

Synthesis of Amino Acids with Pyridoxal 5'-phosphate Enzymes

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We here describe enzymatic synthesis of various optically active amino acids with pyridoxal 5'-phosphate enzymes including aspartate β -decarboxylase, β -tyrosinase, tryptophanase, cysteine desulhydrase, tryptophan synthase, β -chloro-D-alanine hydrochloride lyase, L-methionine γ -lyase, serine hydroxymethyltransferase, and L-threonine aldolase.

KEY WORDS: Enzymatic synthesis/ Optically-active amino acids/
Amino acid synthesis/ Pyridoxal 5'-phosphate enzymes/
Multifunctional enzymes/

I INTRODUCTION

At present, amino acids have been produced by isolation from natural materials, by microbial or enzymatic procedures, or by chemical synthesis. The first two procedures give optically active (usually L-)amino acids, whereas the chemical methods in general produce the racemates, and an additional optical resolution step is necessary to obtain optically active amino acids. Thus, the microbial procedure, particularly using auxotrophs and regulatory mutants has been developed to industrial scale. In the microbial production (amino acid fermentation), amino acids can be synthesized from simple and cheap raw materials, but the production is time-consuming and the required amino acids have to be separated from the other amino acids formed, and from various impurities including microbial cells. The waste may cause water pollution especially when molasses is used as a carbon source.

On the other hand, the enzymatic procedures for amino acid production have been extensively studied, since Kitahara *et al.*¹⁾ reported the efficient conversion of fumarate and ammonia into L-aspartate with bacterial aspartase. The enzymatic procedure surpasses the microbial one for the following reasons: it is less time-consuming and usually more efficient, and does not produce complex impurities and wastes. However, the enzymatic method is not suitable for the production of amino acids from simple materials. The substrates are either precursors, or other chemicals related to amino acids, and are generally more expensive in comparison with the starting materials required in the microbial methods. The enzymes used are neither cheap nor stable. Immobilization of enzymes has been developed to diminish these demerits of the enzymatic methods. We here describe the enzymatic synthesis of amino acids utilizing pyridoxal 5'-phosphate(=pyridoxal-P) enzymes from microorganisms, although

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other enzymes such as hydrolytic enzymes, ammonia lyases and NAD^+ -dependent L-amino acid dehydrogenases also are used as reviewed by Soda *et al.*²⁾

II PYRIDOXAL-P ENZYME

Various enzymes participating in the metabolism of amino acids require pyridoxal-P as a coenzyme. Pyridoxal-P enzymes catalyze racemizations, transaminations, eliminations and replacements. In catalysis with a coenzyme-requiring enzyme, the apoenzyme is generally responsible for the substrate specificity. However, the function of bound pyridoxal-P is the activation of the substrate amino acids to facilitate the catalysis with the apoenzyme.

The enzyme bound pyridoxal-P reacts with the substrate amino acid by transaldimination to form a coenzyme-substrate Schiff base, in which a proton attached to the imine nitrogen holds the conjugated π -system plane (Fig. 1). The electron attraction of the protonated pyridine nitrogen which conjugated with the $\text{C}=\text{N}$ bond permits

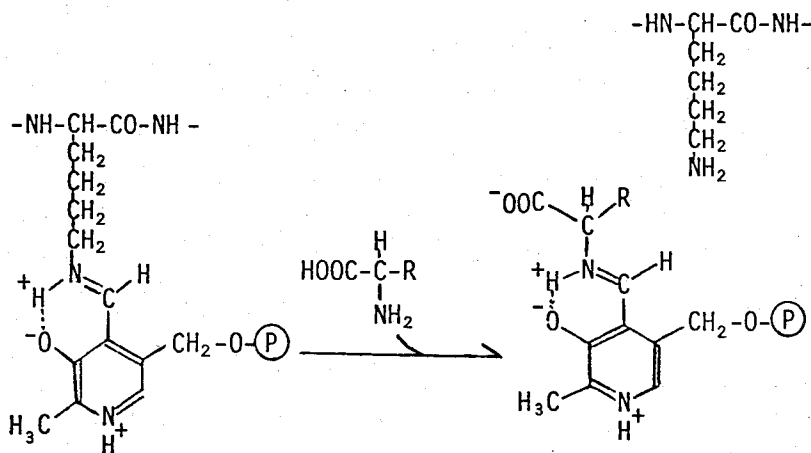


Fig. 1. Reaction of the enzyme-bound pyridoxal-P with an amino acid (transaldimination).

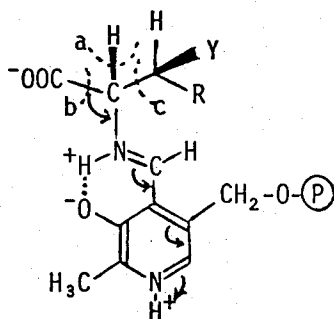


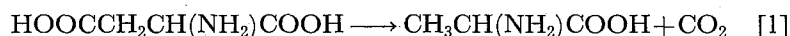
Fig. 2. Reactions catalyzed by pyridoxal-P enzymes. (a) Removal of α -H as H^+ to yield quinoid intermediate; transamination, racemization, α , β -elimination, β -replacement, α , γ -elimination, γ -replacement etc. (b) Decarboxylation. (c) Aldol cleavage (if Y is OH).

electrons to flow from the substrate into the coenzyme. Thus, the pyridine ring of pyridoxal-P functions as an electron sink. The structure of the apoenzyme determines which bond to the α -carbon atom of the activated substrate would be cleaved to release (a) the α -hydrogen, (b) the α -carboxyl group, or (c) a side chain (Fig. 2). Several pyridoxal-P enzymes show multicatalytic functions according to the reaction systems.

