Arogenate (Pretyrosine) Pathway of Tyrosine and Phenylalanine Biosynthesis in Pseudomonas aureofaciens ATCC 15926

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Assays of enzyme activities suggest that arogenate, the product of prephenate transamination, is an intermediate in the biosynthesis of both phenylalanine and tyrosine in Pseudomonas aureofaciens ATCC 15926. In addition to prephenate dehydratase and prephenate dehydrogenase, arogenate dehydratase and arogenate dehydrogenase activities were demonstrated. This pattern of aromatic amino acid biosynthesis in pseudomonads had previously been demonstrated only in P. aeruginosa. Arogenate dehydrogenase from P. aureofaciens differs from that in P. aeruginosa in its utilization of either NAD⁺ or NADP⁺ as cofactor and its inhibition by L-tyrosine. During ammonium sulphate fractionation, arogenate dehydratase co-precipitated with prephenate dehydratase activity and not with prephenate dehydratase II. The pattern of regulation of the arogenate route to tyrosine in P. aureofaciens ATCC 15926 differed from that previously reported for strain ATCC 13986.

INTRODUCTION

In a recent paper, we reported that the biosynthesis of phenylalanine and tyrosine in Pseudomonas aureofaciens ATCC 15926 (Blumenstock et al., 1980) proceeds by a route identical with that previously established in such micro-organisms as Escherichia coli and Bacillus subtilis (Gibson & Pittard, 1968). This route goes via phenylpyruvate and 4-hydroxyphenylpyruvate with a subsequent transamination to phenylalanine and tyrosine, respectively (Fig. 1).

The presence of an alternative pathway for the biosynthesis of tyrosine was originally described in cyanobacteria (Stenmark et al., 1974). In this pathway, prephenate is first transaminated to pretyrosine (now named arogenate), and the last step of tyrosine synthesis is a dehydrogenation. This route was later found in P. aeruginosa (Patel et al., 1977) and more recently in coryneform bacteria (Fazel & Jensen, 1979). In P. aeruginosa there is an enzymic sequence to phenylalanine via pretyrosine in addition to the phenylpyruvate pathway (Patel et al., 1977), hence the adoption of the name arogenate for the common precursor.

The present paper deals with the dual pathways to tyrosine and to phenylalanine in P. aureofaciens and the regulation of the biosynthesis of these aromatic amino acids.

METHODS

Organisms and growth conditions. Pseudomonas aureofaciens ATCC 15926 was grown in a minimal medium and harvested in the late-exponential phase of growth (Blumenstock et al., 1980).

Enzyme assays. Crude extracts of bacteria were prepared as described by Blumenstock et al. (1980) except that cells were suspended in 100 mM-potassium phosphate buffer, pH 8-0 (buffer A) for the dehydratase enzyme, or buffer A supplemented with 0-1 mM-dithioerythritol (buffer B) for the dehydrogenase enzyme, and disrupted in a French press at 110 MPa. Cell debris was removed by centrifuging at 150000 g for 1 h.

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Prephenate dehydratase [prephenate hydro-lyase (decarboxylating); EC 4.2.1.51] was measured by the method of Cotton & Gibson (1970) and prephenate dehydrogenase [prephenate: \( \text{NAD}^+ \) oxidoreductase (decarboxylating); EC 1.3.1.12] by the technique of Cotton & Gibson (1967). Arogenate dehydratase was determined by measuring the enzymic product, phenylalanine, spectrofluorimetrically according to Shapiro et al. (1981).

For the assay of arogenate dehydrogenase, the continuous formation of NADH or NADPH was followed spectrophotometrically at 340 nm. The reaction mixture contained protein (80–130 μg), 4 mM-\( \text{NAD}^+ \) or \( \text{NADP}^+ \), 500 mM-(NH}_4)_2\text{SO}_4, 0.4 mM-prephenate or 0.1 mM-arogenate and buffer B to a final volume of 0.5 ml. A blank containing the same compounds but omitting prephenate or arogenate was used as the control. The reaction was started by the addition of prephenate or arogenate. The reaction mixture was incubated at 30 °C.

Protein was determined by the Lowry method using bovine serum albumin as standard.

**Chemicals.** All reagents were of the highest purity available. Arogenate was prepared from the culture supernatants of a triple mutant of *Neurospora crassa* ATCC 36373 (Jensen et al., 1977). Its purification and isolation was done essentially by the method of Zamir et al. (1980). Arogenate concentrations were determined after acid conversion to phenylalanine as described by Ambrose (1969). Prephenate was prepared as described by Dayan & Sprinson (1970).

