Enzymological Basis of Reluctant Auxotrophy for Phenylalanine and Tyrosine in *Pseudomonas aeruginosa*

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Dual biosynthetic pathways to L-phenylalanine and L-tyrosine exist in *Pseudomonas aeruginosa* (Patel, N., Piersson, D. L., and Jensen, R. A. (1977) *J. Biol. Chem.* 252, 5839-5846). Tightly blocked phenylalanine or tyrosine auxotrophs are not obtained as the result of single mutations. Presumably the presence of a mutation that interrupts one pathway is masked by the presence of the alternative pathway. However, a leaky phenylalanine auxotroph (doubling time of 140 min in minimal glucose medium compared to 57 min with wild type) was isolated which completely lacked aminotransferase DE I. This is one of four aromatic aminotransferases of overlapping specificity, each capable of transamination with prephenate, phenylpyruvate, or 4-hydroxylphenylpyruvate. A suppressor mutation in the genetic background of the phenylalanine bradytroph was equated with the constitutive synthesis of aminotransferase HA I, normally a catabolic enzyme induced in wild type in the presence of either L-tyrosine or L-phenylalanine. The synthesis of aminotransferase HA II is repressed in the presence of phenylalanine and tyrosine, a result which suggests its probable role in aromatic amino acid biosynthesis. Aminotransferase HA III is unregulated by aromatic metabolites and is thought to function primarily in branched-chain amino acid metabolism.

Although the suppressor mutation restores the wild type growth rate in minimal glucose medium, aromatic biosynthesis is highly stressed in this strain as revealed by its hypersensitivity to antimetabolite analogues of phenylalanine and tyrosine. In fact, the DE I aminotransferase is highly expressed in the presence of either L-tyrosine or L-phenylalanine. The suppression of the wild type aminotransferase HA I, normally a catabolic enzyme induced in wild type in the presence of either L-tyrosine or L-phenylalanine, is used.

It is likely that the phenylalanine bradytroph can be utilized as the genetic background for the isolation of otherwise silent mutations that inactivate the various biosynthetic enzymes of tyrosine and phenylalanine biosynthesis.

Although phenylalanine and tyrosine are synthesized exclusively via phenylpyruvate and 4-hydroxyphenylpyruvate in such widely studied microorganisms as *Escherichia coli* or *Bacillus subtilis*, various (and probably all) species of blue-green algae form L-tyrosine from the amino acid intermediate, pretyrosine (1, 2). In some organisms pretyrosine may also function as a precursor of L-phenylalanine, as described recently in *Pseudomonas aeruginosa* (3). This bacterium was shown to possess dual enzymatic routes to convert prephenate to L-phenylalanine via either phenylpyruvate or pretyrosine, and likewise to possess enzymes converting prephenate to tyrosine via either 4-hydroxyphenylpyruvate or pretyrosine. This enzymatic multiplicity for tyrosine and phenylalanine synthesis in *P. aeruginosa* is illustrated in Fig. 1.

Mutagenic treatment of wild type cultures with nitroso-N-nitrosoguanidine has routinely produced nutritional mutants at high frequency in our laboratory. Thus, we readily obtained a full set of tryptophan auxotrophs, determined their enzymological deficiencies (4), and these have now been mapped on the *P. aeruginosa* chromosome. Other aromatic mutants blocked before shikimate or between shikimate and chorismate have been obtained (5) with no difficulty. Nevertheless, we have not managed to isolate mutants having absolute growth requirements for either L-phenylalanine or L-tyrosine. This is consistent with the presence in vivo of a second enzymatic option to either L-tyrosine or L-phenylalanine in any given mutant.

The fractional contributions of the two enzymatic routes to L-phenylalanine or to L-tyrosine under various physiological conditions is uncertain. If any of these four two-step sequences are inadequate in the absence of the alternative pathway to supply the total end product required for protein synthesis, then the imposition of a mutant block in the alternative sequence might produce a phenotype of leaky auxotrophy. Several phenylalanine bradytrophs were indeed obtained, and one was selected for detailed enzymological examination.

