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# MICROBIAL AND ENZYMATIC PROCESSES FOR AMINO ACID PRODUCTION

Hideaki Yamada and Hidehiko Kumagai*

Department of Agricultural Chemistry and Department of Food Science and Technology*, Faculty of Agriculture, Kyoto University, Kitashirakawa, Kyoto, 606, Japan

Abstract - β-Tyrosinase, tryptophanase and cysteine desulfhydrase were shown to catalyze a variety of α,β-elimination, β-replacement and the reverse of the α,β-elimination reactions of amino acids. The mechanism for these reactions was studied using the general mechanism for pyridoxal-dependent reactions. The enzyme-bound α-amino acrylate is the key intermediate. Enzymatic methods for preparing L-tyrosine, L-tryptophan, L-cysteine and related amino acids were developed using bacterial cells with high enzyme activities. These processes are simple and economical for the production of these amino acids.

## INTRODUCTION

Two processes have been established in Japan for the biological production of amino acids. They are:

I. Fermentation
  (a) Methods employing wild strains (producing L-glutamic acid, L- or DL-alanine, L-valine, etc.).
  (b) Methods employing mutant strains (producing L-lysine, L-ornithine, L-citrulline, L-arginine, L-homoserine, L-proline, L-histidine, etc.).
  (c) Methods adding precursors to media (producing L-isoleucine, L-serine, L-tryptophan, etc.).

II. Enzymation
  (a) Methods employing degradative enzymes (producing L-phenylalanine, L-tryptophan, L-alanine, etc.).
  (b) Methods employing synthesizing enzymes (producing L-aspartic acid).

In recently developed enzymatic processes, L-lysine production from DL-α-amino-ε-caprolactam (1-3), L-cysteine production from DL-2-amino-thiazoline-4-carboxylate (4), and the D- forms of phenyl- and hydroxyphenylglycines from the corresponding hydantoins (5) have been reported. During investigation of the biological and enzymatic production of amino acids, we have developed new enzymatic processes to produce L-tyrosine, L-tryptophan, L-cysteine and related amino acids, using microbial multifunctional pyridoxal enzymes.

## CATALYTIC PROPERTIES OF β-TYROSINASE, TRYPTOPHANASE AND CYSTEINE DESULFHYDRASE

β-Tyrosinase (tyrosine phenol-lyase [deaminating]: EC 4.1.99.2), tryptophanase (tryptophan indole-lyase [deaminating]: EC 4.1.99.1) and cysteine desulfhydrase (cysteine hydrogen sulfide-lyase [deaminating]: EC 4.4.1.1) are the enzymes which respectively catalyze the degradation of L-tyrosine, L-tryptophan and L-cysteine, and require pyridoxal 5'-phosphate (PLP) as a cofactor. Crystalline preparations of these enzymes have been prepared in our laboratory from *Escherichia intermedia* (6), *Proteus rettgeri* (7,8) and *Aerobacter aerogenes* (9) and their properties have been established in some detail (6-15).
With the crystalline enzymes we found that these enzymes catalyze a variety of α,β-elimination (eq.1), β-replacement (eq.2) and the reverse of the α,β-elimination (eq.3) reactions (10,12,14,15).

$$\text{L-RCH}_2\text{CHNH}_2\text{COOH} + \text{H}_2\text{O} \longrightarrow \text{RH} + \text{CH}_3\text{COCOOH} + \text{NH}_3 \quad (1)$$

$$\text{L-RCH}_2\text{CHNH}_2\text{COOH} + \text{R'H} \longrightarrow \text{L-R'CH}_2\text{CHNH}_2\text{COOH} + \text{RH} \quad (2)$$

$$\text{R'H} + \text{CH}_3\text{COCOOH} + \text{NH}_3 \longrightarrow \text{L-R'CH}_2\text{CHNH}_2\text{COOH} + \text{H}_2\text{O} \quad (3)$$

| | |
|---|---|
| β-tyrosinase; | R = phenolyl, -OH, -SH, -Cl, R' = phenolyl |
| tryptophanase; | R = indolyl, -OH, -SH, -Cl, R' = indolyl |
| cysteine desulfhydrase; | R = -SH, -OH, -Cl, R' = -SH, mercaptan radicals |

