A Pair of Regulatory Isozymes for 3-Deoxy-d-arabino-
Heptulosonate 7-Phosphate Synthase Is Conserved Within
Group I Pseudomonads

GRAHAM S. BYNG,* ALAN BERRY, AND ROY A. JENSEN

Center for Somatic-Cell Genetics and Biochemistry, State University of New York at Binghamton,
Binghamton, New York 13901

Received 18 April 1983/Accepted 18 July 1983

Two closely related subgroups of group I pseudomonads, which differ from one
another in the overall enzymatic makeup of aromatic amino acid biosynthesis,
possess in common the recently characterized major (tyrosine-sensitive) and
minor (tryptophan-sensitive) isozymes of 3-deoxy-d-arabino-heptulosonate 7-
phosphate synthase of Pseudomonas aeruginosa (17). Since these characteriza-
tions were made for strains whose phylogenetic positions have been determined
by oligonucleotide cataloging, an initial perception of the evolution of aromatic
pathway construction and regulation is emerging.

Until recently, the objective of tracing the evolution of biochemical pathways seemed
largely unapproachable for procaryotes because of the seeming impossibility of establishing firm
phylogenetic relationships. However, the suit-
ability of oligonucleotide cataloging (7, 14) for
construction of definitive phylogenetic trees means that the direction of biochemical pathway
changes in evolutionary time can now be deter-
mined for any set of strains whose evolutionary
relationships with one another are known. A
managable example of such a well-defined bac-
terial group is the group I pseudomonads, which
divide to form two distinct subgroups (13). Sub-
group Ia contains only four species at pres-
ent: Pseudomonas mendocina, P. alcaligenes,
P. pseudoalcaligenes, and P. stutzeri, all of
which are nonfluorescent species. Subgroup Ib,
which makes up the vast majority of group I
species, contains such well-known fluorescent
species as P. aeruginosa, P. putida, P. syringae,
and P. fluorescens.

The pathway of aromatic biosynthesis is di-
verse in nature, varying in enzyme steps used,
allosteric control patterns, and cofactor specific-
ity for pathway dehydrogenases (2, 3). Sub-
groups Ia and Ib differ with respect to phenylala-
nine biosynthesis (16). Subgroup Ib has two
separate pathways to L-phenylalanine, one pro-
ceding through phenylpyruvate and the other
through L-arogenate. The latter flow route (con-
sisting of an unregulated chorismate mutase,
prephenate aminotransferase, and an unregulat-
ed arogenate dehydratase) has been shown to
behave as an overflow pathway to L-phenylala-
nine in P. aeruginosa (6). During the growth of
P. aeruginosa on carbon sources (e.g., glucose
which favor formation of initial substrates enter-
ing the aromatic pathway, an excess level of L-
phenylalanine is generated through the unregu-
lated overflow pathway. When 3-deoxy-d-arabino-
heptulosonate 7-phosphate (DAHP) synthase-tyrosine is rendered insensitive to
feedback inhibition by mutation, prephenate de-
hydratase (a later point of allosteric regulation)
is effectively bypassed by the presence of the
overflow pathway, and considerable L-phenylala-
nine is excreted. Subgroup Ia lacks the over-
flow pathway, since member species lack the
unregulated chorismate mutase (G. S. Byng, A.
Berry, R. J. Whitaker, and R. A. Jensen, Abstr.
p. 166.) as well as arogenate dehydratase (16).
The direction of evolutionary change is deduced
to be that of enzyme loss in subgroup Ia, since
another group of organisms (group V pseudomo-

nads) which diverge from group I pseudomonads at a deeper phylogenetic level (2) are identical to
subgroup Ib pseudomonads with respect to
phenylalanine enzyme arrangement (16).

The regulatory pattern of DAHP synthase has
long been recognized as a relatively conserva-
tive characteristic of microorganisms (3, 11, 12).
In an extensive comparative survey, DAHP synthase activities of all group I pseudomonads
were found to be sensitive primarily to feedback
inhibition by L-tyrosine (15). However, the
DAHP synthase activity of P. aeruginosa was
recently resolved into two regulatory isozymes
(17). A major (>90%) isozyme called DAHP
synthase-tyrosine was found to be highly sensi-
tive to feedback inhibition by L-tyrosine, where-
as a minor (<10%) isozyme called DAHP syn-
thesis-tryptophan was very sensitive to feedback
inhibition by L-tryptophan. The minor isozyme is not readily detected in crude extracts, a circumstance which raised the question of whether the minor regulatory isozyme was characteristic of only subgroup Ib or of the entire assemblage of group I pseudomonads. Hence, a more detailed reevaluation of selected representatives of group I species was carried out, as reported in this paper.

