ENZYMATIC PREPARATION OF L-TYROSINE OR 3,4-DIHYDROXYPHENYL-L-ALANINE FROM PYRUVATE, AMMONIA AND PHENOL OR PYROCATECHOL

H. ENEI, H. NAKAZAWA, H. MATSUI and S. OKUMURA
Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan
and
H. YAMADA*
Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan

Received 23 December 1971

1. Introduction

Tyrosine phenol lyase is an enzyme which catalyzes the stoichiometric conversion of L-tyrosine to pyruvate, ammonia and phenol, and requires pyridoxal phosphate as a cofactor [1–5]. Apparently homogeneous preparations of the enzyme were prepared in our laboratory from cells of *Escherichia intermedia* and *Erwinia herbicola* grown in media supplemented with L-tyrosine [4, 6]. We reported that the crystalline preparations of the enzyme catalyze a series of α,β-elimination [4, 6], β-replacement [7, 8] and racemization [9] reactions. The reverse of the α,β-elimination reaction to synthesize L-tyrosine from pyruvate, ammonia and phenol was also catalyzed by crystalline preparations of the enzyme [10]. In recent studies, we proved that L-tyrosine or 3, 4-dihydroxyphenyl-L-alanine (L-dopa) was synthesized from pyruvate, ammonia and phenol or pyrocatechol with intact cells directly as enzyme, in significantly high yields. We herein report an enzymatic method for the preparation of L-tyrosine and L-dopa.

2. Materials and methods

The cells of *E. herbicola* (ATCC 21434) were selected as a likely source of tyrosine phenol lyase in the present investigation. The cells with higher enzyme activity were prepared by growing them in a medium containing 0.2% L-tyrosine, 0.2% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.01% pyridoxine, 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/dl hydrolyzed soybean protein in tap water, with the pH controlled at 7.5 throughout cultivation.

L-tyrosine and L-dopa were determined using a Yanagimoto model LC-5S automatic amino acid analyzer. L-tyrosine was also determined by microbiological assay with a strain of *Leuconostoc mesenteroides* [11]. Pyruvate was measured by the method of Friedemann and Haugen [12]. Phenol and pyrocatechol were measured with a modification of the method of Porteous and Williams [13].

3. Results and discussion

Since the α,β-elimination reaction catalyzed by tyrosine phenol lyase is reversible [10] and the solubility of L-tyrosine or L-dopa is slight, the L-tyrosine or L-dopa synthesized by this enzymatic method precipitates during incubation. The apparent equilibrium is thought to tend toward the synthetic state when large amounts of substrates, i.e. pyruvate, ammonia and phenol or pyrocatechol, are added to the reaction mixture. Figs. 1 and 2 show this apparent tendency for equilibrium in these experiments.

Conditions for the enzymatic preparation of L-tyrosine or L-dopa were investigated on the basis of this finding. For the synthesis of L-tyrosine, the re-

* To whom requests for reprints should be addressed.

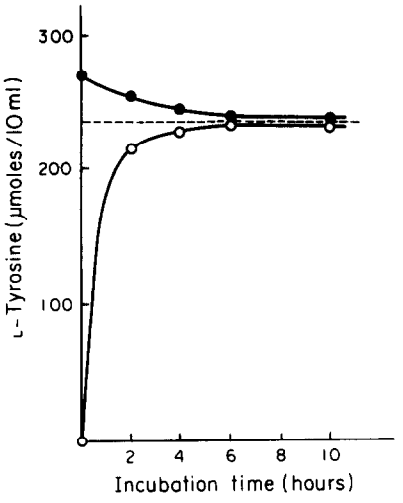

Fig. 1. Apparent equilibrium of synthesis and degradation of L-tyrosine by cells of *E. herbicola*. The reaction mixture for synthesis of L-tyrosine (○–○–○) contained 270 μmoles of phenol, 270 μmoles of sodium pyruvate and 2.7 mmoles of ammonium acetate buffer, pH 8.0, in a total vol of 10 ml. The cells harvested from 10 ml of the cultured broth were suspended in the reaction mixture, then the mixture was incubated at 37° for 10 hr. The reaction mixture for degradation of L-tyrosine (●–●–●) contained 270 μmoles of L-tyrosine and 2.7 mmoles of ammonium acetate buffer, pH 8.0, in a total vol of 10 ml. The cells were suspended as in the synthesis of L-tyrosine, then the mixture was incubated at 37° for 10 hr.