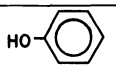
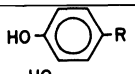
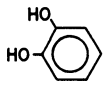
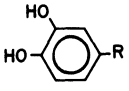
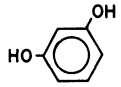
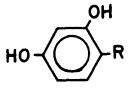
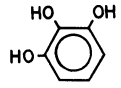
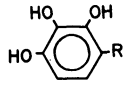
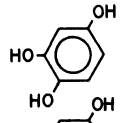
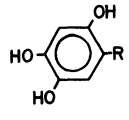
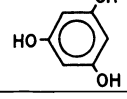
The catalytic property of β-tyrosinase, as an example, will be described in some detail (10). Table 1 shows comparative substrate affinities and activities of β-tyrosinase in the catalysis of α,β-elimination and β-replacement reactions. In these reactions, L-tyrosine, L-serine,

TABLE 1. Comparative substrate affinities and activities of β-tyrosinase in catalysis of different reactions

| Compound | Role | Product measured | Km (mM) | Ki (mM) | Vmax (μmole/min/mg) |
|---|---|---|---|---|---|
| α,β-Elimination reactions | | | | | |
| L-Tyrosine | Substrate | Pyruvate | 0.23 | | 1.9 |
| L-Serine | Substrate | Pyruvate | 34 | | 0.35 |
| S-Methyl-L-cysteine | Substrate | Pyruvate | 1.8 | | 1.2 |
| β-Chloro-L-alanine | Substrate | Pyruvate | 4.5 | | 18.2 |
| L-Alanine | Inhibitor | Pyruvate | | 6.5 | |
| L-Phenylalanine | Inhibitor | Pyruvate | | 2.0 | |
| Phenol | Inhibitor | Pyruvate | | 0.04 | |
| Pyrocatechol | Inhibitor | Pyruvate | | 0.46 | |
| Resorcinol | Inhibitor | Pyruvate | | 0.16 | |
| β-Replacement reactions | | | | | |
| L-Serine | Cosubstrate | L-Tyrosine | 35 | | 0.33 |
| S-Methyl-L-cysteine | Cosubstrate | L-Tyrosine | 1.8 | | 0.82 |
| β-Chloro-L-alanine | Cosubstrate | L-Tyrosine | 4.5 | | 1.4 |
| Phenol | Cosubstrate | L-Tyrosine | 1.2 | | |

S-methyl-L-cysteine and specifically an unnatural amino acid, β-chloro-L-alanine, are substrates: L-alanine and L-phenylalanine are competitive inhibitors. In the β-replacement reaction, phenol is the second substrate to synthesize L-tyrosine. When pyrocatechol, resorcinol, pyrogallol and hydroxyhydroquinone were added to the reaction mixture, in place of phenol, 3,4-dihydroxyphenyl-L-alanine (L-dopa), 2,4-dihydroxyphenyl-L-alanine (2,4-L-dopa), 2,3,4-trihydroxyphenyl-L-alanine (2,3,4-L-topa) and 2,4,5-trihydroxyphenyl-L-alanine (2,4,5-L-topa), respectively, were synthesized (Table 2).

TABLE 2. Relative velocity of synthesis of L-tyrosine related amino acids from β-chloro-L-alanine and phenol derivatives by β-tyrosinase

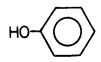
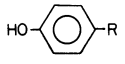
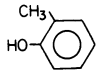
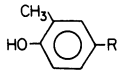
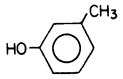
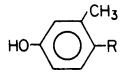
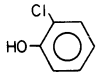
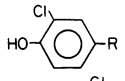
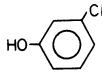
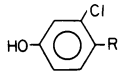
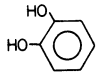
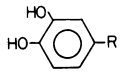
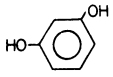
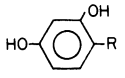
| Phenol derivative | L-Amino acid[a)] synthesized | Relative velocity of synthesis |
|---|---|---|
|  |  | 100 |
|  |  | 26.0 |
|  |  | 30.5 |
|  |  | 9.2 |
|  |  | 3.2 |
|  | — | 0 |

a) R represents L-alanyl moiety.

The synthesis of L-tyrosine from phenol, pyruvate and ammonia is catalyzed by β-tyrosinase through the reverse of the α,β-elimination reaction. This synthesis proceeds as a function of the concentration of phenol, pyruvate and ammonia. Michaelis-Menten kinetics were observed with pyruvate and ammonia, but phenol showed strong substrate inhibition at high concentrations. The Km values for phenol, pyruvate and ammonia, obtained by using three substrate kinetics, were 1.1, 12 and 20 mM, respectively (see Fig. 2), and the maximum velocity was

3.3 μmoles/min/mg of protein, which was about 1.5 times higher than that of L-tyrosine degradation through the α,β-elimination reaction.
When cresols (*m*- and *o*-) and chlorophenols (*m*- and *o*-) were added to the reaction mixture, in place of phenol, methyl-L-tyrosine (2- and 3-) and chloro-L-tyrosine (2- and 3-), respectively, were synthesized (Table 3).

