SHORT COMMUNICATION

Regulation of Phospho-2-keto-3-deoxy-heptonate Aldolase (DAHP Synthase) and Anthranilate Synthase of Pseudomonas aureofaciens

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Phospho-2-keto-3-deoxy-heptonate aldolase (DAHP synthase) of Pseudomonas aureofaciens ATCC 15926 was inhibited by L-tyrosine. The inhibition was competitive with erythrose 4-phosphate as the varied substrate but non-competitive with respect to phosphoenolpyruvate. Anthranilate synthase was inhibited by L-tryptophan. The inhibition was competitive with respect to chorismate but non-competitive with L-glutamine or NH₄⁺ as the varied substrate. DAHP synthase and anthranilate synthase were not repressed when aromatic amino acids were included in the growth medium. In bacteria grown in the presence of L-phenylalanine, the anthranilate synthase activity was enhanced about threefold compared with the control. Similar results were obtained with the mutant strain P. aureofaciens ACN, which produces increased amounts of pyrrolnitrin.

INTRODUCTION

The biosynthesis of aromatic amino acids starts with the synthesis of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) from D-erythrose 4-phosphate and phosphoenolpyruvate. The first reaction specific for tryptophan synthesis is catalysed by anthranilate synthase (EC 4.1.3.27) which uses chorismate as substrate together with L-glutamine or NH₄⁺ as source of the amino group. Although information is available about the properties of DAHP synthase (EC 4.1.2.15) (Jensen et al., 1967; Lingens, 1968) and anthranilate synthase (Zalkin, 1973) from many pseudomonads, we have now studied the regulatory pattern of these enzymes from Pseudomonas aureofaciens because of the role of tryptophan as the precursor in pyrrolnitrin biosynthesis by this organism (Floss et al., 1971).

METHODS

Organisms and growth conditions. Pseudomonas aureofaciens ATCC 15926 and the mutant strain P. aureofaciens ACN were 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 Salcher & Lingens (1980b). DAHP synthase was assayed in 10 mm-K/Na-phosphate buffer, pH 7.0, containing 0.8 mm-phosphoenolpyruvate, 0.4 mm-erythrose 4-phosphate, 0.2 mm-CoCl₂ and crude extract (200 to 400 µg protein) in a total volume of 500 µL. A control lacking phosphoenolpyruvate was included. In feedback inhibition studies with tryptophan, this amino acid (0.1 mM) was also added to the control assay. The DAHP formed was detected by the method of Gollub et al. (1970) and Eberspächer & Lingens (1970).
Table 1. **Comparison of kinetic properties of DAHP synthase and anthranilate synthase from Pseudomonas aureofaciens ATCC 15926**

Enzyme activities were assayed as described in Methods. The $K_m$ values were determined from Lineweaver-Burk plots and the $K_i$ values from Dixon plots.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Varied substrate</th>
<th>$K_m$ value (mM)</th>
<th>Inhibitor</th>
<th>Type of inhibition with respect to corresponding substrate</th>
<th>$K_i$ value ($\mu M$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAHP synthase</td>
<td>Phosphoenolpyruvate</td>
<td>0.45</td>
<td>L-Tyrosine</td>
<td>Non-competitive</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Erythrose 4-phosphate</td>
<td>0.42</td>
<td>L-Tyrosine</td>
<td>Competitive</td>
<td>140</td>
</tr>
<tr>
<td>Anthranilate</td>
<td>Chorismate (in glutamine-dependent reaction)</td>
<td>0.037</td>
<td>L-Tryptophan</td>
<td>Competitive</td>
<td>10</td>
</tr>
<tr>
<td>synthase</td>
<td>Chorismate (in $NH_4^+$-dependent reaction)</td>
<td>0.14</td>
<td>L-Tryptophan</td>
<td>Competitive</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>L-Glutamine</td>
<td>25</td>
<td>L-Tryptophan</td>
<td>Non-competitive</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$NH_4^+$</td>
<td>32</td>
<td>L-Tryptophan</td>
<td>Non-competitive</td>
<td>7</td>
</tr>
</tbody>
</table>

Anthranilate synthase was assayed according to Tamir & Srinivasan (1970). In the glutamine-dependent reaction 0.01 M-Tris/HCl buffer, pH 7.8, was used, and in the $NH_4^+$-dependent reaction 0.01 M-glycine/NaOH buffer, pH 8.9, was used. The assays (total volume 1 ml) contained 500 $\mu$g protein from an ($NH_4$)$_2$SO$_4$ fraction (30 to 60% saturation; Blumenstock et al., 1980).

