SYNTHESIS OF *L*-TYROSINE FROM PYRUVATE, AMMONIA AND PHENOL BY CRYSTALLINE TYROSINE PHENOL LYASE

Hideaki Yamada, Hidehiko Kumagai, Nobukazu Kashima and Hiroshi Torii
Research Institute for Food Science, Kyoto University, Kyoto, Japan

and

Hitoshi Enei and Shinji Okumura
Central Research Laboratories, Ajinomoto Co., Ltd., Kawasaki, Japan

Received October 21, 1971

Summary

Synthesis of *L*-tyrosine from pyruvate, ammonia and phenol is catalyzed by crystalline tyrosine phenol lyase prepared from cells of *Escherichia intermedia*. The synthetic reaction proceeds optimally at pH 8.5-9.0, as a function of enzyme concentration and incubation time. The *Km* values for pyruvate and phenol are determined to be 5.1×10^{-3} M and 4.4×10^{-3} M, respectively, and the maximal velocity was 7.2 μmoles /min./mg of protein. Phenol is replaced by pyrocatechol and resorcinol to synthesize 3,4-dihydroxyphenyl-*L*-alanine and 2,4-dihydroxyphenyl-*L*-alanine, respectively. Addition of pyruvate, ammonia and phenol to holotyrosine phenol lyase results in the appearance of a new spectral band near 500 mμ which has been ascribed to the intermediates in many pyridoxal phosphate dependent reactions.

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[1-4]. 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[3,5]. We reported that the crystalline preparation of the enzyme catalyzes a series of α,β-elimination[3,5], β-replacement[6-8] and racemization reactions[9]. Recently, we proved that this enzyme catalyzes the synthesis

of *L*-tyrosine from pyruvate, ammonia and phenol by reversal of the elimination reaction. We herein describe the enzymatic synthesis of *L*-tyrosine by crystalline tyrosine phenol lyase.

Crystalline tyrosine phenol lyase was prepared from cells of *Escherichia intermedia* grown in a bouillon-peptone medium supplemented with *L*-tyrosine according to the method of Kumagai *et al*[3]. *L*-Tyrosine synthesized was determined by two methods: (a) ion-exchange chromatography with a Yanagimoto model LC-5S automatic amino acid analyzer; and (b) microbiological assay with a strain of *Leuconostoc mesenteroides*[10]. The two methods showed good agreement.

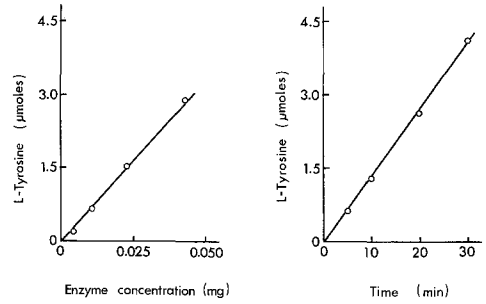

Fig. 1. Synthesis of *L*-tyrosine as a function of enzyme concentration and incubation time. The reactions were carried out at 30° in reaction mixtures containing 300 μmoles of potassium pyruvate, 150 μmoles of ammonium sulfate, 220 μmoles of phenol, 0.6 μmole of pyridoxal phosphate, 200 μmoles of ammonium chloride buffer, pH 9.0, and enzyme in a total volume of 4 ml. In Fig. 1A, incubation was made for 10 minutes and in Fig. 1B, 0.05 mg of enzyme protein was used.

When pyruvate, ammonia and phenol were incubated with crystalline tyrosine phenol lyase in the presence of pyridoxal phosphate, the synthesis of *L*-tyrosine proceeded as a function of enzyme concentration and incubation time (Fig. 1A and 1B). Without pyridoxal phosphate, or with boiled enzyme the synthesis of *L*-tyrosine was not observed.

Tyrosine phenol lyase, in the presence of ammonium chloride buffer, has an optimal reactivity in the pH range of 8.5-9.0 for the synthesis of *L*-tyrosine. The *Km* values for pyruvate and phenol were determined from

the Lineweaver and Burk plots[11], to be 5.1 x 10^{-3} M and 4.4 x 10^{-3} M, respectively, and the maximal velocity of synthesis was 7.2 μmoles/min./mg of enzyme protein.

Table I. Synthesis of 3,4-*L*-Dopa and 2,4-*L*-Dopa by Tyrosine Phenol Lyase

The reaction was carried out under the standard reaction conditions described in Fig.1, except that phenol was replaced by H_2S, CH_3SH, pyrocatechol or resorcinol. Incubation was carried out for 10 minutes with 0.10 mg of enzyme protein.

Reagent	Amino acid synthesized	μmoles
None		0
H_2S		0
CH_3SH		0
Pyrocatechol	3,4-*L*-dopa	1.87*
Resorcinol	2,4-*L*-dopa	0.94*
Phenol	*L*-tyrosine	2.95

*3,4- or 2,4-*L*-Dopa was determined by the automatic amino acid analyzer.