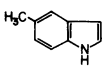
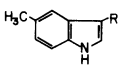
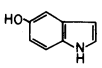
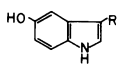
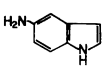
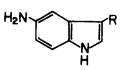
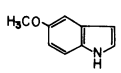
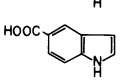
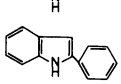
TABLE 3. Relative velocity of synthesis of L-tyrosine related amino acods from pyruvate, ammonia and phenol derivatives by β-tyrosinase

| Phenol derivative | L-Amino acid[a] synthesized | Relative velocity of synthesis |
|---|---|---|
|  |  | 100 |
|  |  | 45.4 |
|  |  | 52.7 |
|  |  | 47.3 |
|  |  | 32.7 |
|  |  | 60.0 |
|  |  | 58.2 |

a) R represents L-alanyl moiety.

All the tyrosine related amino acids synthesized by β-tyrosinase were isolated by ion exchange chromatography. Their chemical structures were identified directly or as acetyl derivatives by the analyses of pmr, mass spectra and optical rotation.
Like β-tyrosinase, tryptophanase and cysteine desulfhydrase catalyze the synthetic reactions

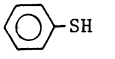
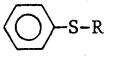
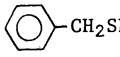
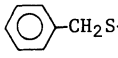
TABLE 4. Relative velocity of synthesis of L-tryptophan related amino acids from pyruvate, ammonia and indole derivatives by tryptophanase

| Indole derivative | L-Amino acid[a] synthesized | Relative velocity of synthesis |
|---|---|---|
|  |  | 100 |
|  |  | 60.7 |
|  |  | 39.6 |
|  |  | 19.6 |
|  | – | 0 |
|  | – | 0 |
|  | – | 0 |
|  | – | 0 |

a) R represents L-alanyl moiety.

through the β-replacement and the reverse of the α,β-elimination reactions.
Table 4 shows the tryptophanase-catalyzed synthesis of L-tryptophan related amino acids through the reverse of the α,β-elimination reactions. L-Tryptophan was synthesized by tryptophanase from indole, pyruvate and ammonia. When indole was replaced by 5-methyl-, 5-hydroxy- and 5-amino-indoles, the 5-methyl-, 5-hydroxy- and 5-amino-L-tryptophans, respectively, were synthesized (12).
Table 5 shows the cysteine desulfhydrase-catalyzed synthesis of L-cysteine related amino acids through the β-replacement reactions between β-chloro-L-alanine and sulfide or mercaptans.

TABLE 5. Relative velocity of synthesis of L-cysteine related amino acids from β-chloro-L-alanine and mercaptans by cysteine desulfhydrase

| Mercaptan | L-Amino acid[a] synthesized | Relative velocity of synthesis |
|---|---|---|
| $H_2S$ | HS-R | 100 |
| $CH_3SH$ | $CH_3S$-R | 0.6 |
| $CH_3CH_2SH$ | $CH_3CH_2S$-R | 2.0 |
| $CH_3(CH_2)_2SH$ | $CH_3(CH_2)_2S$-R | 0.49[b] |
| $CH_2$=CH-$CH_2SH$ | $CH_2$=CH-$CH_2S$-R | 7.2 |
| $CH_3(CH_2)_3SH$ | $CH_3(CH_2)_3S$-R | 0.13[b] |
| $(CH_3)_2CHCH_2SH$ | $(CH_3)_2CHCH_2S$-R | 0.09[b] |
| $CH_3CH_2(CH_3)CHSH$ | $CH_3CH_2(CH_3)CHS$-R | 0.05[b] |
| $(CH_3)_3CSH$ | $(CH_3)_3CS$-R | 0.04[b] |
|  |  | 0.02[c] |
|  |  | 0.17 |

a) R represents L-alanyl moiety.
b) S-Ethyl-L-cysteine was used as the standard for the determination.
c) S-Benzyl-L-cysteine was used as the standard for the determination.

L-Cysteine, S-methyl-, S-ethyl-, S-propyl-, S-butyl-, S-phenyl- and S-benzyl-L-cysteines were synthesized (14).
Table 6 shows the cysteine desulfhydrase-catalyzed synthesis of S-alkyl-L-cysteines through the reverse of the α,β-elimination reactions (15).