**MATERIALS AND METHODS**

**Microbiological Aspects**

Strain 1 of *P. aeruginosa* (wild type) was originally obtained from B. W. Holloway (6). Strain 1 is the parent strain from which phenylalanine bradytroph NP7 was derived through *N*-methyl-*N*-nitro-*N*-nitrosoguanidine mutagenesis (100 µg/ml, 30 min) as previously described (7). Mutant NP 76 was derived from mutant NP 72 following its selection as a spontaneous revertant capable of growth at the wild type rate on minimal glucose medium.

1. B. W. Holloway, manuscript in preparation.
2. N. Patel, S. Stenmark-Cox, and R. A. Jensen, unpublished data.

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beled amino acids CL-[ "CIglutamate or L-I 14C11eucine) were used in
phenylpyruvate at 331 nm was used. In most experiments, radiola-
diated (9). Extracts of 2 to 5 ml were prepared from
PLP, pyridoxal 5'-phosphate.

200-ml volumes of culture in the late exponential phase of growth.

Prephenate aminotransferase,” for example, refers to the combined
and HA III. Each has overlapping specificities for prephenate,
nate is supplied as the keto substrate.

extracts with various keto acceptor substrates under the above assay
obtained with 5 mM of the keto substrate reactant and 0.25 rnM L-
in aminotransferase HA II. The suppressed revertant NP 76 differs
tracts owing to interfering enzymes which react with the various
substrate combination cannot be accurately assessed in crude ex-

HA II, and HA III to total aminotransferase activity in crude
fractional contributions of aromatic aminotransferases DE I, HA I,
which enzyme studies with crude extracts are exceedingly
difficult owing to the presence of two enzymatic pathways

Pretyrosine is an amino acid intermediate which is one enzymatic
step from phenylalanine or tyrosine, and is formed by transamina-
tion of prephenate. Enzyme denotations: III, prephenate dehydra-
tase, 171, pretyrosine dehydrogenase (NAD+-dependent).

Notransferase; 151, prephenate aminotransferase; 161, pretyrosine
dehydrogenase (NAD+-dependent). PLP, pyridoxal 5'-phosphate.

4-HYDROXY-PHENYLPYRUVATE

Aromatic Aminotransferase Assays

Four molecular species of aromatic aminotransferases have been
characterized in P. aeruginosa and designated as DE I, HA I, HA II,
and HA III. Each has overlapping specificities for prephenate,
phenylpyruvate, and 4-hydroxyphenylpyruvate. For convenience,
"prephenate aminotransferase," for example, refers to the combined
activities of all four transaminases in crude extracts when prephe-
nate is supplied as the keto substrate.

Comparison of Aromatic Aminotransferase Activities in Crude
Extracts

Activities of prephenate aminotransferase, phenylpyruvate ami-
notransferase, and 4-hydroxyphenylpyruvate aminotransferase are
expressed as specific activities (nanomoles/min/mg of crude extract)
obtained with 5 mm of the keto substrate reactant and 0.25 mm L-
[14C]Glutamate. Even total aminotransferase activity with a given
substrate combination cannot be accurately assessed in crude ex-
tracts owing to interfering enzymes which react with the various
substrates or products (or both). The method of determination of the
fractional contributions of aromatic aminotransferases DE I, HA I,
HA II, and HA III to total aminotransferase activity in crude
extracts with various keto acceptor substrates under the above assay
conditions was detailed before (Table I of Ref. 3).

Prephenylalanine bradytroph NP 72 differs from wild type in the
absence (by mutation) of aromatic aminotransferase DE I. If grown in mini-
mal glucose medium, the bradytroph also differs from wild type in
its 3-fold decrease of aminotransferase HA I and its 6-fold increase
in aminotransferase HA II. The suppressed revertant NP 76 differs
from wild type in the absence of aminotransferase DE I and a 2.5-
fold elevation of aminotransferase HA I (in minimal glucose
medium).

