ENZYMATIC PREPARATION OF L-TRYPTOPHAN AND 5-HYDROXY-L-TRYPTOPHAN

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1. Introduction

Tryptophanase is known to catalyze the stoichiometric conversion of L-tryptophan to pyruvate, ammonia and indole eq. (1), and requires pyridoxal phosphate as a cofactor [1].

$$\text{L-Tryptophan} + H_2O \rightarrow CH_3COCOOH + NH_3 + \text{indole} \quad (1)$$

A homogeneous preparation of the enzyme was prepared by Newton et al. from cells of *Escherichia coli* B [2, 3]. They reported that the crystalline preparation of the enzyme catalyzes a series of α,β-elimination, eq. (2) and β-replacement, eq. (3) reactions.

$$R_ICH_2CHNH_2COOH + H_2O \rightarrow R_IH + CH_3COCOOH + NH_3 \quad (2)$$

$$R_ICH_2CHNH_2COOH + R_{II}H \rightarrow R_IH + R_{II}CH_2CHNH_2COOH \quad (3)$$

In equations (2) and (3), R_I may represent –OH, –SH, $-OCH_3$, $-SCH_3$ or indolyl radicals, and R_{II} represents indolyl radicals.

Subsequent to these investigations. we showed that [4–8] β-tyrosinase from *E. intermedia* and *Erwinia herbicola* catalyzes the conversion of tyrosine to pyruvate, ammonia and phenol, eq. (4), but not that of L-tryptophan.

$$\text{L-Tyrosine} + H_2O \rightarrow CH_3COCOOH + NH_3 + \text{phenol} \quad (4)$$

It catalyzes a series of α,β-elimination, eq. (5) and β-replacement, eq. (6), reactions, by a similar reaction mechanism as that of tryptophanase.

$$R_{III}CH_2CHNH_2COOH + H_2O \rightarrow R_{III}H + CH_3COCOOH + NH_3 \quad (5)$$

$$R_{III}CH_2CHNH_2COOH + R_{IV}H \rightarrow R_{III}H + R_{IV}CH_2CHNH_2COOH \quad (6)$$

In equations (5) and (6), R_{III} represents –OH, –SH, or phenolyl radicals, and R_{IV} phenolyl radicals. In recent studies, we [9] proved that β-tyrosine catalyze the synthesis of L-tyrosine from pyruvate, ammonia and phenol, apparently by the reversal of α,β-elimination reaction.

In appropriate studies, it has been proved that the crystalline tryptophanase from *E. coli* [10] and *Proteus rettgeri* [11] also catalyzes the synthesis of L-tryptophan by the reversal of α,β-elmination reaction; at rates similar to the forward reaction. We herein describe an enzymatic method for the preparation of L-tryptophan or 5-hydroxy-L-tryptophan from pyruvate, ammonia and indole or 5-hydroxy-indole, respectively.

2. Materials and methods

Tryptophanase has been found in growing cells of

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various bacteria belonging to genera of *Escherichia*, *Kluyvera*, *Enterobacter*, *Erwinia* and *Proteus*. The cells of *Proteus rettgeri* (Aj 2770) were selected as a likely source of enzyme for the present investigation. The cells with higher enzyme activity were prepared by growing them in a medium containing 0.6% L-tryptophan, 6% Sorpol W-200, 0.3% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% succinic acid, 0.06% L-cystine, 0.03% DL-methionine, 0.03% L-proline, 0.06% L-arginine, 6 ml of corn steep liquor, 1% hydrolyzed soybean protein and 0.3% yeast extract in tap water. Sorpol W-200 (polyoxyethylene alkyl phenol ether) was added to the medium to remove indole liberated from L-tryptophan. The pH of the medium was adjusted to 7.0. The culture was carried out at 28° for 16 hr in 500 ml shaking flasks containing 60 ml of the medium under shaking. The cultured broth was used in the present work, directly as the enzyme for the synthesis of L-tryptophan and 5-hydroxy-L-tryptophan.

L-Tryptophan and indole were determined by a modification of the method of Kupfer and Atkinson [12] and pyruvate by the method of Friedemann and Haugen [13].

3. Results and discussion

For the synthesis of L-tryptophan, a reaction mixture contained 6.0 g of indole in 10 ml of methanol, 8.0 g of sodium pyruvate, 8.0 g of ammonium acetate, 0.01 g of pyridoxal phosphate, 0.1 g of Na_2SO_3 and 100 ml of the cultured broth in a total volume of 120 ml. After the pH of the mixture was adjusted to 8.8 by 6 N KOH, it was incubated at 34° for 48 hr. Under these conditions, 7.5 g of L-tryptophan was synthesized, as shown in fig. 1.

The synthesized tryptophan was isolated from the reaction mixture. After incubation, 2 ml of 2 N HCl was added to the mixture and the precipitate formed was removed by centrifugation. Then, the supernatant was applied to an active charcoal column (4 × 20 cm). The column was first eluted with water, then with 0.3 N ammonia. The ammonia eluate was evaporated to dryness at 50° under reduced pressure, then the solid obtained was crystallized from water. Colorless crystals of tryptophan weighing 5.2 g were isolated from the reaction mixture. The isolated tryptophan

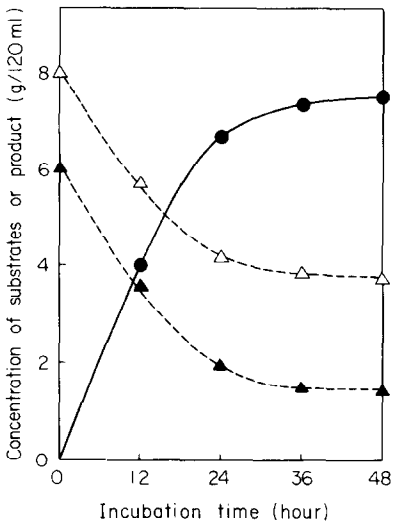

Fig. 1. Synthesis of L-tryptophan by tryptophanase. The reaction conditions were described in the text. In the figure, substrates were sodium pyruvate (△- - -△- - -△) and indole (▲- - -▲- - -▲); product was L-tryptophan (●—●—●).

was identical in all respects (melting point, IR- and NMR-spectra) with authentic L-tryptophan. The isolated tryptophan gave $[\alpha]_D^{20} = -31.5$ (c = 1 in H_2O).

Similarly, 5-hydroxy-L-tryptophan was synthesized from 5-hydroxyindole, pyruvate and ammonia. The reaction mixture contained 3 g of 5-hydroxyindole, 3 g of sodium pyruvate, 8.0 g of ammonium acetate, 0.01 g of pyridoxal phosphate, 0.1 g of Na_2SO_3 and 100 ml of the cultured broth in a total volume of 120 ml (pH adjusted to 8.8 with 6 N KOH). The mixture was incubated at 34° for 48 hr. Under these conditions, 2.8 g of 5-hydroxy-L-tryptophan was synthesized.

The synthesized 5-hydroxytryptophan was isolated from the reaction mixture by the same procedure as that for L-tryptophan. Crystals of 5-hydroxytryptophan of 1.5 g were isolated. The isolated 5-hydroxytryptophan was identical with authentic 5-hydroxy-L-tryptophan. The isolated 5-hydroxytryptophan gave $[\alpha]_D^{20} = -32.5$ (c = 1 in H_2O).

The enzymatic method described above is simple and is one of the most economical processes to date for the preparation of L-tryptophan and 5-hydroxy-L-tryptophan from synthetic starting materials such as sodium pyruvate, indole and 5-hydroxyindole.

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