ENZYME INDUCTION IN THE TRYPTOPHAN BIOSYNTHETIC PATHWAY IN BACILLUS SUBTILIS

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Abstract. A single mutation resulting in a non-functional chorismate mutase simultaneously leads to increased activity of anthranilate synthetase and phosphoribosyl transferase in Bacillus subtilis. This mutant produces a concentration of chorismate that is significantly greater than that found in another mutant which has an unaltered chorismate mutase and is constitutive for the enzymes of the tryptophan pathway. We propose that the high internal concentration of chorismate induces the synthesis of these enzymes. It is inferred that alteration of the internal level of chorismate dramatically changes the specific activity of enzymes in the tryptophan pathway.

Introduction. Although the chemical reaction sequence for aromatic amino acid biosynthesis is invariant in microorganisms studied thus far, the control patterns of the pathway vary from one microorganism to another (1). In Bacillus subtilis the branch point metabolite, prephenate, is a feedback inhibitor of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthetase; chorismate, another intermediate, is a relatively poor feedback inhibitor of DAHP synthetase activity (2). Another indication of the key role of branch point metabolites in governing the regulation of aromatic amino acids in B. subtilis is our evidence suggesting that the synthesis of all of the enzymes of the tryptophan pathway is induced by chorismic acid.

Methods. The strains of B. subtilis used in these experiments are indicated in Table 1. Growth conditions and preparations of enzyme extracts are detailed elsewhere (3).

DAHP synthetase (2), prephenate dehydrogenase (4), prephenate dehydratase (5), anthranilate synthetase (3) and 5-phosphorylribose 1-pyrophosphate phosphoribosyl transferase (PR transferase) (3) were assayed as previously described. Chorismate mutase was assayed by continuously following the disappearance of chorismate at 274 nm for 4 min at 37° in a reaction mixture containing Tris buffer, pH 7.2, 400 nmoles of barium chorismate and enzyme in a final volume of 1.0 ml.

The excretion of tryptophan into the growth medium was determined as previously described (3). The accumulation of chorismate in the supernatants of cultures of NP 100 and NP 40 was followed using the conditions described by Gibson (6). The presence of chorismate in supernatants of strain NP 100 was verified by the following observations: (i) a peak absorbance at 274 nm; (ii) heating the supernatant at 70° for 70 min produced a substance (prephenate) which when acid treated for 10 min resulted in a compound that was identified as phenylpyruvate by its strong absorbance peak at 320 nm in N NaOH; (iii) the pre-heated supernatant served as a substrate for anthranilate synthetase whereas the post-heated supernatant did not serve as a substrate.

Conditions for mutagenesis with nitrosoguanidine and DNA-mediated transformation have been previously detailed (7).

Results and Discussion. The key observations implicating a novel control governing the level of tryptophan biosynthetic enzymes centered about the properties of mutant strain NP 100 (see Table 1). This mutant, as a consequence of a single-

Table 1

Strain	Origin	Growth Requirement	Aromatic Enzyme Block[a]
NP 40	Spontaneous full revertant of strain 168 (trpC) (7)	None	
NP 16	E.W. Nester (SB 167) (8)	Aro[b]	DHQS
NP 100	Spontaneous mutant of NP 40 isolated on growth inhibitory concentrations of chorismate.	phe	CM
E-67	Nitrosoguanidine mutagenesis of NP 100	phe + arg	CM
E-68	NP 16 ——x E-67[c]	aro	DHQS + CM
E-69	Spontaneous revertant of E-68 selected on phenylalanine	phe	CM

[a]DHQS - 5-dehydroquinate synthetase; CM - chorismate mutase.

[b]Aro - tryptophan, tyrosine and phenylalanine.

[c]Genetic notations are used according to Nester et al (17).

step mutation, is both deficient in activity for chorismate mutase and is derepressed for the enzymes of the tryptophan pathway (Table 2). These characteristics are the pleiotropic effect of a single mutation since the two phenotypes are inseparable in genetic crosses using DNA transformation (unpublished observations). One would expect such a mutant to require both phenylalanine and tyrosine. However, the mutant is quite leaky and grows fairly well on phenylalanine alone. Presumably, the low amount of prephenate formed is preferentially utilized to produce tyrosine, possibly because of the more favorable kinetic properties of prephenate dehydrogenase in competition with prephenate dehydratase. Although strain NP 100 is phenotypically a phenylalanine auxotroph, the specific activity of prephenate dehydratase is identical to that of the wild type strain, NP 40 (Table 2). The specific activities of five additional enzymes are compared in strains NP 40 and NP 100 in Table 2.

Table 2

Comparison of Enzymatic Activities of NP 40 and NP 100

	Relative Specific Activity[a]					
Strain	DAHP Synthetase	Chorismate Mutase	Prephenate Dehydratase	Prephenate Dehydrogenase	Anthranilate Synthetase	PR Transferase
NP 40	1	1	1	1	1	1
NP 100	2	0	1	2	15	18

[a]The specific activity of NP 40 was arbitrarily given a value of 1.0 for all enzymes. NP 40 was grown in minimal medium and NP 100 was grown in minimal media plus phenylalanine at 50 μg/ml. Growth of NP 40 on phenylalanine does not significantly influence the levels of any of these enzymes.