TABLE 6. Relative velocity of synthesis of S-alkyl-L-cysteines from pyruvate, ammonia and alkylmercaptans by cysteine desulfhydrase

| Mercaptan | L-Amino acid[a] synthesized | Relative velocity of synthesis |
|---|---|---|
| $CH_3SH$ | $CH_3S$-R | 100 |
| $CH_3CH_2SH$ | $CH_3CH_2S$-R | 131.2 |
| $CH_3CH_2CH_2SH$ | $CH_3CH_2CH_2S$-R | 43.3 |

a) R represents L-alanyl moiety.

## REACTION MECHANISM

The mechanisms for these multifunctional pyridoxal enzymes can be explained by the general mechanism for pyridoxal-dependent reactions, in which the enzyme-α-amino acrylate complex is a common key intermediate for all the α,β-elimination, β-replacement and reverse reactions (Fig. 1) (10,16).
Some details of the mechanism for the reverse reaction which was investigated with β-tyrosinase follow (10).

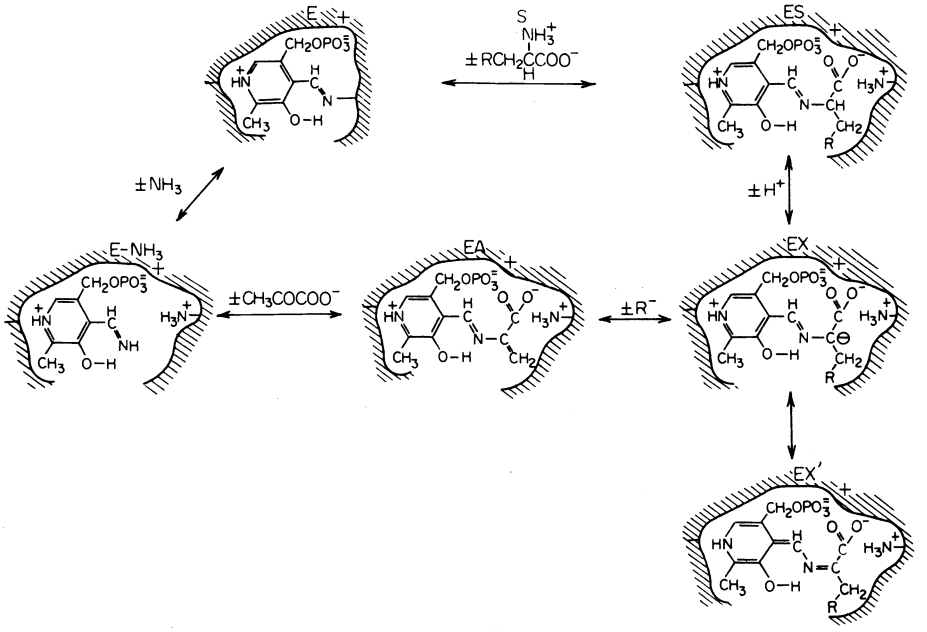


Fig. 1. Schematic representation of the mechanism for the reactions catalyzed by β-tyrosinase. E, ES, etc., represent species of the enzyme and enzyme-substrate complexes.

Kinetic studies of this three-substrate reaction show that the reaction proceeds with the ordered Ter-Uni mechanism of Cleland (17) and that pyruvate may be the second substrate to combine with the enzyme. When the concentration of pyruvate was set for infinity, a parallel

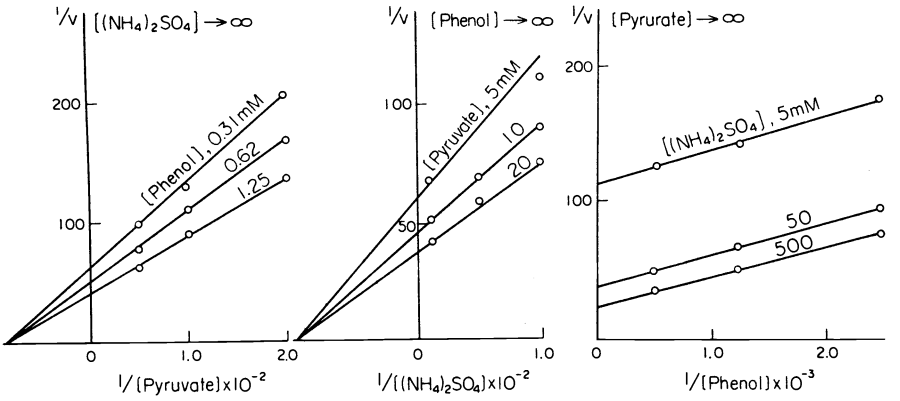



Fig. 2. Kinetic attempts to establish the order of addition of phenol, pyruvate and ammonia to β-tyrosinase during synthesis of L-tyrosine.

set of the plots was obtained (Fig. 2). A spectral study shown in Fig. 3 suggests that ammonia is the first substrate and that phenol should be the third.
This mechanism was further confirmed by proton exchange during incubation of the enzyme with ammonia and pyruvate, but without phenol. If the enzyme-bound α-amino acrylate is formed from these two substrates, proton exchange at the C-3 of pyruvate should occur during incubation of the enzyme with ammonia and pyruvate (Fig. 4). The β-tyrosinase-dependent incorporation of tritium or deuterium into pyruvate did, in fact, occur when the reaction was carried out in $T_2O$ or $D_2O$.

