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Regulation of Enzyme Synthesis in the Aromatic Amino Acid Pathway of *Bacillus subtilus*

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The control of the synthesis of certain key enzymes of aromatic amino acid biosynthesis was studied. Tyrosine represses the first enzyme of the 3-deoxy-D-*arabino* heptulosonic acid 7-phosphate pathway, DAHP synthetase, as well as shikimate kinase and chorismate mutase about fivefold in cultures grown under conditions limiting the synthesis of the aromatic amino acids. A mixture of tyrosine and phenylalanine represses twofold further. Tryptophan does not appear to be involved in the control of these enzymes. The specific activity of at least one early enzyme, dehydroquinase, remains essentially constant under a variety of nutritional supplementations. Two enzymes in the terminal branches are repressed by the amino acids they help to synthesize: prephenate dehydrogenase can be repressed fourfold by tyrosine, and anthranilate synthetase can be repressed over 200-fold by tryptophan. There is no evidence that phenylalanine represses prephenate dehydratase. Regulatory mutants have been isolated in which various enzymes of the pathway are no longer repressible. One class is derepressed for several of the prechorismate enzymes, as well as chorismate mutase and prephenate dehydrogenase. In another mutant, several enzymes of tryptophan biosynthesis are no longer repressible. Thus, the rate of synthesis of enzymes at every stage of the pathway is under control of various aromatic amino acids. Tyrosine and phenylalanine control the synthesis of enzymes involved in the synthesis of the three aromatic amino acids. Each terminal branch is under the control of its end product.

In those microorganisms in which the control of aromatic amino acid biosynthesis has been most thoroughly studied, the efficient regulation of the pathway is in large part dependent on the isoenzymatic nature of the first enzyme of the pathway, 3-deoxy-D-*arabino* heptulosonic acid 7-phosphate (DAHP) synthetase (4, 9, 21). Each of the isoenzymes is controlled by one of the end products of the pathway, tryptophan, tyrosine, or phenylalanine. Such control affects the activity of the enzymes as well as their synthesis. In addition, each of the terminal branches is under the feedback and repression control of the single aromatic amino acid end product.

In *Bacillus subtilis*, only one species of DAHP synthetase exists. Therefore, the physiological control of this enzyme and the pathway of aromatic acid synthesis must be considerably different than in organisms which possess multiple forms of DAHP synthetase. Indeed, it has already been shown that in *B. subtilis* the activity of this enzyme in vitro is controlled by two intermediate metabolites of the pathway, chorismate and prephenate, rather than by the aromatic amino acids themselves (10). The present report is concerned with the regulation of synthesis of DAHP synthetase, and with certain key enzymes in each of the three terminal branches.

MATERIALS AND METHODS

Bacterial strains. All of the bacterial strains used in this study are derivatives of *B. subtilis* strain 168 (22); details are given in Table 1.

Growth of cells and extract preparation. The cells were grown and extracts prepared as previously described (16). The glucose-salts medium of Spizizen (22) was routinely supplemented with (per liter): Fe^{+3}, 0.2 mg; Mn^{+2}, 0.2 mg; Mo^{+6}, 0.02 mg; Zn^{+2}, 2 mg. The detailed procedure of extract preparation was dictated by the particular assay to be done.

Chemicals. 3-Fluorotyrosine was a gift from R. Somerville, Purdue University. All other chemicals were obtained from commercial sources and were of the highest purity available; they were used without further purification.

Enzyme assays. Assay for DAHP synthetase was

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TABLE 1. *Strains of B. subtilis*

Strain	Description
WB746	Prototroph
SB167	Dehydroquinate synthetase-negative, grows poorly on shikimate
WB1000	Multiple auxotroph lacking prephenate dehydrogenase, prephenate dehydratase, anthranilate synthetase
SB455	5-Methyl tryptophan-resistant prototroph derepressed for tryptophan enzymes
WB740	Prototroph; fluorophenylalanine-resistant
SB443	Tyrosine bradytroph, low in prephenate dehydrogenase activity
WB698b	Derivative of SB443, genetically derepressed for several enzymes
WB2055	Prototroph containing regulatory locus of WB698b and a wild-type prephenate dehydrogenase locus
WB2058	3-Fluorotyrosine-resistant; prephenate dehydrogenase insensitive to tyrosine inhibition
WB2593	Prephenate dehydrogenase locus of WB-2508 in the genetic background of WB698b
WB2070	Double mutant, dehydroquinate synthetase and prephenate dehydrogenase-negative
168	Tryptophan auxotroph

done according to the method described by Srinivason et al. (24), as modified by Jensen and Nester (10).