Analytical Procedures

Aromatic Aminotransferase Assays—In some experiments, a spe-
trophotometric assay for phenylpyruvate at 320 nm or 4-hydroxy-
phenylpyruvate at 331 nm was used. In most experiments, radia-

3. Enzymology of aromatic pathways in bacteria. Ph.D. thesis, Harvard Uni-
versity, Cambridge, Mass. (1970.

Results

Enzymological Deficiency of Phenylalanine Bradytroph NP 72—When crude extracts of the mutant were compared with
wild type extracts for differences in enzyme activities, none
were apparent. However, in view of the recent appreciation that
genome studies with crude extracts are exceedingly
complicated owing to the presence of two enzymatic pathways
to L-tyrosine and L-phenylalanine (3), fractionation procedures
were essential. Using procedures described before (3), mutant
NP 72 was found to possess unaltered enzymes for both species
of chorismate mutase, prephenate dehydrogenase, pretyrosine
dehydrogenase, 4-hydroxyphenylpyruvate, and pretyrosine
dehydrogenase.

However, when appropriate chromatographic procedures
were used to separate the four aromatic aminotransferase
activities previously characterized in wild type, aminotrans-
ferase DE I was clearly absent in phenylalanine bradytroph
NP 72 (Fig. 2). A revertant derivative of the phenylalanine
bradytroph, isolate number NP 76, is seen to have arisen as a

RESULTS

Recovery of Aromatic Auxotrophs in P. aeruginosa—
Tightly blocked phenylalanine or tyrosine auxotrophs were
not obtained. We did, however, encounter leaky mutants
whose growth was restored to the rate of wild type in the
presence of L-phenylalanine. Such mutants are recognized if
survivors of mutagenesis are screened for faint growth as
early as possible following the replica plating technique.
Growth data obtained with one such phenylalanine brady-
troph, our isolate number NP 72, are illustrated in Table I. In
minimal glucose medium, the bradytroph grows about 40% as
fast as wild type. The wild type rate of growth was achieved in
the presence of L-phenylalanine or phenylpyruvate. L-Tyros-
ine and its keto acid precursor, 4-hydroxyphenylpyruvate,
also increased the growth rate significantly, but did not
restore the growth rate seen in the wild type parent. Sponta-
neous revertants were readily obtained.

Enzyme Fractionation Procedures

Procedures of DEAE-cellulose chromatography, hydroxyapatite
chromatography, and Sephadex G-200 gel filtration used for the
separation of the four aromatic aminotransferases (DE I, HA I, HA
II, and HA III) were detailed before (3). When column fractions
were pooled and concentrated, an Amicon Diaflow Cell (PM 10 filter) was
used.

Source of Biochemicals

Pyridoxal-5'-P, NAD, NADP, L-amino acids, keto acids, 2-amino-
2-hydroxyethyl-1,3-propanediol (Trizma) buffer, 4-aminophenyl-
alanine, 8-2-thienylalanyline, and Sephadex G-200 were obtained
from Sigma Chemical Co. DE52 cellulose was from Whatman and
hydroxyapatite (Bio-Gel HTP) was from Bio-Rad. L-[14C]Glutamate
(270 mCi/mmol) and L-[14C]Leucine (324 mCi/mmol) purchased from
Amerham/Searle were purified by an organic solvent extraction
method (11) before use. Aquasol was obtained from New England
Nuclear.

Barium prephenate and either ammonium pretyrosine or barium
pretyrosine were isolated from the 5-day accumulation medium of a
multiply blocked mutant strain of Neurospora crassa (12).
TABLE I

| Strain phenotype | Nutritional additions | Doubling time |
|------------------|-----------------------|--------------|
| Wild type        | Minimal glucose       | 57           |
| Phe bradytroph   | Minimal glucose       | 140          |
|                  | +4-Hydroxyphenylpyruvate | 110         |
|                  | +L-Tyrosine           | 70           |
|                  | +Phenylpyruvate       | 58           |
|                  | +l-Phenylalanine      | 56           |
| Phe revertant    | Minimal glucose       | 59           |

a Supplements to minimal glucose medium were present at a final concentration of 50 μg/ml.

