Note

Catalytic Action of L-Methionine γ -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.¹⁾ Although its physiological action has been studied extensively,¹⁾ its metabolism, particularly the enzymological aspects, have remained unsettled. L-Methionine γ -lyase (EC 4.4.1.11) is a pyridoxal 5'-phosphate (pyridoxal-P) enzyme catalyzing the conversion of methionine into α -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 β or γ position of amino acids to lead to elimination and replacement reactions.²⁾ 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 γ -lyase was purified to homogeneity from a cell-free extract of *P. putida* ICR 3460 as reported previously.³⁾ The elimination reactions were followed by measurement of the α -keto acid formed with 3-methyl-2-benzothiazolone hydrazone hydrochloride by the method of Soda.⁴⁾ The replacement reactions were measured by the amount of amino acids formed with a Hitachi high performance amino acid analyzer model 835.⁵⁾ The assay systems and conditions were described in our previous paper.⁵⁾ The pH dependence of the α , β -elimination was examined with the following buffers (final concentration, 0.1 M) citrate/NaHPO₄, pH 6.0; potassium phosphate, pH 6.5~8.5; Tricine-KOH, pH 7.2~8.5; sodium pyrophosphate, pH 8.0~9.5; and Na₂CO₃/Na₂BO₃, pH 9.5~10.0.

We have found that L-methionine γ -lyase catalyzes the

α , β -elimination of 4-azaleucine. Pyruvate was reductively aminated to alanine with alanine dehydrogenase (EC 1.4.1.1) and measured by amino acid analyzer.⁶⁾ Ammonia was measured spectrophotometrically with glutamate dehydrogenase.⁷⁾ 4-Azaleucine was measured by amino acid analysis as described above. A balance study showed that 1.9 μ mol of pyruvate and 2.1 μ mol of ammonia were produced with consumption of 2.0 μ mol of 4-azaleucine. Dimethylamine was identified with nitroprusside after paper chromatography with the solvent systems of *n*-butanol-acetic acid-water (4:1:5, v/v/v) (R_f =0.52), phenol saturated water (R_f =0.86), and *n*-hexane-benzene (3:2, v/v) (R_f =0.17). Table I summarizes the kinetic parameters for the enzyme reaction. We showed that D-methionine and D-cysteine are not substrates.⁵⁾ Therefore, the K_m value for 4-azaleucine was estimated on the assumption that only the L-form is active. 4-Azaleucine is rather a poor substrate in terms of both the affinity and velocity. This is probably due to the branched chain of the amino acid, because the enzyme catalyzes the hydrogen exchange of L-norleucine and L-norvaline with solvent hydrogen, but not that of L-leucine and L-valine.⁸⁾ The optimum pH for the α , β -elimination of 4-azaleucine was 8.2, which is similar to those for other substrates.³⁾ We examined also the ability of the enzyme to catalyze elimination reactions of two other nitrogen-containing amino acids, 2,3-diaminopropionate and 2,4-diaminobutyrate. L-2,3-Diaminopropionate is a substrate for rat liver cystathionine γ -lyase (EC 4.4.1.1),⁹⁾ and is decomposed also by a cell-free extract of *Pseudomonas* sp.¹⁰⁾ We have found, however, that none of them were active as substrates for L-methionine γ -lyase.

4-Azaleucine also underwent enzymatic β -replacement

TABLE I. KINETIC PARAMETERS FOR THE ELIMINATION REACTIONS CATALYZED BY L-METHIONINE γ -LYASE

Amino acid	Concentration (mM)	Relative activity (%)	K_m (mM)
L-Methionine	25	100	1.0
S-Methyl-L-cysteine	25	9.0	0.5
4-Azaleucine ^a	50	0.4	18.2
2,3-Diaminopropionic acid	50	0.0	—
2,4-Diaminobutyric acid	50	0.0	—

^a DL-4-Azaleucine was used as a substrate, and the active concentration is assumed to be half of the DL-isomer.

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SCHEME 1. Mechanism of α,β -Elimination and β -Replacement Reactions of 4-Azaleucine Catalyzed by L-Methionine γ -Lyase.

reactions with various thiols (*i.e.*, ethanethiol, *n*-propanethiol, and benzenethiol). The products were isolated as described previously,⁵⁾ 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.⁵⁾

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.

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