DAHP synthase was assayed as described by Calhoun et al. (5), and protein concentrations of extracts were estimated by the method of Bradford (1). All cultures were grown, harvested, and used to prepare crude extracts free of small molecules, as previously described (17). Strains representing three subgroup Ia species (P. alcaligenes, P. mendocina, and P. stutzeri) were compared with P. aeruginosa (ATCC 15692) and P. fluorescens (ATCC 13525) the latter two chosen as representative species of subgroup Ib. Isozymes of DAHP synthase were fractionated by ion-exchange chromatography with DEAE-cellulose, as illustrated in Fig. 1 for P. alcaligenes, P. fluorescens, and P. aeruginosa. Two distinct isozymes were discerned for all five species, one eluting as a major peak and the other as a minor peak. Except for P. fluorescens, each major isozyme was recovered in the wash elute, whereas each minor isozyme eluted in the salt gradient.

Appropriate fractions recovered after DEAE-cellulose chromatography were characterized for sensitivity of DAHP synthase to potential feedback inhibitors (Tables 1 and 2). Each isozyme was assayed in the presence of a 0.5 mM concentration of L-tyrosine, L-tryptophan, phenylpyruvate, chorismate, or L-phenylalanine. Chorismate was prepared by the method of Gibson (8). Neither isozyme from any of the species studied was inhibited by L-phenylalanine. Although phenylpyruvate has been shown to be a weak competitive inhibitor of DAHP synthase-tyrosine in P. aeruginosa (17), a fact which may be physiologically significant (10),

![FIG. 1. Elution profiles of DAHP synthase-tyrosine and DAHP synthase-tryptophan isozymes resolved by DEAE-cellulose chromatography. Crude extracts prepared from the three species shown and containing up to 100 mg of protein were applied individually at 4°C to Whatman DE-52 ion-exchange columns (1.5 by 20 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.0) and 1 mM dithiothreitol. The loaded columns were washed with 100 ml of equilibration buffer, and bound proteins were eluted with 300-ml volumes of KCl applied as a linear gradient between 0 and 0.5 M KCl. Each reservoir also contained the equilibration buffer. The vertical dashed lines indicate the onset point of gradient elution. Fractions of 2.2 ml were collected and assayed for DAHP synthase activity, expressed here as A549. The distribution of protein elution is shown by dotted lines.](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the DAHP synthase-tyrosine isozyme isolated from group I pseudomonads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ib</td>
</tr>
<tr>
<td>Ib</td>
</tr>
<tr>
<td>Ia</td>
</tr>
<tr>
<td>Ia</td>
</tr>
<tr>
<td>Ia</td>
</tr>
</tbody>
</table>

* The final concentration of L-tyrosine (Tyr), L-tryptophan (Trp), or potassium chorismate (CHA) was 0.5 mM.

* Nanomoles of DAHP formed per minute per milligram of protein at 37°C.

* Fraction of total DAHP synthase activity recovered as DAHP synthase-tyrosine.
TABLE 2. Characteristics of the DAHP synthase-tryptophan isozyme isolated from group I pseudomonads

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Pseudomonas spp.</th>
<th>% Inhibition(^a) by:</th>
<th>Sp act(^b)</th>
<th>% Total activity(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tyr</td>
<td>Trp</td>
<td>CHA</td>
</tr>
<tr>
<td>Ib</td>
<td>P. fluorescens ATCC 13525</td>
<td>0</td>
<td>81</td>
<td>43</td>
</tr>
<tr>
<td>Ib</td>
<td>P. aeruginosa ATCC 15692</td>
<td>0</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>Ia</td>
<td>P. alcaligenes ATCC 14909</td>
<td>21</td>
<td>97</td>
<td>56</td>
</tr>
<tr>
<td>Ia</td>
<td>P. stutzeri ATCC 17588</td>
<td>21</td>
<td>97</td>
<td>56</td>
</tr>
<tr>
<td>Ia</td>
<td>P. mendocina ATCC 25411</td>
<td>33</td>
<td>94</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\) The final concentration of L-tyrosine (Tyr), L-tryptophan (Trp), or potassium chorismate (CHA) was 0.5 mM.
\(^b\) Nanomoles of DAHP formed per minute per milligram of protein at 37°C.
\(^c\) Fraction of total DAHP synthase activity recovered as DAHP synthase-tryptophan.
\(^d\) A minor isozyme of DAHP synthase was recovered from DEAE-cellulose, but activity was too low for determinations of inhibitor sensitivities.