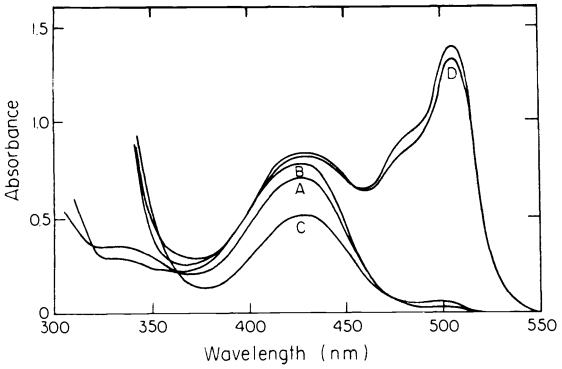



Fig. 3. Absorption spectra of holo-β-tyrosinase in the presence of ammonia, pyruvate and phenol. Curve A: holo-β-tyrosinase; curve B: holo-β-tyrosinase plus ammonia; curve C: holo-β-tyrosinase plus ammonia and pyruvate; curve D: holo-β-tyrosinase plus ammonia, pyruvate and phenol.

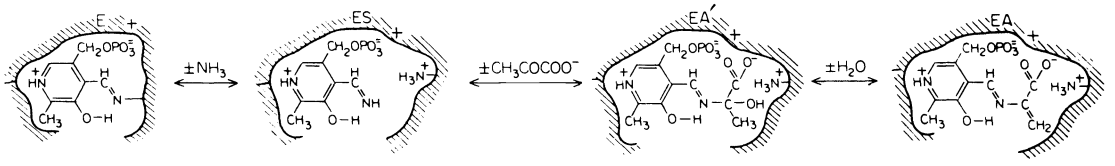



Fig. 4. Schematic representation of the mechanism for the synthesis of enzyme-α-amino acrylate complex catalyzed by β-tyrosinase. E, ES, etc., represent species of the enzyme and enzyme-substrate complexes.

## THE PRODUCTION OF L-TYROSINE, L-TRYPTOPHAN, L-CYSTEINE AND THEIR RELATED AMINO ACIDS

Based on results obtained with crystalline β-tyrosinase, tryptophanase and cysteine desulfhydrase, we developed enzymatic processes to produce L-tyrosine, L-tryptophan, L-cysteine and their related amino acids. As a practical application, bacterial cells which contained high

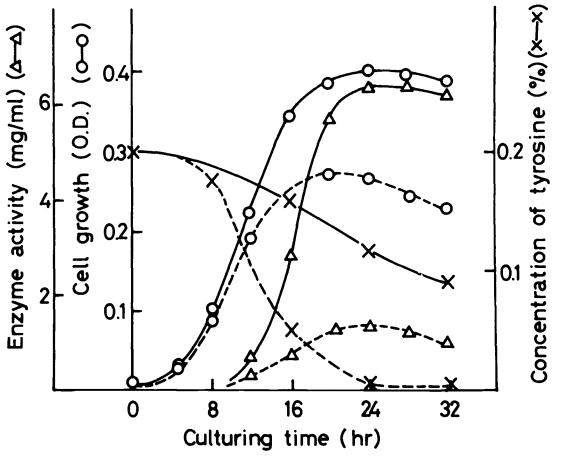



Fig. 5. Time course of the formation of β-tyrosinase in the presence (——) or absence (----) of L-phenylalanine. L-Phenylalanine was added to the medium at the concentration of 0.1%. The growth was determined by measuring the optical density at 562 nm of the culture broth after it was diluted 26 times. The enzyme activity was determined by measuring the amount of L-dopa synthesized in a reaction mixture containing 200 mg of DL-serine, 100 mg of pyrocatechol, 50 mg of ammonium acetate, 20 mg of sodium sulfite, 10 mg of EDTA and the cells harvested from 10 ml of the culture broth in a total volume of 10 ml (pH 8.0). The mixture was incubated at 22°C for 1 hour.

enzyme activities were used directly as enzymes.
Enzymatic processes for the production of L-dopa, L-tyrosine, L-tryptophan and L-cysteine are described below.