III ENZYMATIC SYNTHESIS OF AMINO ACIDS

1. Aspartate β -decarboxylase

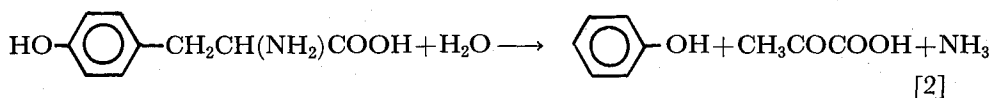
Aspartate β -decarboxylase (EC 4.1.1.12) catalyzes the removal of the β -carboxyl group of L-aspartate to form L-alanine [1].



L-Aspartate is produced industrially from fumarate with aspartase (EC 4.3.1.1) from *Escherichia coli* as described above. Thus, L-alanine can be produced from L-aspartate with aspartate β -decarboxylase. The enzyme has been crystallized from *Alcaligenes faecalis*³⁾ and from *Pseudomonas dacunhae*,⁴⁾ and its molecular and catalytic properties have been studied. Chibata *et al.*⁵⁾ have established an industrial method for the production of L-alanine (about 400 g/L) from L-aspartate with intact cells of *P. dacunhae*.

2. β -Tyrosinase

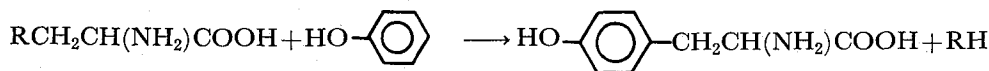
β -Tyrosinase catalyzes the α , β -elimination of L-tyrosine to produce pyruvate, phenol and ammonia [2].



The enzyme has been crystallized from *Citrobacter intermedius* (= *Escherichia intermedia*)⁶⁾ and from *Erwinia herbicola*,⁷⁾ and their physicochemical properties have been presented.

Yamada and his coworkers have established the optimal cultivation conditions for these bacteria for the maximum production of β -tyrosinase. The enzyme is produced inducibly by the addition of L-tyrosine to the medium.⁸⁾ Although L-phenylalanine is not an inducer, it cooperatively enhances the effect of tyrosine.⁹⁾ This is probably due to the inhibition of the β -tyrosinase action by L-phenylalanine, which is observed *in vitro*; phenol formed from L-tyrosine inhibits both bacterial growth and the formation of β -tyrosinase. The concentration of the enzyme can reach as much as 10% of the total soluble protein in *Erwinia herbicola* under optimal conditions.

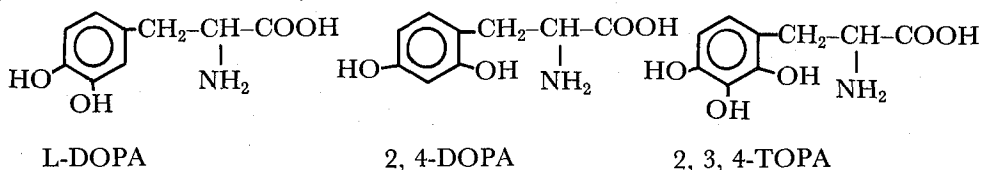
In addition to L-tyrosine, D-tyrosine, L- and D-serine, S-methyl-L-cysteine and β -chloro-L-alanine act as substrates in the α , β -elimination with the formation of pyruvate.⁶⁾ The enzyme also catalyzes the β -replacement reaction [3] between the substrates for α , β -elimination and phenol to yield L-tyrosine.¹⁰⁾



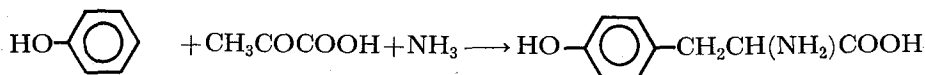
[3]

(R=phenolyl, -OH, -SH, -Cl)

D-Isomers of substrate amino acids also give the L-isomers of tyrosine derivatives.⁶⁾ When phenol is replaced by pyrocatechol, resorcinol, pyrogallol and hydroxyhydroquinone, 3, 4-dihydroxy-L-phenylalanine (L-DOPA), 2, 4-dihydroxy-L-phenylalanine (2, 4-DOPA), 2, 3, 4-trihydroxyphenyl-L-alanine (2, 3, 4-TOPA) and 2, 4, 5-trihydroxyphenyl-L-alanine were synthesized, respectively.^{10,11)}



The synthesis of L-tyrosine from phenol, pyruvate, and ammonia (a reverse reaction of α , β -elimination) is also catalyzed by β -tyrosinase [4].¹²⁾



[4]

The maximum reaction rate is about 1.5 times higher than that of L-tyrosine α , β -elimination. When various derivatives of phenol are used as substituent donors, the corresponding tyrosine derivatives are synthesized; relative velocity is phenol (100), o-cresol (45.4), m-cresol (52.7), o-chlorophenol (47.3), m-chlorophenol (32.7), pyrocatechol (60), and resorcinol (58.2).¹³⁾

The reaction mechanism of the multifunctional β -tyrosinase is similar to the general mechanism for pyridoxal-P enzyme reactions.¹⁴⁾ The enzyme- α -aminoacrylate complex is a common key intermediate for all the α , β -eliminations, β -replacements, and reverse reactions.