- When 50 μg/ml of 4-hydroxyphenylpyruvate or tyrosine was present, growth proceeded at the indicated rates until a turbidity of about 0.35 at 600 nm was reached, and the growth rate decreased to that obtained in minimal glucose. Apparently this amount of tyrosine is degraded in this time span.

- The data given in Fig. 2 are consistent with an inducible regulation of aminotransferase HA I synthesis. The basal, uninhibited level is probably that found in the bradytroph cultured in minimal glucose medium, a condition of end product limitation for the mutant. Presumably, the endogenous level of phenylalanine or tyrosine of wild type (or both) is sufficient to partially induce the synthesis of aminotransferase HA I. The data given in Table II illustrate the variation of aminotransferase HA I activity over a 7-fold range. The suppressed mutant (right column) displays constitutive synthesis of aminotransferase HA I in wild type and in the bradytroph. The presence of L-phenylalanine results in full induction of the HA I enzyme while L-tyrosine promotes partial induction. In the two mutant strains lacking aminotransferase DE I, a certain proportionality between growth rate (Table I) and level of aminotransferase HA I (Table II) exists.

The data given in Fig. 2 are consistent with repression control of aminotransferase HA II synthesis. Thus, in the bradytroph where both phenylalanine and tyrosine are limiting to growth (Table I), the level of aminotransferase HA II was elevated significantly. When the bradytroph was grown in L-phenylalanine-supplemented medium, the level of aminotransferase HA II was repressed to the level of measured in wild type (data not shown). Repression of the synthesis of aminotransferase HA II over about a 6-fold range in the bradytroph is shown in Table III. Apparently in wild type the endogenous levels of end products during growth in minimal glucose are sufficient for maximal repression of aminotransferase HA II.

| Strain phenotype | Nutritional additions | Doubling time |
|------------------|-----------------------|--------------|
| Wild type        | Minimal glucose       | 57           |
| Phe bradytroph   | Minimal glucose       | 140          |
|                  | +4-Hydroxyphenylpyruvate | 110         |
|                  | +L-Tyrosine           | 70           |
|                  | +Phenylpyruvate       | 58           |
|                  | +l-Phenylalanine      | 56           |
| Phe revertant    | Minimal glucose       | 59           |

- DEAE CELLULOSE - - HYDROXYLAPATITE -

- In revertant NP 76, aminotransferase DE I is absent as is the case with its bradytrophic parent (middle panel). This revertant differs genetically from wild type in its absence of aminotransferase DE I (the primary mutation) and its elevated level of aminotransferase HA I (the suppressor mutation).

Regulation of Synthesis of Aromatic Aminotransferases—The data given in Fig. 2 are consistent with an inducible regulation of aminotransferase HA I synthesis. The basal, uninhibited level is probably that found in the bradytroph cultured in minimal glucose medium, a condition of end product limitation for the mutant. Presumably, the endogenous level of phenylalanine or tyrosine of wild type (or both) (Fig. 2) are sufficient to partially induce the synthesis of aminotransferase HA I. The data given in Table II illustrate the variation of aminotransferase HA I activity over a 7-fold range. The suppressed mutant (right column) displays constitutive synthesis of aminotransferase HA I. In wild type and in the bradytroph, the presence of L-phenylalanine results in full induction of the HA I enzyme while L-tyrosine promotes partial induction. In the two mutant strains lacking aminotransferase DE I, a certain proportionality between growth rate (Table I) and level of aminotransferase HA I (Table II) exists.
Total prephenate aminotransferase activity is similar in all three strains, actually being greater in the phenylalanine bradytroph than in wild type. In the phenylalanine bradytroph, the level of phenylpyruvate aminotransferase is 23% of its wild type parent, while the level of 4-hydroxyphenylpyruvate aminotransferase in the bradytroph is 32% of that measured in wild type. Total phenylpyruvate aminotransferase in the revertant is nearly double that of the bradytroph, total 4-hydroxyphenylpyruvate aminotransferase activities of the bradytroph and its revertant are similar.