Limitation of tyrosine has been shown to result in physiological derepression of prephenate dehydrogenase and DAHP synthetase (8). The two-fold elevation of these enzymes reflects the tyrosine limitation in mutant NP 100 grown in the presence of

phenylalanine. That these derepressions are physiological in nature was indicated by the observation that the enzymes were repressed when tyrosine was added to the medium (unpublished observations). The derepression of DAHP synthetase in the aromatic pathway in strain NP 100 as well as the negligible level of prephenate present to inhibit DAHP synthetase would tend to accentuate the accumulation of chorismate behind the enzyme block. Both anthranilate synthetase and PR transferase were highly derepressed in strain NP 100, and these levels were not repressed by growth in 50 μg/ml of tryptophan.

Two general categories of explanations exist to explain the dual phenotype of strain NP 100. (i) The mutation in chorismate mutase has consequences which lead to a physiological derepression (or induction), or (ii) the mutation in chorismate mutase may also cause a true constitutive level of the enzymes in the tryptophan pathway. The latter alternative could arise, for example, (a) by a deletion covering both the chorismate mutase structural gene and a gene specifying an operator or apo-repressor site for tryptophan genes, or (b) by a mutation in a gene coding a common sub-unit for chorismate mutase and an apo-repressor protein for the tryptophan pathway.

Possibility (ii) has been ruled out by the demonstration that the level of tryptophan biosynthetic enzymes can be altered in strain NP 100 by changing the level of early intermediary metabolites. We introduced a mutation specifying a block in the second enzyme of the aromatic pathway (Table 1) into strain E-67, a derivative of strain NP 100 requiring arginine. The recombinant strain, E-68, lacked chorismate mutase and 5-dehydroquinate synthetase. After 24 hours on agar plates E-68 showed a good growth response on shikimate plus phenylalanine and a poor growth response on either shikimate or phenylalanine alone. The enzymatic profile of strains NP 100, E-67 and E-68 is shown in Table 3. By introducing a block in the early part of the pathway (E-68) the specific activities of both of the tryptophan biosynthetic enzymes

Table 3

Specific Activities of Tryptophan Biosynthetic Enzymes in NP 100 and Its Derivatives[a]

Strain Designation	Specific Activity	
	Anthranilate Synthetase[b]	PR Transferase[c]
NP 40	0.007	0.002
NP 100	7.00	4.80
E-67	7.00	4.10
E-68	0.007	0.002
E-69	7.00	4.10

[a] All strains were grown on glucose-minimal media supplemented with 50 μg/ml each of tryptophan, tyrosine and phenylalanine.

[b] Specific activity expressed as nmoles anthranilate formed/min/mg protein.

[c] Specific activity expressed as nmoles anthranilate utilized/min/mg protein.

were significantly reduced to levels characteristic of strain NP 40. E-69, a revertant of E-68 which was selected on phenylalanine medium, regained the enzymatic profile of the parental strain, NP 100. Hence, the chorismate mutase block per se is not sufficient to cause elevated enzyme levels in the tryptophan pathway. Chorismate must accumulate behind this block in mutants NP 100 and E-69. We have observed a 3-fold increase in the amount of chorismate in the culture medium of NP 100 when compared to the culture medium of NP 40 (unpublished observations). We have previously demonstrated that strain NP 100 has a high internal concentration of chorismate and excretes large amounts of tryptophan (50-fold greater than strain NP 40 and 12-fold greater than a genetically derepressed strain which has an unaltered chorismate mutase) (3). The tremendous excretion of tryptophan by strain NP 100 reflects the increased amount of chorismate available as substrate. It is probable that chorismate

induces the tryptophan biosynthetic enzymes coordinately. The possible sequential induction of each enzyme by its substrate seems unlikely for the following reasons: (i) growth of an anthranilate synthetase-deficient mutant on anthranilate does not result in a higher level of PR transferase than that found in wild type NP 40; (ii) when an anthranilate synthetase block is introduced into strain NP 100, PR transferase is still induced at a level comparable to that of the parental strain NP 100; (iii) the operon-like arrangement of the genetic loci and the coordinate expression of the tryptophan enzymes (9) favors the induction of all the enzymes as a unit in response to a single metabolite.

Our experiments show that the increased levels of two tryptophan biosynthetic enzymes in strain NP 100 can be decreased to wild type levels by interrupting the synthesis of precursors of chorismate. Hence a small molecule that almost certainly is chorismate is an inducer which signals the synthesis of tryptophan enzymes. Several examples (10-14) offer a precedent for induction in a biosynthetic pathway. Nevertheless, it is obvious that addition of tryptophan to the growth medium represses the tryptophan biosynthetic enzymes in wild type B. subtilis (8). Two possible explanations occur to us. (i) Chorismate, the initial substrate, is an inducer molecule; tryptophan, the end-product, is a repressor molecule. The critical determinant of enzyme levels is the ratio of these two metabolites. Such a control would resemble the significance of metabolite ratios in several catabolic pathways (15,16). (ii) The levels of tryptophan enzymes are solely dependent upon the intracellular concentration of chorismate. Endproduct control is then achieved indirectly by some unknown negative effect of tryptophan upon chorismate concentrations. Experiments are in progress which should permit a decision between these alternatives.

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