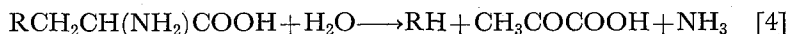
Synthesis of L-DOPA by β -replacement reaction between DL-serine and pyrocatechol was studied with *Erwinia herbicola* cells.¹⁵⁾ Phenol derivatives, particularly at high concentrations, inhibit β -tyrosinase. Therefore, limited concentrations of phenol derivatives are added to the reaction system in portions to increase the yield; 5.5 g of L-DOPA was synthesized per 100 mL of the reaction mixture. More than 6.0 g of L-tyrosine per 100 mL of the reaction mixture was synthesized from phenol, pyruvate and ammonia.¹⁶⁾

Ikedo *et al.*¹⁷⁾ immobilized β -tyrosinase on Sepharose in order to synthesize L-tyrosine continuously. *Erwinia herbicola* cells, immobilized in collagen matrices, are more resistant to pH, heat, and contact with phenolic compounds than the intact cells.¹⁸⁾

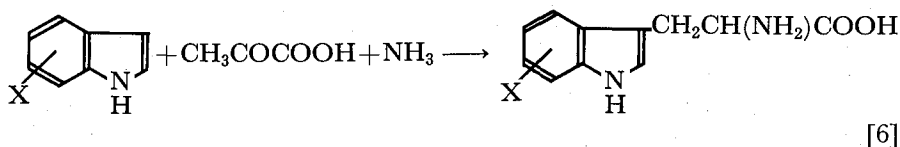
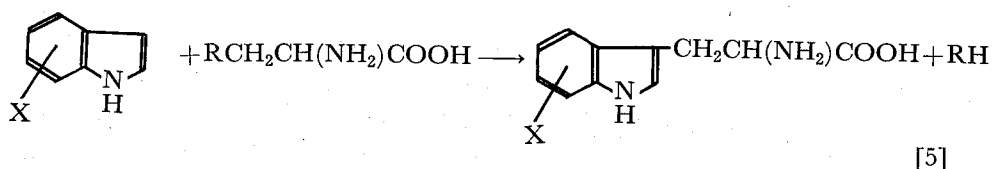
3. Tryptophanase

Tryptophanase (EC 4.1.99.1) is found widely in bacteria and has been purified from *Escherichia coli* B,¹⁹⁾ *E. coli* K12,²⁰⁾ *Bacillus albei*²¹⁾ and *Proteus rettgeri*.²²⁾ Crystalline enzyme preparations have been obtained from *E. coli* B¹⁹⁾ and *Proteus rettgeri*.²²⁾ The physicochemical properties of the enzyme have been studied in detail.

Tryptophanase catalyzes α , β -eliminations [4], β -replacements [5], and reverse reaction of α , β -eliminations [6], similarly to β -tyrosinase.



(R=indolyl, -OH, -SH and -Cl)

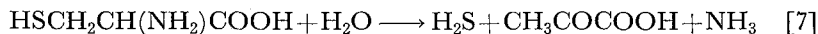


In addition to L-tryptophan, L-cysteine, S-methyl-L-cysteine, β -chloro-L-alanine and L-serine serve as substrates for the α , β -elimination and β -replacement reactions.²³⁾ L-Tryptophan is synthesized from indole, pyruvate and ammonia by a reverse reaction of α , β -elimination.²²⁾ When indole is replaced by 5-methyl-, 5-hydroxy- or 5-aminoindoles, 5-methyl-, 5-hydroxy- or 5-amino-L-tryptophan, respectively, is synthesized.²²⁾ The mechanism of the tryptophanase reaction is similar to that of β -tyrosinase.

Nakazawa *et al.*²⁴⁾ have studied the culture conditions for *Proteus rettgeri*, the best producer of tryptophanase. L-Tryptophan has to be added as an inducer of enzyme formation, but indole produced inhibits bacterial growth and enzyme production. However, indole can be removed from the medium by the addition of Sorpol W-200 (Polyoxyethylene alkyl phenol ether) which forms micelles with indole.²⁵⁾ Thus, tryptophanase can be accumulated in the cells of *P. rettgeri* at a concentration of 6% of the total soluble protein. Synthesis of L-tryptophan based on reverse reaction of α , β -elimination was studied with these cells and a reaction mixture containing inosine. L-Tryptophan synthesized formed an insoluble complex with inosine so that the yield was enhanced. Indole added to the reaction mixture was converted almost quantitatively to L-tryptophan with a yield of 100 g/L.²⁵⁾ 5-Hydroxy-L-tryptophan was synthesized from 5-hydroxyindole, pyruvate and ammonia in a similar manner.²⁵⁾

4. Cysteine desulphydrase

Cysteine desulphydrase (EC 4.4.1.1) catalyzes the α , β -elimination of L-cysteine to produce hydrogen sulfide, pyruvate and ammonia [7].



It has been found in bacteria, yeasts and plants, and purified to homogeneity from *Salmonella typhimurium*²⁶⁾ and from *Aerobacter aerogenes*.²⁷⁾ *A. aerogenes* enzyme has a molecular weight of about 25,0000, and contains six identical subunits (M.W., 41,000).