FIG. 2. Evolution of biochemical pathway for aromatic biosynthesis in pseudomonad groups I and V. An abbreviated scheme showing the relevant sections of the pathway is illustrated at the top of the figure. The isozymes of DAHP synthase and the dual routing to L-phenylalanine are suggested as the ancestral arrangement existing at the evolutionary time shown at the far left of the dendogram drawn at the bottom of the figure. This ancestral arrangement is retained unchanged in subgroup Ib. Enzyme denotations are: [1], the multifunctional “P” protein (6) consisting of chorismate (CHA) mutase and prephenate (PPA) dehydratase; [2], phenylpyruvate (PPY) aminotransferase; [3], chorismate mutase; [4], prephenate aminotransferase; and [5], arogenate (AGN) dehydratase. Enzymes [3] through [5] constitute an unregulated overflow pathway to L-phenylalanine, which is lost in subgroup Ia. The circled numbers in the dendogram are \(S_{AB}\) (similarity coefficient) values obtained by oligonucleotide cataloging (7, 14).
this sensitivity is not readily detected at the saturating substrate concentrations used. Table 1 shows that each major isozyme (recovered as >80% of total DAHP synthase activity) was feedback-inhibited by L-tyrosine but not by L-tryptophan or chorismate. Therefore, each major isozyme can be designated as DAHP synthase-tyrosine.

Table 2 shows the corresponding analysis for the minor isozymes, the results of which identify these as DAHP synthase-tryptophan. L-Tryptophan was a potent inhibitor of the minor isozymes. DAHP synthase-tryptophan from P. fluorescens and P. alcaligenes also showed the same sensitivity to inhibition by chorismate that was established in P. aeruginosa (17). The subgroup Ia species studied both possess DAHP synthase-tryptophan isozymes, which show low but significant sensitivity to inhibition by L-tyrosine. Perhaps this reflects the ancestral origin of these isozymes from a common gene (9).

Figure 2 is a representation of feasible interpretations that can be made about the evolution of aromatic amino acid biosynthesis in one small portion of the procaryotic phylogenetic tree. The groups considered include pseudomonads from group I and V, related to one another by a similarity coefficient, \( S_{AB} \) (7) of 0.48. Subgroups Ia and Ib diverge from one another at an \( S_{AB} \) of 0.77. The logical ancestral pathway of aromatic biosynthesis common to contemporary organisms that are the end products of this phylogeny is shown at the top of Fig. 1. Details relevant to L-tyrosine synthesis are not shown, since the dual pathways to L-tyrosine present in P. aeruginosa are conserved to a phylogenetic depth which includes pseudomonad groups I and V. (However, note that even relatively subtle differences in tyrosine branchlet regulation can enable us to differentiate reliably (4) between group I and group V pseudomonads.)

We suggest that the group V lineage lost an ancestral DAHP synthase-tyrosine (rather than the group I lineage having acquired it) because the enteric lineage which branches off (7) at a deeper phylogenetic level \( S_{AB} = 0.42 \) possesses (9) tyrosine-sensitive and tryptophan-sensitive isozymes of DAHP synthase (in addition to a phenylalanine-sensitive isozyme). Although the single DAHP synthase of group V organisms has been characterized by its feedback sensitivity to chorismate (15), it is also sensitive to feedback inhibition by L-tryptophan. It differs from DAHP synthase-tryptophan isozymes only in having a quantitatively greater relative sensitivity to chorismate inhibition than to L-tryptophan inhibition (R. J. Whitaker, G. S. Byng, and R. A. Jensen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, R260, p. 175).

Since group V pseudomonads (mainly species of Xanthomonas) possess the overflow pathway to L-phenylalanine that exists in the subgroup Ib lineage, it follows that contemporary subgroup Ia species probably pursued an evolutionary direction in which the overflow pathway was lost.

This study was supported by grant DEB 78-12099 from the National Science Foundation.

LITERATURE CITED