Production of L-dopa and L-tyrosine

A strain of *Erwinia herbicola* (ATCC 21434) was selected as a likely source of β-tyrosinase (18). Cells were grown at 28°C for 28 hours in a medium containing 0.2% L-tyrosine, 0.2% $K_2HPO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 2 ppm $Fe^{2+}$ ($FeSO_4 \cdot 7H_2O$), 0.01% pyridoxine-HCl, 0.6% glycerol, 0.5% succinic acid, 0.1% DL-methionine, 0.2% DL-alanine, 0.05% glycine, 0.1% L-phenylalanine and 12 ml of hydrolyzed soybean protein in 100 ml of tap water, with the pH maintained at 7.5 throughout cultivation. β-Tyrosinase is an inducible enzyme and the addition of L-tyrosine to the medium was essential for its formation. However, when large amounts of L-tyrosine were added, both enzyme formation and cell growth were repressed by the phenol liberated from L-tyrosine. L-Phenylalanine did not induce the enzyme by itself, but it had a strong synergistic effect on the induction of the enzyme by L-tyrosine (19). L-Phenylalnine is a competitive inhibitor of the enzyme (see Table 1) and maintained the concentration of L-tyrosine in the medium during cultivation (Fig. 5). Under these culture conditions, β-tyrosinase was effectively accumulated in the cells of *E. herbicola*, and made up about 10% of the total soluble cellular protein.
Synthesis of L-dopa through the β-replacement reaction between DL-serine and pyrocatechol was carried out at 22°C in the reaction mixture shown in Table 7. Under these conditions, about 5.5 g of L-dopa was synthesized in 100 ml of the reaction mixture (Fig. 6) (20). Synthesis of L-tyrosine through the reverse of the α,β-elimination reaction was carried out at 37°C for 10 hours in the reaction mixture shown in Tables 8 and 9. More than 6.0 g of L-tyrosine was synthesized in 100 ml of the reaction mixture (Table 9) (21).

TABLE 7. Reaction mixture for enzymatic synthesis of L-dopa

| | |
|---|---|
| DL-Serine[a)] | 2.0 g/100 ml |
| Ammonium acetate | 1.0 |
| Pyrocatechol[a)] | 0.7 |
| $Na_2SO_3$ | 0.2 |
| EDTA | 0.1 |
| Cells[b)] | |
| pH 8.0 | |

a) At 4 hour intervals, pyrocatechol was fed to maintain the initial concentration, then after incubation for 8 and 16 hours 1 g of DL-serine was added.
b) The cells harvested from 100 ml of the cultured broth were added.

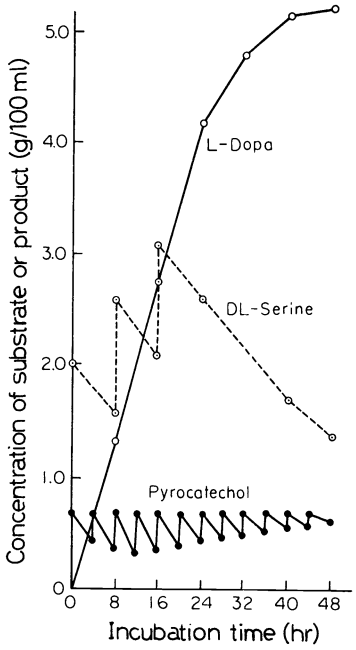



Fig. 6. Synthesis of L-dopa from DL-serine and pyrocatechol by the cells of *Erwinia herbicola*.

TABLE 8. Reaction mixture for enzymatic synthesis of L-tyrosine

| | |
|---|---|
| Sodium pyruvate[a)] | |
| Ammonium acetate | 5.0 g/100 ml |
| Phenol[b)] | 1.0 |
| Cells[c)] | |
| pH 8.0 | |

a) Sodium pyruvate was added as indicated in Table 9.
b) Phenol was fed at 2 hour intervals to maintain the initial concentration.
c) The cells harvested from 100 ml of the cultured broth were added.

TABLE 9. Effect of pyruvate concentration on the synthesis of L-tyrosine

| Sodium pyruvate added (g) | | | | Phenol consumed (g) | L-Tyrosine synthesized (g) |
|---|---|---|---|---|---|
| 0 | 2 | 4 | 6 | | |
| | (hours) | | | | |
| 0.4 | 0 | 0 | 0 | 3.90 | 5.80 |
| 0.2 | 0 | 0.2 | 0 | 3.50 | 6.05 |
| 0.1 | 0.1 | 0.1 | 0.1 | 2.33 | 3.50 |

At high concentrations phenol and its derivatives showed strong substrate inhibition in the β-tyrosinase-catalyzed reactions, as stated previously. They were added at intervals during incubation to maintain the optimum concentration for the synthesis of L-tyrosine related amino acids.