Protein concentration was determined by the methods of Layre (1957) and Lowry.

**Estimation of molecular weights.** The molecular weights of anthranilate synthase and DAHP synthase were estimated by gel filtration (Andrews, 1964; Blumenstock et al., 1980). Glycine/NaOH buffer (0.05 M; pH 8.9) was used throughout.

**Reagents.** These were of the highest purity grade available. Chorismate (Gibson, 1970), prephenate (Dayan & Sprinson, 1970) and erythrose 4-phosphate (Ballou et al., 1955) were prepared by standard methods.

**RESULTS AND DISCUSSION**

**Regulation of DAHP synthase and anthranilate synthase activities**

Different patterns of regulation of DAHP synthase activity by aromatic amino acids have been reported among pseudomonads (Jensen et al., 1967). Inhibition by L-tyrosine is common and also occurred in *P. aureofaciens* (Table 1). In *P. aeruginosa*, phenylpyruvate and L-tryptophan were also inhibitory (Jensen et al., 1973); however, neither these compounds nor D-tyrosine, anthranilate or prephenate (each at 0.1 mM) influenced the DAHP synthase activity of *P. aureofaciens*.

Anthranilate synthase activity of *P. aureofaciens* was inhibited by L-tryptophan (Table 1) and by 7-chloro-L-tryptophan, an early prospective intermediate in pyrrolnitrin biosynthesis, but not by L-tyrosine, L-phenylalanine or the corresponding D-amino acids.

Results similar to those shown in Table 1 for *P. aureofaciens* ATCC 15926 were also obtained with mutant strain ACN.

The inhibition of anthranilate synthase by L-tryptophan and of DAHP synthase by L-tyrosine was not enhanced by guanosine tetraphosphate (350 $\mu$M) as was observed for the ATP phosphoribosyltransferase from *Salmonella typhimurium* (Morton & Parsons, 1977).

**Molecular weight estimation**

Molecular weights were estimated by gel filtration with Sephadex G-100. DAHP synthase was eluted at a position corresponding to a molecular weight of 66000. Enzyme activities of crude extracts and eluates were enhanced by addition of Co$^{2+}$. The glutamine-dependent and the $NH_4^+$-dependent activities of anthranilate synthase co-eluted at a position corresponding to a molecular weight of 90000.
Aromatic acid biosynthesis and pyrrolnitrin biosynthesis

Although activities of DAHP synthase and anthranilate synthase were influenced by L-tyrosine or L-tryptophan, respectively, repression by aromatic amino acids, which were added at 3 mM to the growth medium, did not occur. Similar results were obtained with the enzymes for the biosynthesis of phenylalanine and tyrosine (Blumenstock et al., 1980). Also, the kinetic properties of the corresponding enzyme activities were not altered when extracts prepared from bacteria grown under conditions of potential repression were tested.

The anthranilate synthase activity of bacteria grown in 3 mM-L-phenylalanine was considerably enhanced [1.39 μmol min⁻¹ (mg protein)⁻¹] compared with a control grown on minimal medium [0.38 μmol min⁻¹ (mg protein)⁻¹]. Increased activities were obtained in both the glutamine- and the NH₄⁺-dependent reaction. An activation of the synthesis of anthranilate synthase by L-phenylalanine has not been reported in other pseudomonads. The concentration of extracellular L-tryptophan in cultures grown in the presence of L-phenylalanine was 20 μg ml⁻¹ and without L-phenylalanine was 0.7 μg ml⁻¹ (Salcher & Lingens, 1980b). In addition, pyrrolnitrin production in phenylalanine-grown cells was doubled, when assayed by the agar-well diffusion technique (Salcher & Lingens, 1980a). On the other hand, the increased amount of tryptophan produced was not sufficient for induction of the catabolic branch of tryptophan metabolism, e.g. tryptophan 2,3-dioxygenase (Salcher & Lingens, 1980b). The latter pathway is preferred in the presence of 3 mM-L-tryptophan. The important regulatory role of the tryptophan catabolic pathways on pyrrolnitrin biosynthesis is shown by the results obtained with the mutant strain P. aureofaciens ACN. The properties of some enzymes of its primary metabolism, e.g. DAHP synthase, anthranilate synthase (results not shown) as well as the enzymes of phenylalanine and tyrosine biosynthesis (Blumenstock et al., 1980), were similar to the properties of the wild-type enzymes. Anthranilate 1,2-dioxygenase activity, however, was absent from the mutant strain. The mutant was therefore unable to use the benzene moiety of L-tryptophan.

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REFERENCES


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