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<特 別 講 演 要 旨>

Microbiological Synthesis of L-Tyrosine and 3, 4-Dihydroxy-Phenyl-L-Alanine

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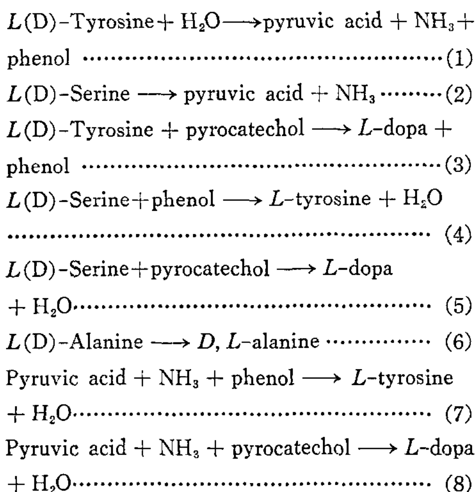
Tyrosine phenol lyase is an enzyme which catalyzes the stoichio-metric conversion of *L*-tyrosine to pyruvic acid, ammonia and phenol, and requires pyridoxal phosphate as a cofactor. Apparently homogeneous preparations of the enzyme were prepared in our laboratory from cells of *Escherichia intermdia* and *Erwinia herbicola* grown in media supplemented with *L*-tyrosine. We reported that crystalline preparations of the enzyme catalyze a series of α, β-elimination (1, 2), β-replacement (3, 4, 5) and racemization (6) reactions. The reverse of the α, β-elimination reaction to synthesize *L*-tyrosine and 3, 4-dihydroxypheny 1-*L*-alanine(*L*-dopa) (7, 8) was also catalyzed by crystalline preparations of the enzyme.



L(D)-Tyrosine + H_2O ⟶ pyruvic acid + NH_3 + phenol ....(1)

L(D)-Serine ⟶ pyruvic acid + NH_3 ....(2)

L(D)-Tyrosine + pyrocatechol ⟶ *L*-dopa + phenol ....(3)

L(D)-Serine + phenol ⟶ *L*-tyrosine + H_2O ....(4)

L(D)-Serine + pyrocatechol ⟶ *L*-dopa + H_2O ....(5)

L(D)-Alanine ⟶ *D*, *L*-alanine ....(6)

Pyruvic acid + NH_3 + phenol ⟶ *L*-tyrosine + H_2O ....(7)

Pyruvic acid + NH_3 + pyrocatechol ⟶ *L*-dopa + H_2O ....(8)

In recent studies, we proved that this enzyme catalyzes the synthesis of *L*-tyrosine or *L*-dopa from *D, L*-serine and phenol or pyrocatechol or from pyruvic acid, ammonia and phenol or pyro catechol; in significantly high yields. We herein report the enzymatic methods for preparations of *L*-tyrosine and *L*-dopa.

Tyrosine phenol lyase has been found in growing cells of the following bacteria: *Erwinia herbicola*, *Escherichia coli*, *E. intermedia*. *E. freundii*, *Proteus morganii*, *aerobacter aerogenes*, *etc.* Cells of *Erwinia herbicola*(ATCC 21434) were selected as a likely source of enzyme for the present investigation. The culture was carried out at 27° for 28 hours in 500 ml flasks with reciprocal shaking, using 60 ml of a culture medium supplemented with *L*-tyrosine as inducer. The synthesis of *L*-tyrosine was done for 10-15 hours in a reaction mixture containing phenol, *D, L*-serine or pyruvic acid and ammonia as substrate, with intact cells directly as enzyme. The synthesis of *L*-dopa was done for 24-48 hours in a reaction mixture containing pyrocatechol, *D, L*-serine or pyruvic acid and ammonia as substrate, with intact cells as enzyme.

A summary of the results of present investigation follows.

(A) Culture conditions for prrparation of cells containing high tyrosine phenol lyase activity. Since intact cells were used directly as the enzyme in this synthetic reaction, we considered it necessary to establish the culture conditions under which cells would grow well and the enzyme would be sufficiently accumlated in growing cells. The addition of *L*-tyrosine, as inducer, in the culture medium was essential for formation of the enzyme. However, when large amounts of *L*-tyrcsine were added, inhibiton of enzyme forma-

tion was caused by phenol liberated from *L*-tyrosine. In fact, the formation of enzeym was enhanced by removal of phenolt during cultivation. *L*(*D*)-Phenylalanine or phenylpyruvic acid gave a synergistic effect on the induction of enzyme by *L*-tyrosine. Addition of pyridoxine to the medium enhanced enzyme formation, suggesting that it was utilized as a precursor of the coenzyme, pyridoxal phosphate. Metallic ions, ferrus ion; amino acids, *D*, *L*-methionine, *D*, *L*-alanine and glycine; and glycerol plus succinic acid promoted enzyme formation as well as cell growth.

Cells with higher enzyme activity were prepared by growing the cells in a medium containing 0. 2 % *L*-tyrosine, 0. 2% KH_2PO_4, 0. 1% $MgSO_4.7H_2O$ 0. 0001% $FeSO_4$ $7H_2O$, 0. 01% pyridoxine, 0. 6 % glycerol, 0. 5% succinic acid, 0. 1% *D*. *L*-methionine, 0. 2% *D*, *L*-alanine, 0. 05% glycine, 0. 1% *L*-phenylalanine and 12 m*l*/d*l* hydrolyzed soybean protein in tap water; controlling the pH at 7. 5 throughout cultivation.

(B) Reaction conditions to synthesize *L*-tyrosine an d*L*-dopa. The optimum pH for this synthetic reaction was around 8. 0. The optimal temperature ranges of the reaction were at 37-40° for the synthesis of *L*-tyroine and at 15-25° for that of *L*-dopa. To keep the synthesized *L*-dopa stable in the reaction mixture, sodium sulfite and EDTA were added. As the high concentration of phenol or pyrocatechol denatured the enzyme, phenol or pyrocatechol was fed to maintain the optimum concentration for the reaction during incubation.

In the synthetic reaction with *D*, *L*-serine as substrate, a reaction mixture (100ml) containing 4. 0 g of *D*, *L*-serine, 1. 0 g of phenol or 0. 7 g of pyrocatechol, 0. 5 g of ammonium acetate and the cells, was fed to maintain the initial concentration. Under these conditions, 5. 35 g of *L*-tyrosine or 5. 10 g of *L*-dopa was synthesized in the reaction mixture. For the synthetic reaction with pyruvic acid as substrate, a reaction mixture(100ml) containing 0. 5-2. 0 g of pyruvic acid, 0. 8 g of phenol or pyrocatechol, 5. 0 g of ammonium acetate and the cells, was incubated. At intervals pyruvic acid and phenol or pyrocatechol were added. Under these conditions, 6. 05 g of *L*-tyrosine or 5. 85 g of *L*-dopa was synthesized. When a higher concentration of pyruvic acid was added to the reaction mixture of *L*-dopa, a spontaneous condensation product, *L*-dopa with pyruvic acid, was formed.

The enzymatic method described here is simple and is one of the most economical processes to date, for the preparation of *L*-tyrosine and *L*-dopa from the synthetic starting materials *D*, *L*-serine, pyıuvic acid, phenol and pyrocatechol.

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