In addition to L-cysteine, L-serine, S-methyl-L-cysteine and β -chloro-L-alanine serve as substrates for α , β -elimination.²⁷⁾ The enzyme also catalyzes the β -replacement reaction between β -chloro-L-alanine and various thiols to produce the corresponding S-substituted L-cysteines.²⁸⁾ Reverse reaction of α , β -elimination is also catalyzed; S-alkyl-L-cysteines are produced from pyruvate, ammonia and alkane thiols.²⁹⁾

Kumagai *et al.*³⁰⁾ also have studied the synthesis of L-cysteine from β -chloro-L-alanine by a replacement reaction with *Enterobacter cloacae* cells selected as the best producer of cysteine desulfhydrase. L-Cysteine was produced efficiently by the addition of acetone to the reaction mixture.³¹⁾ Thus, β -chloro-L-alanine was converted to L-cysteine at a concentration of 50 g/L giving a molar yield of more than 80%. L-Cysteine is readily isolated as L-cystine after oxidation with FeCl_3 .³¹⁾

5. Tryptophan synthase

Tryptophan synthase (EC 4.2.1.20) is found in various bacteria, yeasts, molds and plants, and catalyzes reactions [8], [9], [10], and [12] (Table I).³²⁾ Reaction [10] is the physiological one, and reactions [8] and [9] are regarded as partial reactions of reaction [10]. Tryptophan synthase of *Escherichia coli*, which has been extensively studied,³²⁾ is composed of two kinds of proteins called α and β . Two α -subunits combine with one β_2 -dimer to form an $\alpha_2\beta_2$ -complex. Each of the subunits catalyzes reactions shown in Table I. The crystalline $\alpha_2\beta_2$ -complex was obtained after a six fold purification from *E. coli* tryR⁻ Δ trpED102/F' Δ trpED102.³²⁾ In this strain about 16% of the total soluble protein is the tryptophan synthase complex.

Table I. Reactions Catalyzed by *E. coli* Tryptophan Synthase and Its Subunits

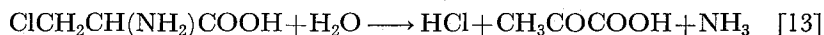
Reactions	Catalyzed by
8. Indole-3-glycerol phosphate $\rightleftharpoons$ indole + D-glyceraldehyde 3-phosphate	α ; $\alpha_2\beta_2$
9. Indole + L-serine $\rightleftharpoons$ L-tryptophan + H_2O	β_2 ; $\alpha_2\beta_2$
10. Indole-3-glycerol phosphate + L-serine $\rightleftharpoons$ L-tryptophan + D-glyceraldehyde 3-phosphate + H_2O	$\alpha_2\beta_2$
11. L-serine $\rightleftharpoons$ pyruvate + NH_3	β_2
12. β -Mercaptoethanol + L-serine $\rightleftharpoons$ S-(β -hydroxyethyl)L-cysteine + H_2O	β_2 ; $\alpha_2\beta_2$
13. β -Mercaptoethanol + L-serine + Pyridoxal-P $\rightleftharpoons$ S-pyruvylmercaptoethanol + Pyridoxamine-P + H_2O	β_2

Reactions [9] and [12] are applicable to the production of L-tryptophan and S-substituted L-cysteines, respectively. Recently, Esaki *et al.*³³⁾ have demonstrated that various selenols also serve as substituent donors in the β -replacement reaction [12]. Various selenium amino acids are found to be physiologically active, and probably can be used as antineoplastic and antiinflammatory agents.

6. β -Chloro-D-alanine hydrogenchloride lyase

The growth of most microorganisms is inhibited by the addition of β -chloro-DL-alanine to the medium. β -Chloro-D-alanine rapidly inactivates an active transport system coupled to a membrane-bound D-alanine dehydrogenase in *Escherichia coli*.³⁴⁾ Manning *et al.*³⁵⁾ have suggested that the antibacterial action of β -chloro-D-alanine is due to the inactivation of D-amino acid transaminase and alanine racemase, which results in the inhibition of biosynthesis of the peptidoglycan layer of the bacterial cell wall.

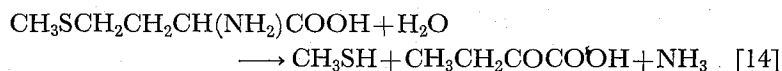
Recently, Nagasawa *et al.*³⁶⁾ have isolated a strain of *Pseudomonas putida* CR 1-1 that can grow in the presence of β -chloro-DL-alanine. They have studied the resistance mechanism of the strain and demonstrated a novel enzyme, β -chloro-D-alanine hydrogenchloride lyase, which catalyzes α , β -elimination of β -chloro-D-alanine to form pyruvate, ammonia and hydrogen chloride [13].



β -Chloro-D-alanine hydrogenchloride lyase is inducibly formed only by addition of β -chloro-D-alanine to the medium. It has a molecular weight of about 76,000 and consists of two identical subunits of molecular weight 38,000. In addition to β -chloro-D-alanine, which is the preferred substrate, D-cysteine and D-cystine also serve as substrates. The enzyme catalyzes the β -replacement reaction between β -chloro-D-alanine and hydrogen sulfide to yield D-cysteine. Nagasawa *et al.*³⁷⁾ have studied the conditions for the cultivation of *P. putida* CR 1-1 and for D-cysteine production by the β -replacement reaction with the resting cells. Under optimal conditions, 20.6 mg of D-cysteine per mL of the reaction mixture was produced from β -chloro-D-alanine, with a molar yield of 100%.

