BIOSYNTHESIS OF β-PHENETHYL ALCOHOL IN CANDIDA GUILLIERMONDII

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SUMMARY: Candida guilliermondii produced β-phenethyl alcohol and β-phenyllactic acid when grown in a synthetic medium containing L-phenylalanine as sole source of nitrogen. The cell-free preparations from these cells showed the following enzymes: phenylalanine aminotransferase, phenylpyruvate decarboxylase, phenylpyruvate reductase and phenylacetaldehyde reductase. The cell-free preparations of C. guilliermondii grown in medium with ammonium sulfate, lacked these enzyme activities, indicating the inducible nature of these enzymes. The results indicate the role of β-phenylpyruvate as a key intermediate in the pathway of biosynthesis of β-phenethyl alcohol and β-phenyllactic acid from L-phenylalanine.

INTRODUCTION

L-Phenylalanine is converted to either L-tyrosine or β-phenylpyruvate in bacteria (1,2), to cinnamate in most of the plants and fungi, and to tyrosine in mammalian systems. Thus, the pathway involved in the catabolism of L-phenylalanine varies widely in diverse organisms. However, in phenylketonuric patients, phenylalanine metabolism leads to accumulation of β-phenylpyruvate, β-phenyllactate and phenylacetylglutamine (3).

The biosynthetic abilities of different species of Saccharomyces and Candida have been exploited in the production of alcohols (4-7), vitamins (8), and enzymes (9). Candida albicans excretes β-phenethyl alcohol (β-PEA) when grown in Sabouraud's broth (10) or defined media (11). Several species of Candida have been reported to produce aromatic alcohols like β-PEA (11), β-(4-hydroxyphenyl)-ethanol (HOPEA) (12) and β-indoleethanol (β-IEA) (13) when L-phenylalanine, L-tyrosine or L-tryptophan

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served as sole sources of nitrogen, respectively. Though the pathways involved in the biosynthesis of these aromatic amino acids in yeasts have been extensively studied, little is known about the enzymology of their catabolism. The enzymology of the biosynthesis and regulatory aspects of β-PEA and β-IEA are of great interest in view of their reported autoantibiotic activity (14). In this communication, we report the demonstration of the activities of the enzymes which convert L-phenylalanine to β-PEA and β-PLA in C. guilliermondii.

MATERIALS AND METHODS

Chemicals: L-Phenylalanine, β-phenylpyruvate, β-phenyllactate, β-phenethyl alcohol, α-ketoglutarate, NADH, NADPH, PLP, and TPP were obtained from Sigma Chemical Company, St. Louis, Mo, U.S.A. Phenylacetaldehyde obtained locally was purified by distillation.

Organism: Candida guilliermondii Z55 was obtained from the London School of Hygiene and Tropical Medicine, London. Stock cultures were maintained by bimonthly subculturing on Sabouraud's glucose agar slants.

Growth: The medium used for growing cells was of the composition: glucose, 2%; $(NH_4)_2SO_4$, 0.25% or phenylalanine, 0.1%; $CaCl_2.2H_2O$, 0.25%; $MgSO_4.7H_2O$, 0.25%; KH_2PO_4, 0.35%; and biotin, 3 μg/100 ml.

Erlenmeyer flasks of 500 ml capacity each containing 200 ml of medium were inoculated with 20 ml of 10 h culture of C. guilliermondii in the same medium, and grown for 12 to 24 h on a rotary shaker at 30±1°C. The cells were harvested by centrifugation in Sorvall RC2-B centrifuge at 4,000xg at 0-4°C. The cell-pellet was washed thrice with ice-cold 0.05M phosphate buffer, pH 7.0.

Cell-free preparations: Cells of C. guilliermondii were ground for 15-20 min with twice the amount of acid-washed sand (60-80 mesh) in a precooled mortar. The slurry was extracted with 0.05M Tris-HCl buffer, pH 7.4. The suspension was centrifuged at 18,000xg for 30 min. The supernatant was used to assay various enzyme activities. Protein content was estimated by the method of Lowry et al. (15).

Enzyme assays: Phenylalanine aminotransferase activity was assayed according to the procedure of Lin et al. (16).

Phenylpyruvate formed was estimated as its enol-borate complex, which absorbs at 300 nm.

Phenylpyruvate decarboxylase activity was assayed using standard manometric technique (17), by measuring carbon dioxide released.

Table 1. Enzymes involved in biosynthesis of β-phenethyl alcohol in *Candida guilliermondii*

Enzyme	Substrate	System	Specific activity
1. Phenylalanine amino-transferase[a]	L-Phenylalanine	Complete	210
		- α-Keto-glutarate	-
		- Phe	-
		- PLP	-
2. Phenylpyruvate decarboxylase[b]	β-Phenyl-pyruvate	Complete	47
		- TPP	-
		- Mg^{2+}	-
		- Substrate	-
3. Phenylpyruvate reductase[c]	β-Phenyl-pyruvate	Complete	18
4. Phenylacetaldehyde reductase[d]	Phenyl-acetaldehyde	Complete	1600

a. Complete system, in a final volume of 2.5 ml, contained L-phenylalanine, 30 μmoles; α-ketoglutarate, 30 μmoles; pyridoxal phosphate, 10 nmoles; potassium phosphate buffer, pH 8.0, 250 μmoles; and enzyme protein (1.5 mg). Assay temperature was 37°C.

b. Complete system, in a final volume of 3.2 ml, contained β-phenylpyruvate, 20 μmoles; $MgCl_2$, 1 μmole; thiamine pyrophosphate, 2 μmoles, potassium phosphate buffer, pH 6.0, 1 mmole; and enzyme protein (1.5 mg). Assay temperature was 37°C.

c and d. Complete system, in a final volume of 2 ml, contained β-phenylpyruvate, 20 μmoles, or phenylacetaldehyde, 10 μmoles; NADH or NADPH, 0.2 μmoles; potassium phosphate buffer, pH 6.5, 50 μmoles; and enzyme protein (0.5 mg). Assay temperature was 25°C.