Dehydroquinate synthetase assay was done according to Srinivason et al. (23). The substrate (DAHP) was isolated as previously described (15).

Assay for dehydroquinase (14), shikimate kinase (17), chorismate mutase (13), prephenate dehydrogenase (20), and anthranilate synthetase (16) were done as previously described.

Anthranilate was measured in an Aminco-Bowman spectrophotofluorometer. Tryptophan synthetase was assayed according to the method described by Schwartz and Bonner (19), and prephenate dehydratase was assayed according to Coats and Nester (3).

The specific activities of dehydroquinate synthetase and tryptophan synthetase are expressed as nanomoles of substrate utilized per minute per milligram of protein. All other specific activities are expressed as nanomoles of product formed per minute per milligram of protein.

RESULTS

The pathway of aromatic amino acid biosynthesis can be conveniently subdivided into five segments (Fig. 1). These components include the enzyme reactions common to the synthesis of all aromatic amino acids (DAHP to chorismate),

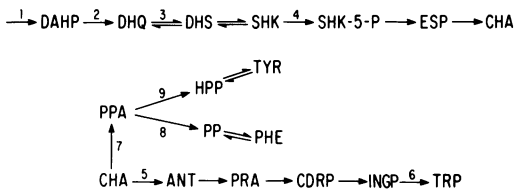

FIG. 1. *Pathway of aromatic amino acid synthesis. (1) DAHP synthetase, (2) dehydroquinate synthetase, (3) dehydroquinase, (4) shikimate kinase, (5) anthranilate synthetase, (6) tryptophan synthetase, (7) chorismate mutase, (8) prephenate dehydratase, (9) prephenate dehydrogenase. Abbreviations: DHQ, dehydroquinate; DHS, dehydroshikimate; SHK, shikimate; SHK-5-P, shikimate-5-phosphate; ESP, enolpyruvylshikimate-5-phosphate; CHA, chorismate; PPA, prephenate; HPP, p-hydroxyphenylpyruvate; TYR, tyrosine; PP, phenylpyruvate; PHE, phenylalanine; ANT, anthranilate; CDRP, 1-(o-carboxyphenylamino)-1-deoxyribulose-5-phosphate; InGP, indole glycerol phosphate; TRP, tryptophan.*

the enzymatic conversion of chorismate to prephenate, and the three terminal branches for tyrosine, phenylalanine, and tryptophan biosynthesis. We present information on the regulation of each of these segments and then collate these data for a picture of the overall pattern of control of this pathway. We did not observe any regulatory control by the vitamins derived from chorismate (5) and will not consider this portion of the pathway.

Enzymes leading to chorismate. This sequence comprises seven enzyme steps. In *B. subtilis* there is only one molecular species of DAHP synthetase (11), in contrast to the multiple forms of this enzyme observed in a wide variety of other organisms (9). There is evidence for two species of shikimate kinase, and one species of each of the other enzymes concerned with chorismate synthesis (15).

The synthesis of three of these early enzymes, DAHP synthetase, dehydroquinate synthetase, and shikimate kinase, by the prototrophic strain WB746 is repressed by a combination of the aromatic amino acids. Another enzyme, dehydroquinase, is not repressed (Table 2). The above three repressible enzyme activities are partially repressed in the prototroph growing in minimal medium without supplementation. They are elevated several-fold in a mutant (SB167) grown on a supplement, shikimate, which limits the synthesis of the end metabolites, tyrosine, phenylalanine, and tryptophan (Table 2). This mutant (SB167), deficient in dehydroquinate synthetase, has a generation time of 84 min when grown on shikimate and 60 min when grown on a medium supplemented with the three aromatic amino

TABLE 2. *Enzyme activity in a wild type and bradytrophic strain*

Strain[a]	Supplementation	Specific activity			
		DAHP synthetase	Dehydroquinate synthetase	Dehydro-quinase	Shikimate kinase
WB746	0	70	15	4.3	562
WB746	Aro + shikimate	19	6	8.9	140
SB167	Shikimate	163	<1	5.8	708
SB167	Aro + shikimate	22	<1	5.8	150