The total specific activities expressed in Fig. 3 are given in Table IV, together with a detailed breakdown of the fractional contribution of the four aromatic aminotransferase species to each of the three aminotransferase activities involved in aromatic amino acid biosynthesis of P. aeruginosa. Aminotransferase DE I of wild type is the major contributor to phenylpyruvate aminotransferase and 4-hydroxyphenylpyruvate aminotransferase activities. In the phenylalanine bradytroph, species HA II accounts for about 60% of all three aminotransferase activities, a result in marked contrast to the low fractional contribution of species HA II to the three aminotransferase activities, of either wild type or the revertant.

![Figure 3](http://www.jbc.org/)

**Figure 3.** Levels of aromatic aminotransferase activities in wild type and mutant strains of P. aeruginosa. Prephenate (PPA) aminotransferase (left), phenylpyruvate (PPY) aminotransferase (upper right), and 4-hydroxyphenylpyruvate (HPP) (lower right) are represented by solid arrows. Other enzyme reactions shown are detailed in Fig. 1. The bar graphs indicate the specific activities of the indicated aminotransferase in wild type (WT), phenylalanine bradytroph NP 72 (PHE), or a suppressed revertant (PHE REV) NP 76 derived from NP 72. Specific activities expressed as nanomoles/min/mg of crude extract protein and determined as described under "Materials and Methods." [WT] phenylalanine bradytroph NP 72; 2.49, 0.50, and 0.95 with prephenate, phenylpyruvate, and 4-hydroxyphenylpyruvate, respectively in wild type; 2.49, 0.50, and 0.95 with prephenate, phenylpyruvate, and 4-hydroxyphenylpyruvate, respectively, in phenylalanine bradytroph NP 72; 2.02, 0.77, and 1.15 with prephenate, phenylpyruvate, and 4-hydroxyphenylpyruvate, respectively, in the suppressed revertant. PRT, pretyrosine.

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| TABLE II | Inducibility of aromatic aminotransferase HA I |
|---|---|
| Extracts were prepared from cultures in the late exponential phase of growth as described under "Materials and Methods." Aromatic aminotransferase HA I was isolated as described under Fig. 2 and the appropriate fractions were pooled. Assays were carried out at 37°C for 20 min in 200-μl reaction mixtures containing 50 mM Tris buffer, pH 7.9, 50 mM 2-ketoglutarate, 2.5 mM L-phenylalanine, and enzyme. Reactions were terminated with 0.8 ml of 2.5 N NaOH and absorbance of phenylpyruvate was read at 320 nm. |

| Growth condition | Specific activity<sup>a</sup> | Prephenate aminotransferase/min/mg crude extract |
|---|---|---|
| Minimal glucose | 3.2 | 1.1 | 8.0 |
| +L-Phenylalanine<sup>b</sup> | 8.0 | 7.2 | |
| +L-Tyrosine<sup>b</sup> | 4.0 | 3.6 | |

<sup>a</sup> Appropriate calculations were made to extrapolate activities measured in partially purified preparations back to specific activities in crude extracts originally prepared from whole cells.

<sup>b</sup> Added to a final concentration of 50 μg/ml.

| TABLE III | Repression of aromatic aminotransferase HA II in phenylalanine bradytroph NP 72 |
|---|---|
| Aminotransferase HA II was recovered from hydroxylapatite as described under Fig. 2. |

| Growth condition | Specific activity<sup>a</sup> |
|---|---|
| Minimal glucose | 0.68 |
| +L-Phenylalanine<sup>b</sup> | 0.18 |
| +L-Tyrosine<sup>b</sup> | 0.11 |

<sup>a</sup> Expressed as nanomoles of L-2-keto[14C]glutarate formed/min/mg of crude extract. The enzyme activity was assayed with prephenate and L-[14C]glutamate as described under "Materials and Methods."