The activities of phenylpyruvate reductase and phenylacetaldehyde reductase were assayed by determining the decrease in absorbancy of reduced pyridine nucleotides at 340 nm in a Carl Zeiss spectrophotometer.

One unit of activity is defined as equivalent to that amount of the enzyme which catalyzes the conversion of one nmole of substrate per minute. Specific activity is expressed as units of enzyme activity per milligram protein.

RESULTS AND DISCUSSION

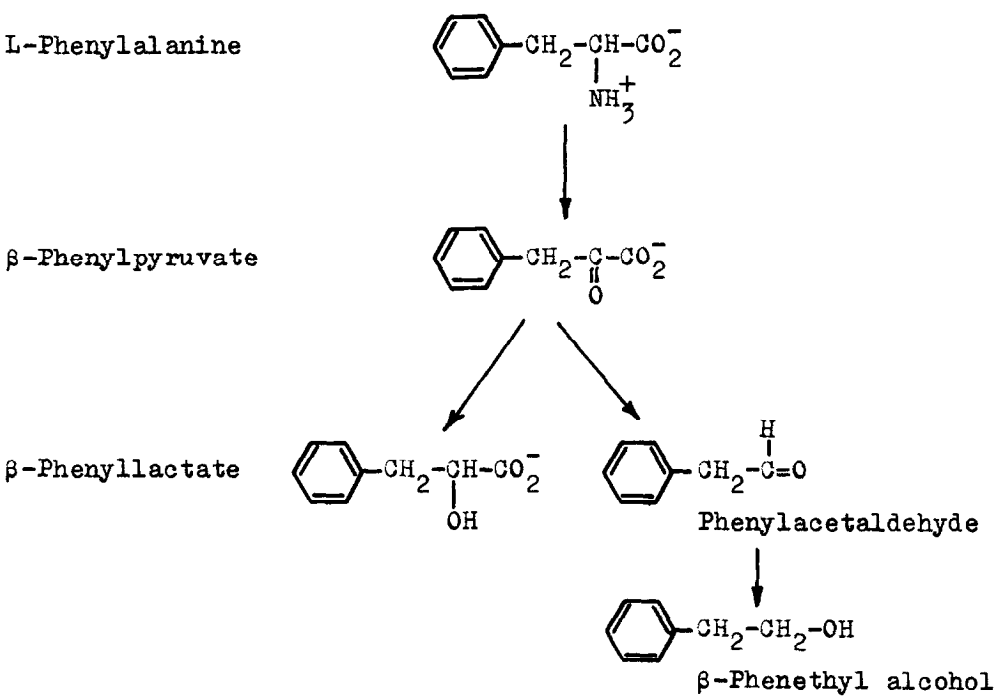
The ability of C. guilliermondii to grow in synthetic media containing L-phenylalanine as sole source of nitrogen and to produce β-PEA has been reported earlier from our Laboratory (11). Cell-free preparations from these cells showed phenylalanine aminotransferase (PAT) activity (Table 1). The enzyme displayed an absolute requirement for pyridoxal phosphate for its activity. The occurrence of PAT in several organisms (1) including yeasts viz., S. cerevisiae (18), and in S. fragilis (19) was reported and its role in the metabolism of L-phenylalanine reviewed (1). Phenylpyruvate decarboxylase which catalyzes the non-oxidative decarboxylation of β-phenylpyruvate to phenylacetaldehyde, was also detected in the cell-free preparations. It required both thiamine pyrophosphate and Mg^{2+} for its activity. The occurrence of this enzyme in bacteria was reported (20). Cell-free preparations of C. guilliermondii also showed activity of phenylpyruvate reductase which catalyzes the reduction of β-phenylpyruvate to β-PLA, and the reaction is reversible. Both NADH and NADPH served as electron donors. The product of the reaction catalyzed by this enzyme. viz., β-PLA was isolated and identified by paper chromatography (11). The presence of this enzyme was also reported in phenylketonurics, who excrete β-PLA, L-phenylalanine, β-phenylpyruvate and phenylacetylglutamine in urine (21). Conversion of β-phenylpyruvate to β-PLA has not so far been reported in microorganisms including yeasts.

Phenylacetaldehyde reductase which reduces phenylacetaldehyde to β-PEA requiring either NADH or NADPH for its activity was also detected in cell-free preparations of C. guilliermondii. Though a possible mechanism of its conversion in plants has been

proposed by earlier workers (22), its activity has not been demonstrated. However, an enzyme converting p-hydroxyphenylacetaldehyde to tyrosol in S. cerevisiae was reported (18).

Cell-free preparations of C. guilliermondii grown on ammonium sulfate as nitrogen source failed to show these enzyme activities indicating the inducible nature of these enzymes. The results presented here indicate the role of β-phenylpyruvate as a key intermediate in the conversion of L-phenylalanine to β-PEA and β-PLA by the pathway proposed below. A similar pathway might be operating in the biosynthesis of HOPEA and β-IEA from L-tyrosine and L-tryptophan, respectively, in Candida species. Studies on purification and characterization of these enzymes, and their role in the regulation of the pathway are in progress.

The proposed pathway for the biosynthesis of β-phenethyl alcohol and β-phenyllactate from L-phenylalanine in Candida guilliermondii is as follows:



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