to be closely related to—if not identical with—the system effecting aerobic hydroxylation of alkanes to give the primary alcohol.

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PN 10061

## Induced, soluble phenylalanine hydroxylase from Pseudomonas sp. grown on phenylalanine or tyrosine

The formation of tyrosine by the phenylalanine hydroxylase (EC 1.99.1.2) of mammalian liver has been well studied[1–4]. The biosynthesis of tyrosine in microorganisms is generally via an entirely different pathway[5,6]. Although it has been reported that intact cells of certain species of microorganisms can form tyrosine from phenylalanine under the proper conditions[7–9], the enzyme or enzymes involved have not been obtained in cell-free extracts. This communication describes a soluble, cell-free preparation from Pseudomonas sp. which hydroxylates phenylalanine to form tyrosine.

Pseudomonas sp. (ATCC 11299a) was grown on minimal media containing 0.1% $K_2HPO_4$, 0.1% $KH_2PO_4$, 0.02% $MgSO_4$, 0.2% $NH_4Cl$, and either 0.2% L-phenylalanine, 0.1% L-tyrosine, or 0.2% L-asparagine (pH 6.8). The cells were harvested during log phase and washed five times with 0.2% phosphate buffer containing 0.02% $MgSO_4$ (pH 6.8). The concentrated cell suspension was lysed in an osmotic pressure cell and the lysate centrifuged in a Spinco centrifuge, Model L, at 40 000 rev./min for 1 h. The clear supernatant fraction from this preparation was used for these experiments.

The supernatant fraction was incubated in air at 30° with DPNH and L-phenylalanine. At the end of the incubation period trichloroacetic acid was added to a final concentration of 6%. Tyrosine[10] and phenylalanine[11] were determined fluorometrically in aliquots of the protein-free filtrate.

High-speed supernatant preparations from cells grown on phenylalanine con-

verted phenylalanine to tyrosine (Table I). The reaction was completely dependent on exogenous phenylalanine and was stimulated by added DPNH. D-Phenylalanine was not hydroxylated. TPNH was inactive and, in fact, inhibited the reaction. The enzyme was saturated with respect to DPNH and phenylalanine at the concentrations used.

The preparation did not metabolize tyrosine under these conditions. The conversion of phenylalanine to tyrosine was stoichiometric and linear for at least 30 min. The formation of tyrosine was linear with enzyme concentration above a minimal protein content of about 2 mg per 0.8-ml incubation. All the experiments presented were carried out with protein concentrations between 5 and 7 mg per incubation.

TABLE I

TYROSINE FORMATION BY HIGH-SPEED SUPERNATANT FRACTION FROM PSEUDOMONAS SP.

The complete system consisted of 2.5 μmoles of DPNH, 6.06 μmoles of L-phenylalanine, and 0.5 ml of supernatant in 0.2% phosphate buffer containing 0.02% $MgSO_4$ (pH 6.8). The total volume was 0.8 ml. Experiments 1 and 2 contained 6.85 and 5.36 mg of protein, respectively. The reaction mixtures were shaken for 20 min at 30° and the reactions stopped with 0.2 ml of 30% trichloroacetic acid.

| *Incubation* | *μmoles Tyrosine* | |
|---|---|---|
| | *No. 1* | *No. 2* |
| Complete | 1.81 | 1.23 |
| — DPNH | 0.99 | 0.81 |
| — Phenylalanine | 0.55 | 0.45 |
| — Enzyme | 0.03 | 0.02 |
| Complete (enzyme boiled 30 sec) | 0.55 | 0.44 |
| Complete (0 min) | 0.61 | 0.44 |

The addition of components from the mammalian phenylalanine hydroxylase system, *i.e.*, concentrates containing the pteridine cofactor or preparations of the "sheep enzyme"[12] had no effect on this activity. The relationship of this hydroxylase to the mammalian phenylalanine hydroxylase or to the other hydroxylases elaborated by this organism[13,14] remains to be studied.

Extracts from cells grown on asparagine were devoid of hydroxylase activity (Table II). Extracts from cells grown on tyrosine, however, possessed about the same

TABLE II

TYROSINE FORMATION BY HIGH-SPEED SUPERNATANT FRACTIONS FROM CELLS GROWN ON VARIOUS MEDIA

The conditions of incubation were as indicated in Table I. Figures for tyrosine formation were obtained by subtracting the amounts of tyrosine in the zero-time samples from that in the complete system. The numbers represent two separate experiments with each culture medium.

| *Culture medium* | *μmoles tyrosine formed/mg protein* |
|---|---|
| L-Phenylalanine (0.2%) | 0.18, 0.15 |
| L-Tyrosine (0.1%) | 0.25, 0.18 |
| L-Asparagine (0.2%) | 0.003, 0.003 |

amount of hydroxylase activity as did extracts of cells grown on phenylalanine. This observation has been confirmed in studies on the metabolism and incorporation of phenylalanine in whole cells[15]. Other similar examples of the induction of the initial enzyme of a degradative pathway by the product of its action have been reported[16,17].

Data are available to indicate that the tyrosine formed by this inducible system can be used for protein synthesis[15]. A study of the relationship between the alternate routes of tyrosine formation in the induced organism is in progress.

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## The involvement of sulfhydryl sites in dopamine-β-hydroxylase activity

The enzyme dopamine-β-hydroxylase catalyzes the β-hydroxylation of dopamine[1] and other structural related phenylethylamines and phenylpropylamines[2–4]. As cofactors, ascorbic and fumaric acids are required. While it was shown that the enzymatic β-hydroxylation of the amines is coupled to a stoichiometrically equivalent oxidation of ascorbic acid, the requirement for fumarate is still unexplained[1]. The enzymatic hydroxylation is stimulated by ATP, and it was suggested that ATP functions as a chelating agent rather than as a donor of high energy[2]. EDTA in-