<sup>b</sup> Added to a final concentration of 50 μg/ml.

**TABLE IV**: Comparison of fractional contributions of various molecular species of aromatic aminotransferases to enzyme reactions in different strains

Aminotransferases DE I, HA I, HA II, and HA III were separated by DEAE-cellulose and hydroxylapatite chromatography, and appropriate calculations were made to extrapolate these completely stable activities back to specific activities of crude extracts originally prepared from whole cells.

| Strain phenotype | Total specific activity<sup>a</sup> | Prephenate aminotransferase<sup>a</sup> | Phenylpyruvate aminotransferase<sup>a</sup> | 4-Hydroxyphenylpyruvate aminotransferase<sup>a</sup> |
|---|---|---|---|---|
| | DE I | HA I | HA II | HA III | DE I | HA I | HA II | HA III | DE I | HA I | HA II | HA III |
| Wild type | 2.18 | 36 | 19 | 12 | 33 | 2.14 | 81 | 11 | 2 | 6 | 2.94 | 76 | 10 | 3 | 11 |
| Phe' bradytroph | 2.49 | 0 | 6 | 65 | 29 | 0.50 | 0 | 16 | 60 | 24 | 0.95 | 0 | 10 | 57 | 33 |
| Phe' revertant | 2.02 | 0 | 50 | 14 | 36 | 0.97 | 0 | 78 | 6 | 16 | 1.16 | 0 | 65 | 8 | 21 |

<sup>a</sup> Keto acid used at 5 mM; L-[14C]glutamate at 0.25 mM.

<sup>b</sup> Expressed as nanomoles/min/mg of crude extract (see "Materials and Methods").
ant. In the suppressed mutant, species HA I is the major fractional component of all three aminotransferase activities. Hence, in each of the three strains compared in Table IV, a different species of aromatic aminotransferase contributes the major share of transaminase activity with each of the three aromatic keto substrates.

**Recruitment of Catabolic Aminotransferase to Biosynthetic Function—**Aminotransferase DE I is reasonably deduced to be a biosynthetic enzyme since its loss by mutation leads to slowed growth in the absence of end products. Aminotransferase HFA I is catabolic in function since it is induced by phenylalanine and tyrosine. The kinetic data illustrated in Fig. 4 confirm the opposing equilibria of the enzymes. Aminotransferase DE I exhibits $K_v$ values for the substrate reactants shown that favors catalysis in the rightward directions (top) by virtue of order of magnitude differences.

Aminotransferase HA I actually displays greater affinities for phenylpyruvate and 4-hydroxyphenylpyruvate than does aminotransferase DE I. The relative affinities for glutamate and 2-ketoglutarate, however, are dramatically different in comparison of the two enzymes, suggesting catalysis in the rightward direction for aminotransferase DE I and catalysis in the leftward direction for aminotransferase HA I.

The growth of phenylalanine bradytroph NP 72 may be limited more or less equally by phenylalanine and tyrosine synthesis, as suggested by the analysis depicted in Fig. 3. An apparently greater deficiency in endogenous phenylalanine synthesis is suggested by the better growth response to L-phenylalanine or phenylpyruvate (Table I) than to L-tyrosine or 4-hydroxyphenylpyruvate. However, this probably reflects the excellent induction of aminotransferase HA I synthesis by L-phenylalanine, but not by L-tyrosine (Table I).

Comparison of Responses of Wild Type and Pseudo Wild Type to Analogue Stress—In minimal glucose medium the growth rate of wild type *P. aeruginosa* is retarded weakly by β 2-thienylalanine, which is nevertheless one of the most effective phenylalanine antimetabolites for this organism (8). The pseudo wild type derivative (NP 76) of the phenylalanine bradytroph grows in glucose minimal medium at the same rate as wild type (Table I). However, mutant NP 76 is hypersensitive to growth inhibition by β 2-thienylalanine (Table V), indicating that phenylalanine biosynthesis is more stressed in the revertant strain than in wild type. The growth rates of the two strains are only slightly different in the presence of 4-aminophenylalanine, an antimetabolite of L-tyrosine in *P. aeruginosa* (8).