7. Methionine γ -lyase

L-Methionine γ -lyase (EC 4.4.1.11) catalyzes the conversion of L-methionine into methanethiol, α -ketobutyrate and ammonia [14].



Screening was carried out in order to find bacterial strains that would produce a high activity of methionine γ -lyase under aerobic and anaerobic conditions. *Pseudomonas ovalis*, which is now reclassified as *P. putida*, was shown to have the highest enzyme activity, which was inducibly formed by the addition of L-methionine to the medium.³⁸⁾ It has a molecular weight of 173,000 and consists of non-identical subunits (molecular weights of 40,000 and 48,000). It contains 4 mol of pyridoxal-P/mol of enzyme.^{38, 39)}

In addition to methionine, which is the preferred substrate, several derivatives of L-methionine and L-cysteine are effective substrates, whereas D-methionine, D-cysteine, L-cystathionine and L-norleucine do not react (Table II).³⁸⁾ These results show that the enzyme catalyzes both α , γ - and α , β -elimination reactions.

L-Methionine γ -lyase also catalyzes replacement reactions.³⁸⁾ Ethionine was

Table II. Substrate Specificity of L-Methionine γ -Lyase of *Pseudomonas ovalis*

Substrates	Rel. Act.
L-Methionine	100
D-Methionine	0
L-Ethionine	90
DL-Methionine sulfone	87
DL-Methionine sulfoxide	35
L-Methionine-DL-sulfoximine	27
S-Methyl-L-methionine	8
L-Homocysteine	71
L-Norleucine	0
DL- α -Aminobutyrate	0
L-Cystathionine	0
L-Norvaline	0
L-Cysteine	11
D-Cysteine	0
L-Cystine	0
S-(β -Aminoethyl)L-cysteine	14
S-Methyl-L-cysteine	67

Table III. Relative Velocity of Synthesis of L-Ethionine and S-Ethyl-L-cysteine from Ethanethiol and Derivatives of Homocysteine and Cysteine by L-Methionine γ -Lyase.

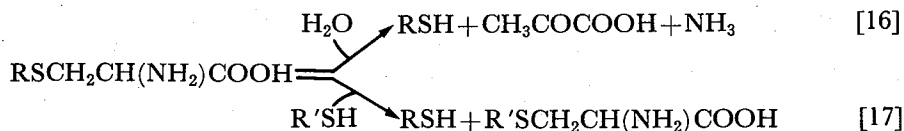
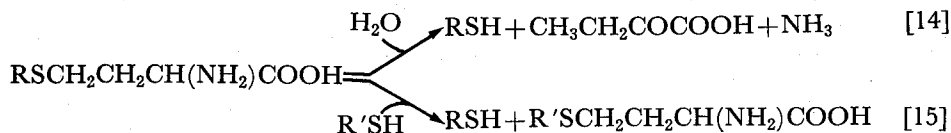
Type	Amino acid substrate	Rel. Velocity
γ -Replacement (L-Ethionine formation)	Methionine	100
	Methionine sulfone	22
	Methionine sulfoxide	7
	Methionine sulfoximine	29
	Homocysteine	93
β -Replacement (S-Ethyl-L- cysteine formation)	S-(β -Aminoethyl)homocysteine	3
	S-Methylcysteine	73
	Cysteine	6

synthesized from L-methionine and ethanethiol by means of γ -replacement reactions. The synthetic reaction proceeded as a function of enzyme concentration and incubation time. Successive additions of enzyme and ethanethiol were very effective, giving a yield of more than 95%. L-Ethionine was also produced by a γ -replacement reaction between the derivatives of methionine (*e.g.*, homocysteine, methionine sulfone or methionine sulfoxide) and ethanethiol (Table III).

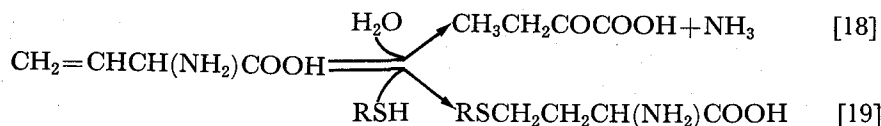
When ethanethiol was replaced by an alkanethiol (C_3 – C_7) or an arylthioalcohol (benzenethiol or β -naphthalenethiol) in the reaction system, a new sulfur amino acid,

corresponding to the thiol used, was enzymatically synthesized. Longer-chain alkanethiols (C_8 – C_{10}), however, were inert as substituent donors. The aminoacids produced from L-methionine and substituted thiols were identified as the corresponding *S*-substituted homocysteines, *e.g.*, 2-mercaptoethanol and cysteamine produced *S*-(β -hydroxyethyl) homocysteine and *S*-(β -aminoethyl) homocysteine, respectively. Thiols with a charged group were generally poor substituent donors.

In addition, the enzyme catalyzes the β -replacement reaction between *S*-methyl-L-cysteine and ethanethiol to yield *S*-ethyl-L-cysteine (Table III). Thus, L-methionine γ -lyase catalyzes the following reactions [14]–[17]:



Vinylglycine is regarded as a common key intermediate in the α , γ -elimination and γ -replacement reactions.¹⁴⁾ L-Methionine γ -lyase catalyzes the deamination of vinylglycine to produce α -ketobutyrate and ammonia [18]⁴⁰⁾ The enzyme also catalyzes the γ -addition reaction of various alkanethiols to vinylglycine to yield the corresponding S-substituted homocysteines [19].



