive} \quad [3]} \quad [3,7] \\
\text{l-β-Chloroalanine} & \quad \text{<3>} \quad [7] \\
\text{NaCl} & \quad \text{<11>, slight inhibition above 600 mM} \quad [26] \\
\text{O-Carbamoyl-d-Ser} & \quad \text{<1>, inhibition of wild type enzyme but not of the O-carbamoyl-d-Ser mutant} \quad [6]
\end{align*} \]
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>, K_m: 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]; <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> (n-Ala, <3>) [5])
- 2.2 (<1,3> (n-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> (n-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>, l-Ala) [27])
- 9 (<1> [6])
- 9.5 (<12>, d-Ala) [27])

### pH-Range
7–9.5 (<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>, 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>, 20 C: about 60% of maximal activity, 50 C, about 35% of maximal activity) [26]

### 4 Enzyme Structure

#### Molecular weight
- 43000 (<3>, gel filtration) [7]
- 50000 (<3>, gel filtration) [5]
- 67000 (<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 D-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**

<table>
<thead>
<tr>
<th>pH</th>
<th>Activity</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>&lt;3&gt;</td>
<td>(&lt;3&gt;, 80 C, stable) [24]</td>
</tr>
<tr>
<td>8.3–10.5</td>
<td>&lt;4,8&gt;</td>
<td>(&lt;4,8&gt;, 1 h, 0 C, stable) [25]</td>
</tr>
</tbody>
</table>

**Temperature stability**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Activity</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>&lt;8&gt;</td>
<td>(&lt;8&gt;, 1 h, stable) [19]</td>
</tr>
<tr>
<td>30°C</td>
<td>&lt;8&gt;</td>
<td>(&lt;8&gt;, over 30 C, 1 h, quick loss of activity [19,25]; &lt;8&gt;, 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]; &lt;4&gt;, 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]</td>
</tr>
<tr>
<td>40–50°C</td>
<td>&lt;11&gt;</td>
<td>(&lt;11&gt;, pH 8.0, 5 min, sharp decrease of activity between 40 C and 50 C) [26]</td>
</tr>
<tr>
<td>60°C</td>
<td>&lt;11&gt;</td>
<td>(&lt;11&gt;, pH 8.0, 5 min, almost complete inactivation) [26]</td>
</tr>
<tr>
<td>70°C</td>
<td>&lt;3&gt;</td>
<td>(&lt;3&gt;, 80 min, pH 7.2, stable) [24]</td>
</tr>
<tr>
<td>75°C</td>
<td>&lt;4&gt;</td>
<td>(&lt;4&gt;, 1 h, inactivation over 75 C) [25]</td>
</tr>
</tbody>
</table>

**General stability information**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3&gt;, NaB₃H₄-reduced DadB holoenzyme is resistant to α-chymotrypsin and trypsin and is labile only towards subtilisin [31]</td>
<td></td>
</tr>
<tr>
<td>&lt;3&gt;, stable in 30% ammonium sulfate. Irreversibly diminished activity by exposure to ammonium sulfate concentrations near or above 40%, where precipitation occurs [5]</td>
<td></td>
</tr>
<tr>
<td>&lt;4&gt;, 5 min, resistant to incubation with 0.08% SDS, 1 M guanidine hydrochloride, 4 M urea, 45% ethanol or 50% dimethyl sulfoxide [25]</td>
<td></td>
</tr>
<tr>
<td>&lt;4&gt;, denatured by 3.5 M urea in one transition phase [25]</td>
<td></td>
</tr>
<tr>
<td>&lt;4&gt;, 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]</td>
<td></td>
</tr>
<tr>
<td>&lt;8&gt;, 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]</td>
<td></td>
</tr>
<tr>
<td>&lt;8&gt;, denatured by urea in two transition phases, dissociation of pyridoxal 5’-phosphate with 4.0 M urea, unfolding with 5.5 M urea [25]</td>
<td></td>
</tr>
</tbody>
</table>

**Storage stability**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3&gt;, -70°C, 10% glycerol, more than 90% of the activity is retained after 2 years [5]</td>
<td></td>
</tr>
<tr>
<td>&lt;8&gt;, -20°C, stable for 1 month [19]</td>
<td></td>
</tr>
<tr>
<td>&lt;8&gt;, 4°C, stable for 1 week [19]</td>
<td></td>
</tr>
<tr>
<td>&lt;11&gt;, enzyme loses about 20% of activity during storage for 20 days at -20°C, 0°C and 5°C [26]</td>
<td></td>
</tr>
</tbody>
</table>
References