[a] WB746 was grown in a glucose salts medium supplemented with 100 μg of tyrosine, 100 μg of phenylalanine, 20 μg of tryptophan (aro), and 50 μg of shikimate per ml where indicated. SB167 was grown on 50 μg of shikimate per ml and on aro-supplemented medium. Extracts were prepared and the assay was performed as described in Materials and Methods. WB746, prototroph; SB167, multiple aro auxotroph.

acids. The poor growth on shikimate is apparently caused by the inability of this strain to take up shikimate readily (Chapman, *unpublished data*). Thus, shikimate serves to limit growth. Excess levels of a combination of the aromatic amino acids repressed enzyme synthesis in both strains to the same level (Table 2). The level of dehydroquinase did not decrease upon aromatic acid supplementation, suggesting that this enzyme is not under the repression control of the aromatic amino acids. The repression control of the first enzyme of the pathway, DAHP synthetase, is of particular interest, since it is reasonable to assume that the synthesis and activity of this enzyme in large part determine the availability of chorismate for aromatic amino acid synthesis.

The rate of synthesis of DAHP synthetase was determined in the mutant SB167 by measuring its level in the mutant grown on shikimate plus each of the aromatic amino acids. The results (Table 3) indicate that tyrosine represses most markedly, then phenylalanine, with tryptophan repressing very little, if at all. The combination of the three represses somewhat more than the tyrosine added alone. However, it is not possible to conclude from these data that the repression by tyrosine can be attributed to tyrosine per se. Its addition would undoubtedly affect the intracellular concentrations of phenylalanine and tryptophan as well as those of the intermediates, chorismate and prephenate. *B. subtilis* does not convert phenylalanine to tyrosine (18).

To vary the concentration of one end product independently of the other two, a triple auxotroph (WB1000) was studied. This mutant is blocked in the synthesis of tyrosine (lacks prephenate dehydrogenase), phenylalanine (lacks prephenate dehydratase), and tryptophan (lacks anthranilate synthetase). This strain was grown in a chemostat under conditions of limiting tyrosine, phenylalanine, or tryptophan (Table 3). The results indicate that, with limiting tyrosine, the enzyme is relatively derepressed. In cells grown in excess tyrosine (with either phenylalanine or tryptophan limiting), the activity is repressed. Although these data show quite conclusively that tyrosine is the single most important amino acid mediating repression control of DAHP synthetase, there is a suggestion that phenylalanine might also play a role. The level of derepression with limiting

TABLE 3. *DAHP synthetase activity in SB167 and WB1000 grown under different aromatic acid supplementations*

Strain[a]	Supplementation	DAHP synthetase (specific activity)
SB167....	Shikimate	163
SB167....	Tyrosine + shikimate	29
SB167....	Phenylalanine + shikimate	80
SB167....	Tryptophan + shikimate	142
SB167....	Aro + shikimate	22
SB167....	Aro (no shikimate)	20
WB1000...	Limiting tyrosine	100
WB1000...	Limiting phenylalanine	22
WB1000...	Limiting tryptophan	12

[a] SB167 was grown in minimal medium supplemented with 50 μg of shikimate per ml and each of the other aromatic amino acids indicated; L-tyrosine, 100 μg/ml; L-phenylalanine, 100 μg/ml; L-tryptophan, 50 μg/ml. WB1000, a triple auxotroph requiring tyrosine, phenylalanine, and tryptophan, was grown in a chemostat with the following level of supplements: limiting tyrosine, 5 μg/ml; excess tyrosine, 50 μg/ml; limiting phenylalanine, 5 μg/ml; excess phenylalanine, 50 μg/ml; limiting tryptophan, 1 μg/ml; excess tryptophan, 5 μg/ml. The flow rate was adjusted to give a generation time of 150 min. Cultures were grown for a minimum of six generations under a limiting nutritional supplement before the cells were harvested. Cultures were grown with one amino acid limiting and the other two in excess.

tyrosine does not reach the level observed in SB167. This suggests that at high concentrations of phenylalanine and limiting tyrosine, there might be repression. The activity is twofold higher in cells grown on limiting phenylalanine than in cells grown on limiting tryptophan, implying that maximal repression requires tyrosine plus phenylalanine.