The relative activity of the enzyme for various alkanethiols in the γ -addition reaction of vinylglycine is close to that obtained for the γ -replacement reaction of methionine (Table IV).⁴⁰⁾

Table IV. Relative Velocity of Synthesis of *S*-Substituted Homocysteines from Methionine or Vinylglycine by L-Methionine γ -Lyase

RSH R=	Vinylglycine (%)	Methionine (%)
Ethyl-	100 (75%)	100 (100%)
<i>n</i> -Propyl-	82	81
<i>n</i> -Hexyl-	23	17
Benzyl-	72	71

Selenium is highly toxic but is also an essential trace element for animals and bacteria. Its biological role has received considerable attention, as reviewed by Stadtman.⁴¹⁾ The selenium analogs of sulfur-containing amino acids occur in nature,

and play important roles in biological systems. L-Methionine γ -lyase catalyzes the α , γ -elimination of selenomethionine to yield α -ketobutyrate, ammonia and methane-selenol, and also its γ -replacement reaction with various thiols to produce S-substituted homocysteines. Selenomethionine is a better substrate than methionine in α , γ -elimination but is less effective in γ -replacement. In addition, L-methionine and its derivatives react with selenols to form the corresponding Se-substituted selenohomocysteines, although selenols are less efficient substituent donors than thiols. Vinylglycine also serve as a substrate in the γ -addition reaction with selenols to form the corresponding Se-substituted selenohomocysteines. This is the first proven instance in which a selenium atom is incorporated into an amino acid.⁴²⁾ These enzymatic replacement and γ -addition reactions facilitate the synthesis of various selenium amino acids labeled with ^{14}C , ^{75}Se and ^3H .

The reaction mechanism for L-methionine γ -lyase is shown in Fig. 3. The formation of the ketimine quinoid intermediate of S-substituted homocysteine is followed by the loss of an α -hydrogen and a γ -substituent. The resulting β , γ -unsaturated compound is also derived from a pyridoxal-P aldimine intermediate of vinylglycine. This intermediate can undergo γ -addition of R'SH to form an amino acid, or tautomerization followed by hydrolysis to form α -ketobutyrate and ammonia.

L-Methionine γ -lyase catalyzes the exchange of the α -H and β -H of L-methionine

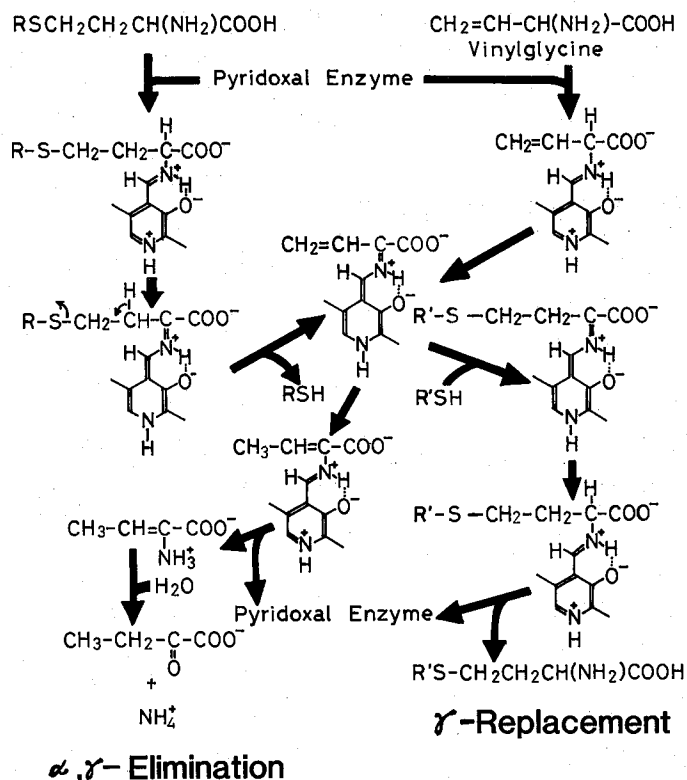


Fig. 3. Mechanism for the reaction of L-methionine γ -lyase.

and *S*-methyl-L-cysteine with ^2H in $^2\text{H}_2\text{O}$.⁴³⁾ The rate of α -H exchange is about 40 times higher than that of the elimination reaction. The enzyme also can exchange the α - and β -H of the following straightchain amino acids that are not substrates for the elimination: L-alanine, L- α -aminobutyrate, L-norvaline, and L-norleucine. The exchange of the two prochiral β -H is not stereospecific. No exchange is observed for D-isomers, L-valine, L-leucine, L-glutamate, L-aspartate, L-lysine and L-arginine, although glycine, L-tryptophan and L-phenylalanine slowly undergo the α -H exchange. This enzymatic proton exchange reaction facilitates the synthesis of various amino acids labeled with ^2H and ^3H .⁴³⁾

8. Serine hydroxymethyltransferase

L-Serine hydroxymethyltransferase (EC 2.1.2.1) catalyzes the reactions in Table V. Reaction [20] is the physiological one for the generation of a one-carbon group, in the form of 5, 10-methylenetetrahydrofolate, from serine. The enzyme has been demonstrated in a variety of tissues and organisms.⁴⁴⁾ It has been purified to homogeneity from mammalian tissues, and characterized in detail.⁴⁴⁾ The microbial enzyme has not yet been purified, but the regulatory mechanism and physiological function of the enzyme have been investigated extensively.

