Note

Catalytic Action of L-Methionine \( \gamma \)-Lyase on 4-Azaleucine

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2-Amino-3-(N,N-dimethylamino)propionic acid, also termed 4-azaleucine, is a nitrogen analogue of leucine, and acts as an antimetabolite against leucine. Although its physiological action has been studied extensively, its metabolism, particularly the enzymological aspects, have remained unsettled. L-Methionine \( \gamma \)-lyase (EC 4.4.1.1) is a pyridoxal 5’-phosphate (pyridoxal-P) enzyme catalyzing the conversion of methionine into \( \alpha \)-ketobutyrate, methanethiol, and ammonia, and is important in the bacterial metabolism of methionine, which is a key metabolite in the metabolism of sulfur amino acids. We have purified the enzyme to homogeneity from Pseudomonas putida, and shown that the enzyme can cleave C-S, C-Se, and C-O bonds at the \( \beta \) or \( \gamma \) position of amino acids to lead to elimination and replacement reactions. We here show that the enzyme catalyzes cleavage of a C–N bond of 4-azaleucine as well.

DL-4-Azaleucine, DL-2,3-diaminopropionate, and DL-2,4-diaminobutyrate were purchased from Nakarai Chemicals Co., Kyoto, Japan. L-Methionine \( \gamma \)-lyase was purified to homogeneity from a cell-free extract of \( P. \) putida ICR 3460 as reported previously. The elimination reactions were followed by measurement of the \( \alpha, \beta \)-elimination and those for other nitrogen-containing amino acids, 2,3-diaminopropionate and 2,4-diaminobutyrate. L-2,3-Diaminopropionate is a substrate for rat liver cystathionine \( \gamma \)-lyase (EC 4.4.1.1), and is decomposed also by a cell-free extract of \( P. \) putida.10 We have found, however, that none of them were active as substrates for L-methionine \( \gamma \)-lyase.

4-Azaleucine also underwent enzymatic \( \beta \)-replacement

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration (mM)</th>
<th>Relative activity (%)</th>
<th>( Km ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Methionine</td>
<td>25</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>S-Methyl-l-cysteine</td>
<td>25</td>
<td>9.0</td>
<td>0.5</td>
</tr>
<tr>
<td>4-Azaleucine</td>
<td>5.0</td>
<td>0.4</td>
<td>18.2</td>
</tr>
<tr>
<td>2,3-Diaminopropionic acid</td>
<td>50</td>
<td>0.0</td>
<td>—</td>
</tr>
<tr>
<td>2,4-Diaminobutyric acid</td>
<td>50</td>
<td>0.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* DL-4-Azaleucine was used as a substrate, and the active concentration is assumed to be half of the DL-isomer.
reactions with various thiols (i.e., ethanethiol, n-propanethiol, and benzenethiol). The products were isolated as described previously, and identified as the corresponding S-substituted L-cysteines. The relative rates of the thiols (e.g., ethanethiol:n-propanethiol:benzenethiol = 100:90:44) are similar to those in the γ-replacement reactions of L-methionine.

Scheme 1 shows a proposed mechanism of the enzymatic α, β-elimination and β-replacement reactions of 4-azaleucine. A Schiff base of 4-azaleucine is first produced, which is converted to a quinoid intermediate through α-proton abstraction by a base at the active site. The quinoid intermediate releases a β-substituent, dimethylamine, to generate an α, β-unsaturated intermediate, which is the common key intermediate in the enzymatic α, β-elimination and β-replacement reactions. The α-aminoacrylate is produced by a subsequent transaldimination with the lysine residue to which pyridoxal-P is bound, and tautomerizes to α-iminopropionate, which is spontaneously hydrolyzed to form pyruvate and ammonia. The addition of thiol to the α,β-unsaturated intermediate yields the S-substituted cysteine. We are currently studying the elimination and replacement reactions of other nitrogen-containing amino acids to clarify the reaction mechanism.

REFERENCES