Production of L-tryptophan

A strain of *Proteus rettgeri* (AJ 1770) was selected as the source of tryptophanase (22). Cells were grown at 28°C for 16 hours in a medium containing 0.6% L-tryptophan, 4% Sorpol W-200 (polyoxyethylene alkyl phenol ether), 1% soybean protein hydrolyzate, 6% corn steep liquor, 0.3% yeast extract, 0.3% succinic acid, 0.06% L-cystine, 0.06% L-arginine-HCl, 0.03% DL-methionine, 0.03% L-proline, 0.3% $KH_2PO_4$ and 0.1% $MgSO_4 \cdot 7H_2O$ in tap water, with the pH adjusted to 7.0. An addition of L-tryptophan was essential for enzyme formation. However, formation was inhibited by the indole liberated from the added L-tryptophan. In fact, formation of the enzyme was enhanced by adding Sorpol W-200 which removed the indole from the medium (Fig. 7) (23). Tryptophanase was accumulated in the cells of *P. rettgeri* as about 6% of the total cellular protein.

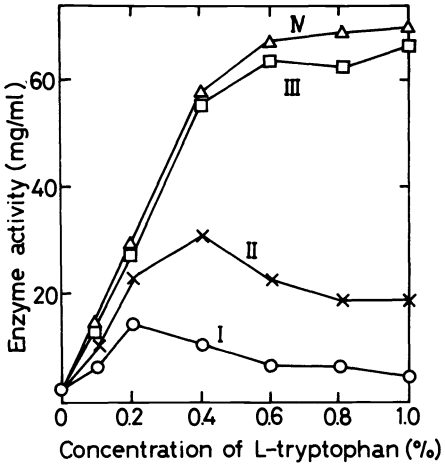



Fig. 7. Effect of L-tryptophan and Sorpol W-200 on the formation of tryptophanase. Sorpol W-200 was added to the basal medium at the concentrations of 0 (I), 1 (II), 3 (III) and 5% (IV). The enzyme activity was determined by measuring the amount of L-tryptophan synthesized in a reaction mixture containing 200 mg of sodium pyruvate, 200 mg of ammonium acetate, 150 mg of indole, 0.5 mg of PLP, 5 mg of sodium sulfite and the cells harvested from 5 ml of the culture broth in a total volume of 5 ml. After the pH was adjusted to 8.8, the mixture was incubated at 37°C for 12 hours.

Synthesis of L-tryptophan through the reverse of the α,β-elimination reaction was carried out at 34°C in the reaction mixture shown in Table 10. Inosine was added to the mixture which then formed an insoluble complex with the synthesized tryptophan. Under these conditions, the indole added to the mixture was almost quantitatively converted to L-tryptophan, producing about 10 g of L-tryptophan in 100 ml of the reaction mixture (Fig. 8) (24).

TABLE 10. Reaction mixture for enzymatic synthesis of L-tryptophan

| | |
|---|---|
| Sodium pyruvate | 8.0 g |
| Ammonium acetate | 8.0 |
| Indole | 6.0 |
| Inosine | 14.0 |
| $Na_2SO_3$ | 0.1 |
| PLP | 0.01 |
| Methanol | 10.0 ml |
| Cultured broth | 100.0 |
| pH 8.8 | |

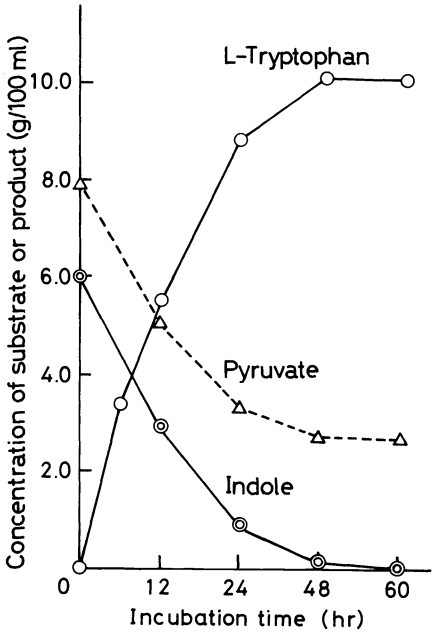



Fig. 8. Synthesis of L-tryptophan from pyruvate, ammonia and indole by the cultured broth of *Proteus rettgeri*.

