Synthesis of L-3,4-Dihydroxyphenylalanine from L-Tyrosine by Microorganisms

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L-3,4-Dihydroxyphenylalanine can be synthesized efficiently from L-tyrosine under acidic conditions by Aspergillus oryzae and other fungi.

Since L-3,4-dihydroxyphenylalanine (L-dopa) attracted notice as a specific drug for Parkinson's disease, reports concerning its microbial production have appeared (2, 4). Sih et al. (4) reported that the microbial conversion of L-tyrosine to L-dopa is possible with about 30% yield by the introduction of N-blocking groups into L-tyrosine to inhibit deaminase activity. L-Dopa is synthesized from L-tyrosine by a one-step oxidation reaction catalyzed by tyrosinase or tyrosine hydroxylase and is an important intermediate in the biological metabolic pathway from L-tyrosine to melanin or to catecholamines. We screened microorganisms which catalyze L-dopa synthesis from L-tyrosine and found several which carry out this reaction efficiently.

Tyrosinase activity in microorganisms is generally very weak. L-Tyrosine and L-dopa are rapidly decomposed to other metabolites; thus stoichiometric accumulation of L-dopa from L-tyrosine was very difficult to achieve. It was found that, when intact cells or an enzyme solution of fungi was used, L-dopa synthesis from L-tyrosine could proceed preferentially under acidic conditions. Of the fungi, Aspergillus species were especially active. A. chevalieri IFO4086 and A. oryzae IAM2625 were used in the following experiments.

Fungal cells were cultured aerobically in shaken flasks in a medium composed of 2% glucose, 0.3% NH₄Cl, 0.3% KH₂PO₄, 0.02% MgSO₄·7H₂O, 1% polypeptide, and 1% yeast extract (Difco) at 30 C for 2 to 3 days. The cells were harvested by filtration, washed with distilled water, suspended in buffer solutions containing L-tyrosine, and incubated aerobically. Figure 1 shows that the pH of the reaction mixture is very important and that its optimum lies in the acidic range (pH 2 to 5). The hydroxylation reaction of L-dopa from L-tyrosine at acidic pH proceeded preferentially, and degradative reaction of the L-dopa formed and of the L-tyrosine substrate scarcely occurred.

In the neutral or alkaline pH range, L-tyrosine as a substrate was decomposed to other metabolites, and L-dopa did not accumulate. In the acidic pH range, the molar ratio of L-dopa synthesis to L-tyrosine consumed was about 80% in the absence of L-ascorbic acid. The addition of L-ascorbic acid (2 mg/ml) to these reaction systems increased the L-dopa formation to about 100%, as was reported by Sih et al. (4).

The reaction temperature was 30 to 60 C, and the reaction occurred with sufficient oxygen supply. The reaction rate was very fast, and the reaction was completed in 1 or several hr. There was no need to supply cofactors to the reaction systems.

Fig. 1. Effect of pH on L-dopa formation. L-Tyrosine (0.1%) and 10% wet cells were used, and the reactions were carried out at 30 C for 2 hr. The following pH solutions were used: 0.1 N HCl at pH 1.0; 0.05 M KCl-HCl buffer at pH 2.0; 0.05 M acetate buffer at pH 3.5 to 5.5; 0.05 M phosphate buffer at pH 6 to 8. L-Dopa was measured by the method of Arnow (1, 3). Symbols: O, Aspergillus oryzae IAM2625; △, A. chevalieri IFO4086.
To identify the product, cells of *A. oryzae* IAM2625 were cultured for 45 hr in 250 ml of the medium mentioned above, 25 g of cells (wet) was suspended in 500 ml of 50 mm acetate buffer solution (pH 3.5) containing 0.25% L-tyrosine and 0.2% l-ascorbic acid, and the reaction was carried out for 2 hr at 45°C. After removal of the cells by filtration, the reaction mixture was passed through a Dowex 50-X (200 to 400 mesh) H⁺-form column (4) and eluted with 0.7 N HCl. L-Dopa was obtained in the 23rd to 49th fractions, and L-tyrosine was in the 57th to 83rd fractions. A 0.88-g amount of the product was obtained in crystalline form and identified. The mp was 275 to 278°C (decomposed); \( [\alpha]_{D}^{2} \) was -12.3° (c = 3.0, 1 N HCl). Elementary analysis: C, 54.76; H, 5.70; N, 6.99; calculated: C, 54.82; H, 5.62; N, 7.10; O, 32.46. The infrared absorption spectrum is shown in Fig. 2. The conditions described here may be employed with advantage for the preparation of this useful product.

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**LITERATURE CITED**