When glucose is replaced with fructose, *P. aeruginosa* grows more slowly (Table VI), and it was previously shown (8) that in the presence of fructose, total aromatic biosynthesis becomes growth-limiting owing to decreased flux of early precursors into the pathway. Under these conditions of growth (minimal fructose), β 2-thienylalanine inhibits the growth rate of wild type better than 4-aminophenylalanine (Table VI), in contrast to results shown in Table V with minimal glucose.

Strikingly, the revertant does not achieve the wild type rate of growth in minimal fructose. In fact, the growth rate of the revertant is as slow as that of the bradytroph. Clearly, the suppressor mutation present in the revertant would never have been detected if selection had been carried out on minimal fructose medium. Presumably aminotransferase HA I cannot operate effectively in the biosynthetic direction under the metabolic conditions that exist during growth on fructose.

![Fig. 4.](http://www.jbc.org/)...
Reluctant Auxotrophy in Pseudomonas

common in nature than previously suspected, and even *E. coli* is not altogether free of such pathway multiplicity (13). The physiological and evolutionary implications of metabolic ambiguity for synthesis of small molecules has been discussed elsewhere (14). It seems likely that difficulties encountered in the isolation of amino acid auxotrophs from various groups of bacteria of wide distribution in nature may be explained by the compensatory presence in "silent" mutants of a second pathway to a given end product. Aromatic biosynthesis in pseudomonad microorganisms is an excellent example. Since the phenylpyruvate and 4-hydroxyphenylpyruvate pathways of *E. coli* were readily demonstrated in *P. aeruginosa*, the additional presence of the pretyrosine pathways to phenylalanine and tyrosine has remained unsuspected until recently. In such systems, single mutations may at best be expressed as leaky auxotrophs (if the presence of only one intact sequence to end product results in a growth-limiting supply of that end product), or perhaps as mutants that are hypersensitive to inhibition of growth by end product analogues (if the presence of only one intact sequence to end product results in a significantly decreased endogenous pool size of end product).

If the presence of a mutation is recognized, either by leaky auxotrophy or by analogue hypersensitivity, a second mutation in the strain should produce tightly blocked derivatives carrying an additional block in the second pathway. When genetic backgrounds containing such combinations of sequentially introduced mutations are subsequently separated by recombination, it is likely that their individual phenotypes may be similar to wild type.

**In Vivo Function of Aminotransferase Reactions**—The extent to which a given aminotransferase may be shared to carry out transamination reactions in different biochemical pathways is not very well understood. The broadly overlapping specificities of most aminotransferase activities in vitro may or may not reflect the spectrum of reactions actually catalyzed in vivo. Even when a particular transaminase reaction can only be catalyzed by one aminotransferase, that aminotransferase may nevertheless function in another pathway, such as that of lysidine phosphate aminotransferase in *B. subtilis* (15). Under particular, specialized conditions an aminotransferase may function in transamination reactions that do not ordinarily occur in wild type, as with prephenate transaminase in *N. crassa* (12).

The combined activities of aminotransferase enzymes, having different, but overlapping specificities has long been assumed in many biochemical systems to account for the lack of mutant phenotypes that might arise from aminotransferase deficiencies. The fractional contribution of various aminotransferases to particular transamination reactions in vivo can best be approached through the systematic and sequential elimination of individual aminotransferases by mutation. Thus, *E. coli* each of three aminotransferases (specified by genes *tyrB*, *ileE*, and *aspC*) is sufficient alone to function as phenylpyruvate aminotransferase (16). Absence of aminotransferases specified by *tyrB* or *aspC* does not lead to nutritional requirements unless the genetic background is also deficient in *ileE* (16). A fourth aminotransferase of *E. coli* appears to take place primarily in *L*-valine and *L*-alanine biosynthesis (17).