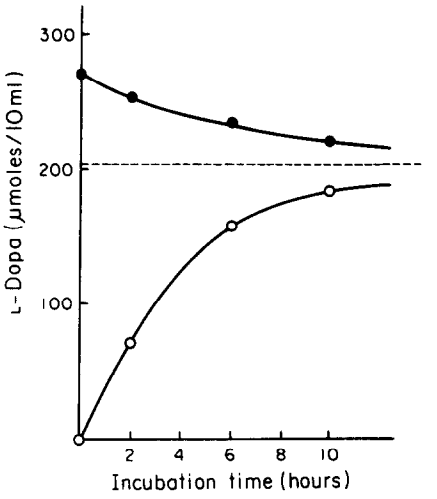

Fig. 2. Apparent equilibrium of synthesis and degradation of L-dopa by cells of *E. herbicola*. The reaction mixture for synthesis of L-dopa (○–○–○) contained 270 μmoles of pyrocatechol, 270 μmoles of sodium pyruvate, 2.7 mmoles of ammonium acetate buffer, pH 8.0, 80 μmoles of sodium sulfite and 25 μmoles of EDTA in a total vol of 10 ml. The cells harvested from 10 ml of the cultured broth were suspended in the reaction mixture, then the mixture was incubated at 15° for 10 hr. The reaction mixture for degradation of L-dopa (●–●–●) contained 270 μmoles of L-dopa, 2.7 mmoles of ammonium acetate buffer, pH 8.0, 80 μmoles of sodium sulfite and 25 μmoles of EDTA in a total vol of 10 ml. The cells were suspended as in the synthesis of L-dopa, then the mixture was incubated at 15° for 10 hr.

action mixture contained 1.0 g of phenol, 4.0 g of sodium pyruvate and 5.0 g of ammonium acetate in a total vol of 100 ml. The cells harvested from 100 ml of the cultured broth were suspended in the reaction mixture, then the pH was adjusted to 8.0 by adding ammonia. The mixture was incubated at 37° for 10 hr and at hourly intervals during incubation, phenol was fed to maintain the initial concentration. Under these conditions, 5.80 g of L-tyrosine was synthesized in the mixture, as shown in fig. 3.

In the synthesis of L-dopa, the reaction mixture contained 0.8 g of pyrocatechol, 0.5 g of sodium pyruvate, 5 g of ammonium acetate, 0.2 g of sodium sulfite and 0.1 g of EDTA in a total vol of 100 ml. The cells were suspended, then the pH was adjusted as in the synthesis of L-tyrosine. The mixture was incubated at 15° for 48 hr. At 2 hr intervals, pyrocatechol and sodium pyruvate were added to maintain the initial concentrations. To keep the synthesized L-dopa stable during incubation sodium sulfite and EDTA were added, and the temperature for incubation was kept lower than that of L-tyrosine. Under these conditions, 5.85 g of L-dopa was synthesized as shown in fig. 4.

Enzymatically synthesized L-tyrosine or L-dopa was isolated from a large-scale reaction mixture of 500 ml. The isolated tyrosine or dopa was identical in all respects (melting point, IR–, NMR-spectra and specific optical rotation) with authentic L-tyrosine or L-dopa. The isolated tyrosine afforded $[\alpha]_D^{25} = -11.9$ (c=5 in 1 N HCl) and dopa $[\alpha]_D^{25} = -11.8$ (c=3.7 in 4% HCl).

The enzymatic method described here is simple and efficient, and is the most economical process to date for the preparation of L-tyrosine and L-dopa from the starting materials of sodium pyruvate, phenol and pyrocatechol.