Production of L-cysteine

A strain of *Enterobacter cloacae* (NCTC 10005) was selected as the source of cysteine desulfhydrase. Cells were grown at 30°C for 20 hours in a medium containing 0.2% L-cysteine, 0.2% $KH_2PO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.05% pyridoxine-HCl, 1.0% glucose, 1.0% yeast powder and 4 ml of hydrolyzed soybean protein in 100 ml of tap water, with the pH adjusted to 7.2 (25). L-Cysteine was synthesized through the β-replacement reaction between β-chloro-L-alanine and $Na_2S$ at 30°C in the reaction mixture shown in Table 11.

TABLE 11. Reaction mixture for enzymatic synthesis of L-cysteine

| | |
|---|---|
| β-Chloro-L-alanine | 6.0 g/100 ml |
| $Na_2S \cdot 9H_2O$ | 12.0 |
| Acetone | 8.0 |
| $NH_4Cl$ | 4.0 |
| PLP | 0.003 |
| Sodium dodecylsulfate | 0.05 |
| Cells[a)] | |
| pH 9.5 | |

a) The cells harvested from 100 ml of the cultured broth were added.

As stated previously, enzyme-bound α-amino acrylate is the key intermediate. The synthesized L-cysteine reacts with this intermediate to form a complex, 2-methyl-1,4-thiazolidine dicarboxylate (Fig. 9) (26) and, therefore, inhibits the further synthesis of L-cysteine. The synthesis of L-cysteine was markedly enhanced by adding acetone to the mixture (Fig. 10). Acetone may repress the formation of this complex between L-cysteine and the key intermediate.

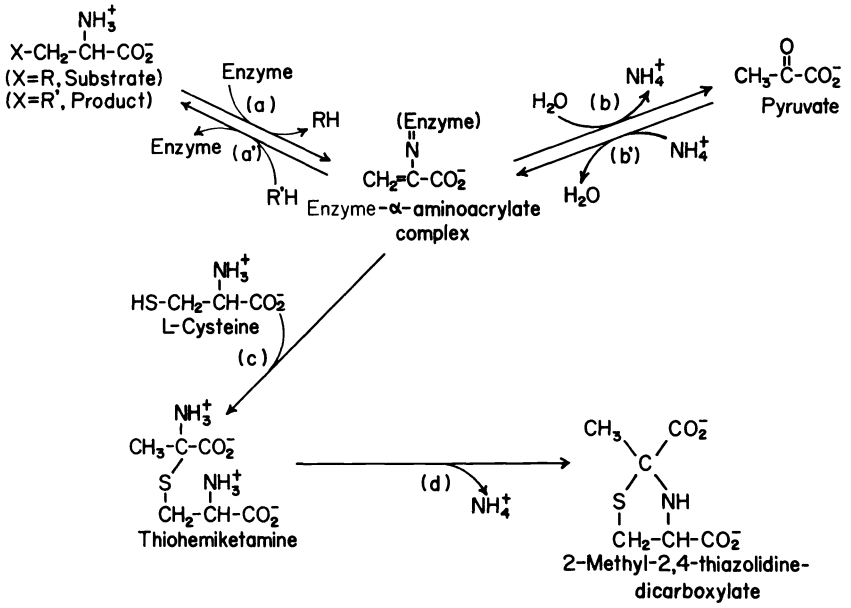



Fig. 9. Schematic representation of the mechanism for the formation of 2-methyl-2,4-thiazolidine dicarboxylic acid catalyzed by cysteine desulfhydrase.

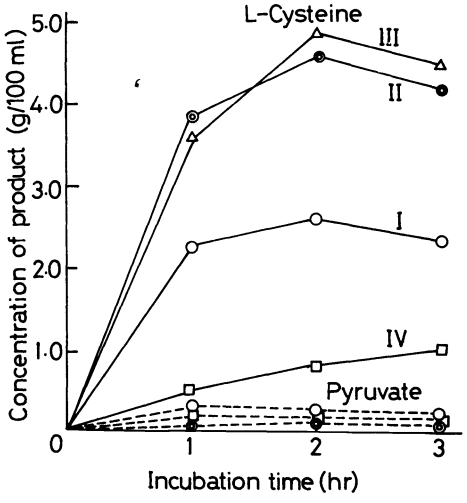



Fig. 10. Effect of acetone concentration on the synthesis of L-cysteine and on the formation of pyruvate by the cells of *Enterobacter cloacae*. Acetone was added to the reaction mixture at the concentrations of 0 (I), 4 (II), 8 (III) and 16% (IV).

Under the conditions used, the β-chloro-L-alanine added to the mixture was effectively converted to L-cysteine with a yield of more than 80% (see Fig. 10) (25).
The synthesized cysteine was isolated as L-cystine after the reaction mixture had been oxidized by bubbling oxygen in the presence of $FeCl_3$.

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