Table V. Reactions Catalyzed by Serine Hydroxymethyltransferase

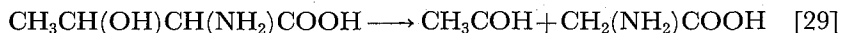
Reactions	
20.	L-Serine + H_4 -folate $\rightleftharpoons$ Glycine + 5, 10-methylene- H_4 -folate
21.	L- α -Methyl serine + H_4 -folate $\rightleftharpoons$ D-alanine + 5, 10-methylene- H_4 -folate
22.	Allothreonine $\rightleftharpoons$ glycine + acetaldehyde
23.	L-Threonine $\rightleftharpoons$ glycine + acetaldehyde
24.	<i>erythro</i> - β -Phenylserine $\rightleftharpoons$ glycine + benzaldehyde
25.	<i>threo</i> - β -Phenylserine $\rightleftharpoons$ glycine + benzaldehyde
26.	ϵ -Trimethyl-3-hydroxylysine $\rightleftharpoons$ glycine + γ -butyrobetaine aldehyde
27.	D-Alanine + pyridoxal-P $\rightleftharpoons$ pyruvate + pyridoxamine-P
28.	Aminomalonate $\rightleftharpoons$ glycine + CO_2

H_4 -folate represents tetrahydrofolate.

Although tetrahydrofolate was considered to be absolutely required in reaction [20], the enzyme also catalyzes the interconversion of glycine and serine in the absence of tetrahydrofolate.⁴⁴⁾ Therefore, L-serine can be enzymatically produced from glycine and formaldehyde, cheaply available starting materials.

9. L-Threonine aldolase

L-Threonine aldolase (EC 4.1.2.5) catalyzes the conversion of L-threonine into acetaldehyde and glycine [29].



It occurs in various microorganisms, *e.g.*, *Klebsiella*, *Escherichia*, *Arthrobacter*, *Bacterium*, *Xanthomonas*, *Proteus* and *Candida*.⁴⁵⁾ These microorganisms grow in media containing

L-threonine as the sole source of carbon, and the enzyme is induced by L-threonine. Strict anaerobes such as *Clostridium pasteurianum* and *Selenomonas ruminatum* also produce this enzyme, but it is synthesized constitutively and functions in the biodegradation of L-threonine into glycine in these microorganisms.⁴⁶⁾ Evidence has been obtained to show that microbial L-threonine aldolase and *allo*-threonine aldolase are identical to each other, but distinct from L-serine hydroxymethyl-transferase (cf. reaction [23] in Table V).⁴⁷⁾ In mammals, however, L-threonine aldolase is identical to L-serine hydroxymethyltransferase.⁴⁴⁾ Tetrahydrofolate, a cofactor of L-serine hydroxymethyltransferase, is rather inhibitory to the microbial threonine aldolase.⁴⁸⁾

L-Threonine aldolase, crystallized from *Candida humicola*, has a molecular weight of about 277,000, and contains 6 mol of pyridoxal-P per mol of enzyme.⁴⁸⁾ In addition to L-threonine, L-*allo*-threonine and L-serine serve as substrates producing glycine. These reactions proceed reversibly: L-threonine is synthesized from acetaldehyde and glycine, and L-serine from formaldehyde and glycine. Thus, with this enzyme, L-serine and L-threonine can be synthesized from cheaply available materials.

REFERENCES

- (1) K. Kitahara, S. Fukui, and M. Misawa, *Nippon Nogeikagaku Kaishi*, **34**, 44 (1960).
- (2) K. Soda, H. Tanaka, and N. Esaki, "Biotechnology" (Rehm, H. -J. and Reed, G., eds), Vol. 3, Verlag Chemie, Weinheim, 1983, pp. 479-530.
- (3) E. W. Wilson, and H. L. Kornberg, *Biochem. J.*, **88**, 578 (1963).
- (4) T. Kakimoto, J. Kato, T. Shibatani, N. Nishimura, and I. Chibata, *J. Biol. Chem.*, **244**, 353 (1969).
- (5) I. Chibata, T. Kakimoto, and J. Kato, *Appl. Microbiol.*, **13**, 638 (1965).
- (6) H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi, and K. Ogata, *J. Biol. Chem.*, **245**, 1767 (1970).
- (7) H. Kumagai, N. Kashima, H. Yamada, H. Enei, and S. Okumura, *Agric. Biol. Chem.*, **36**, 472 (1972).
- (8) H. Enei, H. Matsui, K. Yamashita, S. Okumura, and H. Yamada, *Agric. Biol. Chem.*, **36**, 1861 (1972).
- (9) H. Enei, K. Yamashita, S. Okumura, and H. Yamada, *Agric. Biol. Chem.*, **37**, 485 (1973).
- (10) K. Kumagai, H. Matsui, H. Ohkishi, K. Ogata, and H. Yamada, *Biochem. Biophys. Res. Commun.*, **34**, 266 (1969).
- (11) T. Ueno, H. Fukami, H. Ohkishi, H. Kumagai, and H. Yamada, *Biochim. Biophys. Acta*, **206**, 476 (1970).
- (12) H. Yamada, H. Kumagai, H. Kashima, and H. Torii, *Biochem. Biophys. Res. Commun.*, **46**, 370 (1972).
- (13) H. Yamada, and H. Kumagai, *Adv. Appl. Microbiol.*, **19**, 249 (1975).
- (14) L. Davis, and D. E. Metzler, "Enzymes," 3rd Ed., 7, 33 (1972).
- (15) H. Enei, H. Matsui, H. Nakazawa, S. Okumura, and H. Yamada, *Agric. Biol. Chem.*, **37**, 493 (1973).
- (16) H. Enei, H. Nakazawa, S. Okumura, and H. Yamada, *Agric. Biol. Chem.*, **37**, 727 (1973).
- (17) S. Ikeda, and S. Fukui, *Methods Enzymol.*, **62**, 517 (1979).
- (18) H. Yamada, K. Yamada, H. Kumagai, T. Hino, and S. Okumura, "Enzyme Engineering" (K. Pyc and H. H. Weetall, eds), Vol. 3, Plenum Press, New York, 1978, pp. 57.