When phenol was replaced by pyrocatechol or resorcinol in the reaction mixture, 3,4-dihydroxyphenyl-*L*-alanine (3,4-*L*-dopa) or 2,4-dihydroxyphenyl-*L*-alanine[7] (2,4-*L*-dopa) was synthesized, respectively. However, hydrogen sulfide and methylmercaptan were inert in the synthetic reaction by tyrosine phenol lyase (Table I).

Holotyrosine phenol lyase has two absorption maxima at 280 and 430 mμ, and a shoulder at 340 mμ[4]. Addition of pyruvate, ammonia and phenol to the holoenzyme resulted in the appearance of a new spectral band near 500 mμ (Fig. 2).

The appearance of a new absorption peak around 500 mμ on addition of substrate was observed in many pyridoxal phosphate enzymes[12-16]. The similar band at 500 mμ was observed in tyrosine phenol lyase by the

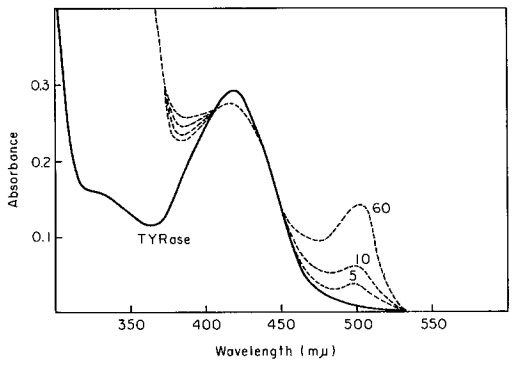

Fig. 2. Absorption spectra of holotyrosine phenol lyase in the presence of pyruvate, ammonia and phenol. The solutions contained 10.2 mg of the holoenzyme in 1 ml of 0.01 M potassium phosphate buffer, pH 8.0. The spectra were taken at 25° at times (in minutes) indicated after 60 μmoles of potassium pyruvate, 30 μmoles of ammonium sulfate and 60 μmoles of phenol were added to the solution. The spectrum of enzyme alone was indicated as TYRase.

addition of *L*-tyrosine, *L*- or *D*-alanine[4,9]. From the spectral studies on tryptophanase of *Escherichia coli*, Morino and Snell[15] concluded that the absorption at 500 mμ can be ascribed to the deprotonated aldimine intermediate or to a species in equilibrium with one of the aldimine and ketimine intermediates. The appearance of the spectral band at 500 mμ on addition of pyruvate, ammonia and phenol to the holotyrosine phenol lyase indicates that it results from accumulation of the aldimine or ketimine intermediate in the synthetic reaction of *L*-tyrosine by tyrosine phenol lyase.

Acknowledgments

We wish to thank Prof. T. Hata and Prof. K. Ogata, Kyoto University, Kyoto, and Mr. R. Akino, Dr. T. Tsunoda and Dr. M. Takahashi, Central Research Laboratories, Ajinomoto Co., Ltd., Kawasaki, for their interest and advice during the course of this work.

References

1. Kakihara, Y. and Ichihara, K., *Med. J. Osaka Univ. (Osaka)*, 3, 497 (1953).
2. Uchida, M., Taketomo, Y., Kakihara, Y. and Ichihara, K., *Med. J. Osaka Univ. (Osaka)*, 3, 509 (1953).
3. Kumagai, H., Yamada, H., Matsui, H., Ohkishi, H. and Ogata, K., *J. Biol. Chem.*, 245, 1767 (1970).

4. Kumagai, H., Yamada, H., Matsui, H., Ohkishi, H. and Ogata, K., *J. Biol. Chem.*, 245, 1773 (1970).
5. Kumagai, H., Kashima, N., Torii, H., Yamada, H., Enei, H. and Okumura, S., *Agr. Biol. Chem.*, in press.
6. Kumagai, H., Matsui, H., Ohgishi, H., Ogata, K., Yamada, H., Ueno, T. and Fukami, H., *Biochem. Biophys. Res. Commun.*, 34, 266 (1969).
7. Ueno, T., Fukami, H., Ohkishi, H., Kumagai, H. and Yamada, H., *Biochim. Biophys. Acta*, 206, 476 (1970).
8. Enei, H., Matsui, H., Okumura, S. and Yamada, H., *Biochem. Biophys. Res. Commun.*, 43, 1345 (1971).
9. Kumagai, H., Kashima, N. and Yamada, H., *Biochem. Biophys. Res. Commun.*, 39, 796 (1970).
10. Tamura, G., Tsunoda, T., Kirimura, J. and Miyasawa, M., *Nippon Nogeikagaku Kaishi*, 26, 464 (1952).
11. Linweaver, H. and Burk, D., *J. Am. Chem. Soc.*, 56, 658 (1934).
12. Jenkins, W. T., *J. Biol. Chem.*, 236, 1121 (1961).
13. Jenkins, W. T., *Fed. Proc.*, 20, 978 (1961).
14. Schirch, L. and Mason, M., *J. Biol. Chem.*, 237, 2578 (1962).
15. Morino, Y. and Snell, E. E., *J. Biol. Chem.*, 242, 2800 (1967).
16. Hudgins, W. R. and Brown, F. C., in K. Yamada, N. Katunuma and H. Wada (Editors), *Symposium on pyridoxal enzymes*, Maruzen Company, Ltd., Tokyo, 1968, p.97.