In *P. aeruginosa*, a comparable systematic approach should be feasible, by exploiting the genetic background of the phenylalanine bradytroph NP 72 for mutagenesis. The absence of aminotransferase DE 1 in bradytroph NP 72 may permit the recognition of newly arisen aminotransferase deficiencies by the expression of phenotypes that may not be apparent when isolated in wild type backgrounds.

**Specialization of Aromatic Aminotransferases in *P. aeruginosa***—Aminotransferase DE 1 is clearly essential for normal aromatic biosynthesis in wild type since its loss by mutation results in growth-limiting rates of phenylalanine or tyrosine synthesis. The DE 1-negative bradytroph is deficient in overall aminotransferase activity with phenylpyruvate and 4-hydroxyphenylpyruvate, but not in overall aminotransferase activity with prephenate. Since intracellular levels of prephenate should be elevated in the bradytroph owing to relaxation of the regulation of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthetase (18) in response to end product limitation, intracellular concentrations of pretyrosine may even exceed that of wild type. If so, then pretyrosine dehydratase and pretyrosine dehydrogenase must be rate-limiting reactions for growth in the bradytroph when cultured in minimal glucose medium.

Aminotransferase HA 1 appears to overlap the catalytic characteristics of aminotransferase DE 1 most effectively. Since the synthesis of species HA 1 is induced in the presence of either *L*-phenylalanine or *L*-tyrosine, its normal function in vivo is undoubtedly catabolic. The inducible regulation of aminotransferase HA 1 prevents the bradytroph lacking species DE 1 from exploiting the potential of species HA 1 to catalyze the reactions normally carried out by species DE 1. This interpretation is further supported by the ability of a mutation to constitutive synthesis of aminotransferase HA 1 to suppress the DE 1 deficiency. Thus, it appears that elevated levels of a degradative aminotransferase in the absence of its normal substrate (*e.g.* *L*-phenylalanine) and the probable increase of its normal product (*e.g.* phenylpyruvate) functions in the backward direction in recruitment to biosynthetic function. The ability of a constitutively synthesized aminotransferase HA 1 to function as the biosynthetic aminotransferase DE 1 may suggest the evolutionary origin of the aminotransferase proteins from a common protein. The identical molecular weights (70,000) of aminotransferases HA 1 and DE 1 (3) are consistent with this possibility. Although a constitutively synthesized aminotransferase HA 1 can replace aminotransferase DE 1 when glucose is the carbon source for growth, it cannot do so when aromatic biosynthesis is further stressed by growth on fructose.

Aminotransferase HA 2 is repressible over a 6-fold range, and this regulation implicates its role in aromatic amino acid biosynthesis. Enzyme HA II works much better as prephenate aminotransferase than as either phenylpyruvate aminotransferase or 4-hydroxyphenylpyruvate aminotransferase (3). The enzymological analysis of the wild type and mutant strains is suggestive of a primary role of aminotransferase HA II as prephenate aminotransferase.

The synthesis of aminotransferase HA III does not appear to vary in response to excess or limitation of aromatic end products. The dramatically better function of *L*-leucine as an amino-donor reactant in comparison with *L*-glutamate (3) suggests that aminotransferase HA III may primarily function in branched-chain amino acid metabolism. (In *E. coli* the *tyrB*-encoded aminotransferase can function as the sole transamination step required for leucine biosynthesis (16).)

Our present interpretations are that the phenylpyruvate and 4-hydroxyphenylpyruvate fractions to phenylalanine and tyrosine synthesis are fractions greater than the pretyrosine pathways to phenylalanine and tyrosine synthesis; that aminotransferase DE 1 is primarily phenylpyruvate/4-
hydroxyphenylpyruvate aminotransferase in vivo while aminotransferase HA II functions primarily as a prephenate aminotransferase; that aminotransferase HA I is an inducible enzyme which functions in vivo for catabolism of l-tyrosine and L-phenylalanine; and that aminotransferase HA III probably is primarily functional in the branched-chain amino acid pathways, rather than in aromatic metabolism.

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