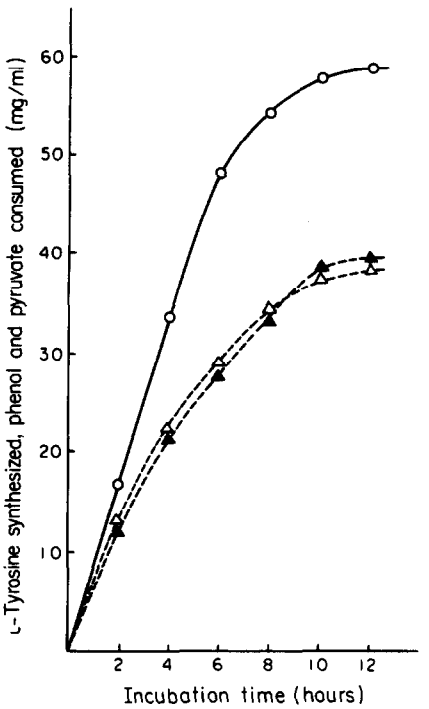

Fig. 3. Synthesis of L-tyrosine from phenol, pyruvate and ammonia by cells of *Erwinia herbicola.* The reaction conditions were described in the text. In the figure, (○—○—○) is L-tyrosine synthesized , (△--△--△) phenol consumed, and (▲--▲--▲) pyruvate consumed.

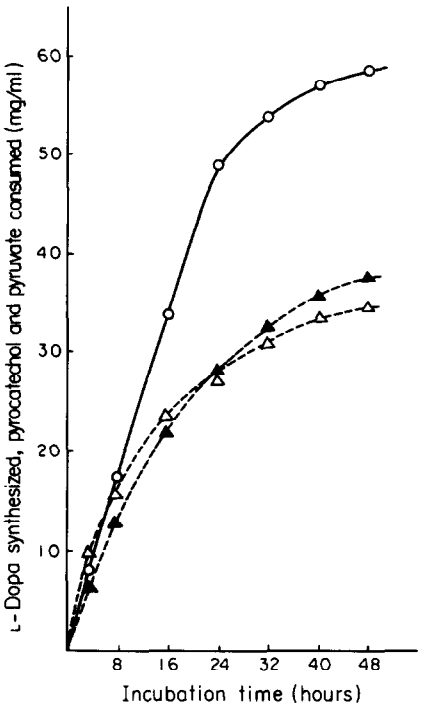

Fig. 4. Synthesis of L-dopa from pyrocatechol, pyruvate and ammonia by cells of *E. herbicola.* The reaction conditions were described in the text. In the figure, (○—○—○) is L-dopa synthesized, (△--△--△) pyrocatechol consumed, and (▲--▲--▲) pyruvate consumed.

Acknowledgements

We thank Dr. Koichi Ogata, Professor of the Department of Agricultural Chemistry, Kyoto University, and Dr. Dr. Toshinao Tsunoda, Director of the Central Research Laboratories, Ajinomoto Co., Inc., for their interest and valuable discussion during the course of this work.

References

[1] Y. Kakihara and K. Ichihara, Med. J. Osaka Univ. 3 (1953) 497.
[2] M. Uchida, Y. Taketomo, Y. Kakihara and K. Ichihara, Med. J. Osaka Univ. 3 (1953) 509.
[3] N. Brot, Z. Smit and H. Weissbach, Arch. Biochem. Biophys. 112 (1965) 1.
[4] H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi and K. Ogata, J. Biol. Chem. 245 (1970) 1767.
[5] H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi and K. Ogata, J. Biol. Chem. 245 (1970) 1773.
[6] H. Kumagai, N. Kashima, H. Torii, H. Yamada, H. Enei and S. Okumura, Agr. Biol. Chem., in press.
[7] H. Kumagai, H. Matsui, H. Ohkishi, K. Ogata, H. Yamada, T. Ueno and H. Fukami, Biochem. Biophys. Res. Commun. 34 (1969) 266.
[8] T. Ueno, H. Fukami, H. Ohkishi, H. Kumagai and H. Yamada, Biochim. Biophys. Acta 206 (1970) 476.
[9] H. Kumagai, N. Kashima and H. Yamada, Biochem. Biophys. Res. Commun. 39 (1970) 796.
[10] H. Yamada, H. Kumagai, N. Kashima, H. Torii, H. Enei and S. Okumura, Biochem. Biophys. Res. Commun., in press.
[11] G. Tamura, T. Tsunoda, J. Kirimura and M. Miyasawa, Nippon Nogeikagaku Kaishi 26 (1952) 464.
[12] T.E. Friedemann and G.E. Haugen, J. Biol. Chem. 147 (1943) 415.
[13] J.W. Porteous and R.T. Williams, Biochem. J. 44 (1949) 46.