## Results and Discussion

**Prephenate and arogenate dehydrogenase**

The regulation of prephenate dehydrogenase has already been described (Blumenstock et al., 1980). As pseudomonads exhibit diverse patterns of enzyme regulation in tyrosine biosynthesis (Byng et al., 1980), we extended our previous studies to include the enzymes of the arogenate pathway to tyrosine in *P. aureofaciens* ATCC 15926.

The regulation of arogenate dehydrogenase was found to be identical with that of prephenate dehydrogenase, i.e. the enzyme was neither repressed by tyrosine nor induced or...
activated by phenylalanine. The sole regulatory effector was tyrosine which at 1 mM caused 60% inhibition.

NAD+ and NADP+ were equally effective as cofactor, with a specific activity of 9 nmol min⁻¹ (mg protein)⁻¹ in a crude extract from cells grown in minimal medium.

The variation of cofactor specificity and regulatory properties of the prephenate/arogenate dehydrogenase allow the family of Pseudomonadaceae to be divided into five groups (Byng et al., 1980) which corresponded strikingly with the five ribosomal RNA (rRNA) homology groups established by rRNA–DNA hybridization (Palleroni et al., 1973). The dehydrogenase pattern observed with P. aureofaciens ATCC 15926 permits its assignment to group II of the pseudomonads (Byng et al., 1980). The characteristics of this group are as follows: an arrogenate dehydrogenase using either NAD+ or NADP+ as hydrogen acceptor and sensitive to feedback inhibition by L-tyrosine. Byng et al. (1980) assigned P. aureofaciens ATCC 13986 to group I of the pseudomonad classification on the basis of an arrogenate dehydrogenase inactive with NADP+. Moreover, group I species have an NAD+‐linked arrogenate dehydrogenase highly sensitive to inhibition by L-tyrosine and an NAD+‐linked prephenate dehydrogenase relatively insensitive to tyrosine inhibition.

Prephenate and arrogenate dehydratase

In P. aureofaciens, Blumenstock et al. (1980) distinguished two prephenate dehydratases (prephenate dehydratases I and II) separable by (NH₄)₂SO₄ fractionation, DEAE‐Sephadex or Sephadex G‐100 chromatography, and exhibiting differences in their molecular weights and regulatory behaviour. Prephenate dehydratase I was found in (NH₄)₂SO₄ fractions up to 50% saturation, whereas prephenate dehydratase II activity was detected in (NH₄)₂SO₄ fractions of 50–90% saturation. Two different prephenate dehydratases were already known in P. aeruginosa PA01 (Calhoun et al., 1973; Patel et al., 1977), but these enzymes were distinguishable by their molecular weights from those detectable in P. aureofaciens. Prephenate dehydratase I associated with chorismate mutase is activated by tyrosine and inhibited by phenylalanine, whereas prephenate dehydratase II is not influenced by either end-product (Blumenstock et al., 1980; Patel et al., 1977). Prephenate dehydratase I from P. aeruginosa did not show any activity with arogenate as substrate, whereas prephenate dehydratase II was able to react with both prephenate and arogenate (Patel et al., 1977).

In a crude extract of P. aureofaciens ATCC 15926 the specific activity of arrogenate dehydratase was determined to be 8 nmol h⁻¹ (mg protein)⁻¹. Under the same conditions the prephenate dehydratase activity was 2000 nmol h⁻¹ (mg protein)⁻¹. After (NH₄)₂SO₄ fractionation arrogenate dehydratase activity was found in the precipitate formed between 0 and 50% saturation; the fraction precipitated between 50 and 90% saturation exhibited only prephenate dehydratase activity. These results demonstrated the co-fractionation of arrogenate dehydratase with prephenate dehydratase I and its non-identity with prephenate dehydratase II.

Phenylalanine and tyrosine synthesis in P. aureofaciens ATCC 15926

The results suggest that P. aureofaciens synthesizes tyrosine via parallel routes, the 4-hydroxyphenylpyruvate and arrogenate pathways, as well as phenylalanine via the phenylpyruvate or the arogenate branchlet (Fig. 1). In the view of the 250-fold excess of prephenate dehydratase I activity, the phenylpyruvate branch seems to be the main route of phenylalanine synthesis.

REFERENCES


_**Arogenate pathway in P. aureofaciens**_ 1201