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- (19) W. Newton, and E. E. Snell, *Proc. Natl. Acad. Sci. U. S. A.*, **51**, 381 (1964).
- (20) J. London, and M. Goldberg, *J. Biol. Chem.*, **247**, 1566 (1977).
- (21) S. Hich, and R. DeMoss, *J. Bacteriol.*, **114**, 341 (1973).
- (22) H. Yoshida, H. Kumagai, and H. Yamada, *Agric. Biol. Chem.*, **38**, 463 (1974).
- (23) W. Newton, Y. Morino, and E. E. Snell, *J. Biol. Chem.*, **240**, 1211 (1965).
- (24) H. Nakazawa, H. Enei, S. Okumura, and H. Yamada, *Agric. Biol. Chem.*, **36**, 2523 (1972).
- (25) H. Yamada, H. Kumagai, H. Enei, H. Nakazawa, and K. Mitsugi, "Enzyme Engineering" (G. B. Broun, G. Manecke, and L. B. Wingard Jr., eds), Vol. 4, Plenum Press, New York, 1978, pp. 25.
- (26) N. M. Kredich, B. S. Keenan, and L. J. Foote, *J. Biol. Chem.*, **247**, 7157 (1972).
- (27) H. Kumagai, S. Sejima, Y. Choi, and H. Yamada, *FEBS Lett.*, **52**, 304 (1975).
- (28) H. Kumagai, H. Tanaka, S. Sejima, and H. Yamada, *Agric. Biol. Chem.*, **41**, 2071 (1977).
- (29) H. Kumagai, Y. Choi, S. Sejima, and H. Yamada, *Biochem. Biophys. Res. Commun.*, **59**, 789 (1974).
- (30) H. Kumagai, Y. Choi, S. Sejima, and H. Yamada, *Agric. Biol. Chem.*, **39**, 387 (1975).
- (31) H. Yamada, and H. Kumagai, *Pure Appl. Chem.*, **50**, 1117 (1978).
- (32) E. W. Miles, *Adv. Enzymol.*, **49**, 127 (1979).
- (33) N. Esaki, H. Tanaka, E. W. Miles, and K. Soda, "The proceedings of Annual Meeting of Agricultural Chemical Society of Japan", 1982, pp. 359.
- (34) G. Kaczorwski, L. Shaw, R. Landa, and C. Walsh, *J. Biol. Chem.*, **250**, 8921 (1975).
- (35) J. M. Manning, and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).
- (36) T. Nagasawa, H. Okishi, B. Kawakami, H. Yamano, H. Hosono, Y. Tani, and H. Yamada, *J. Biol. Chem.*, **257**, 13479 (1982).
- (37) T. Nagasawa, H. Yamano, H. Ohkishi, H. Hosono, Y. Tani, and H. Yamada, *Agric. Biol. Chem.*, **46**, 3003 (1982).
- (38) H. Tanaka, N. Esaki, and K. Soda, *Biochemistry*, **16**, 100 (1977).
- (39) H. Tanaka, N. Esaki, T. Yamamoto, and K. Soda, *FEBS Lett.*, **66**, 307 (1976).
- (40) N. Esaki, T. Suzuki, H. Tanaka, K. Soda, and R. R. Rando, *FEBS Lett.*, **84**, 309 (1977).
- (41) T. C. Stadtman, *Adv. Enzymol.*, **48**, 1 (1979).
- (42) N. Esaki, H. Tanaka, S. Uemura, T. Suzuki, and K. Soda, *Biochemistry*, **18**, 407 (1979).
- (43) N. Esaki, S. Sawada, H. Tanaka, and K. Soda, *Anal. Biochem.*, **119**, 281 (1982).
- (44) L. Schirch, *Adv. Enzymol.*, **53**, 83 (1982).
- (45) H. Yamada, H. Kumagai, T. Nagate, and H. Yoshida, *Agric. Biol. Chem.*, **35**, 1340 (1971).
- (46) R. H. Dainty, *Biochem. J.*, **117**, 585 (1970).
- (47) S. C. Bell, and J. M. Tutner, *Biochem. J.*, **166**, 209 (1977).
- (48) H. Yamada, H. Kumagai, T. Nagate, and H. Yoshida, *Biochem. Biophys. Res. Commun.*, **39**, 53 (1970).