To assess the role of phenylalanine in the repression of DAHP synthetase, we studied the kinetics of derepression of this enzyme in the presence and absence of phenylalanine. In the experiment shown in Fig. 2, a double mutant with blocks in the genes specifying dehydroquinate synthetase and prephenate dehydrogenase was grown in tryptophan-supplemented medium. Samples were then transferred to three flasks of fresh medium supplemented with (i) shikimate plus limiting tyrosine, (ii) shikimate plus excess tyrosine, and (iii) shikimate plus excess tyrosine and excess phenylalanine. In the presence of tyrosine and phenylalanine, there is virtually no derepression, whereas, in the absence of tyrosine, derepression of enzyme activity does occur. Phenylalanine cannot merely be influencing the synthesis of tyrosine because of the block in prephenate dehydrogenase.

We conclude from the data presented in Table 3 and Fig. 2 that the level of tyrosine is paramount in controlling the synthesis of DAHP synthetase. However, the combination of tyrosine plus phenylalanine represses more effectively than does tyrosine alone. Tryptophan does not appear to play any significant role.

Chorismate mutase. Evidence for the repression control of chorismate mutase has already been presented (13). It was found that some strains of *B. subtilis* possess one form of this enzyme; others possess two forms. One form exists in an aggregate with DAHP synthetase, and the regulation of its activity is coordinate with DAHP synthetase. The activity of the second enzyme varies approximately twofold between cells grown with limiting or excess aromatic amino acids.

Enzymes of tryptophan synthesis. We studied the repression control of two enzymes of tryptophan biosynthesis, anthranilate synthetase, and tryptophan synthetase. The results (Table 4) indicate that both activities are strongly repressed by tryptophan. Anagnostopoulos and Crawford (1) showed that an additional enzyme of this pathway, indole glycerol phosphate synthetase, was also repressed by tryptophan. A regulatory mutant (SB455) genetically derepressed for enzymes of tryptophan biosynthesis has been isolated (18). This mutant excretes tryptophan but has the level of DAHP synthetase of the parent strain (Table 4). Apparently this DAHP synthetase activity provides a level of common intermediates sufficient for the increased level of tryptophan synthesis by the mutant.

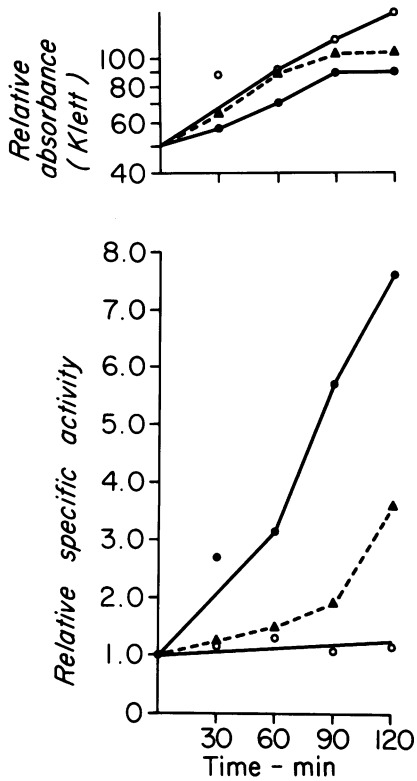

FIG. 2. *Derepression of DAHP synthesis under different aromatic amino acid supplementations. Mutant strain WB2070 was grown for 14 hr in minimal medium (22) supplemented with shikimate, 50 μg/ml; L-tryosine, 100 μg/ml; L-phenylalanine, 100 μg/ml; and L-tryptophan, 50 μg/ml. The cells were harvested in the log phase by centrifugation and dispensed into three different flasks containing minimal medium with the supplements indicated, in the concentrations (μg/ml) indicated. The flasks were incubated at 37 C with shaking. At 30-min intervals, samples were removed from each flask, monitored for cell mass, and extracts were prepared. DAHP synthetase was assayed as described in Materials and Methods. Symbols:* ○, *shikimate (4) plus tyrosine (50) plus phenylalanine (50);* ▲, *shikimate (4) plus tyrosine (50);* ●, *shikimate (4).*

Since both anthranilate synthetase and tryptophan synthetase activities are not repressible in mutants with regulator gene mutations, both enzymes are under the control of at least one common element. However, anthranilate synthetase is derepressed over 200-fold, and tryptophan synthetase B activity is derepressed less than 10-fold under conditions of tryptophan limitation

TABLE 4. *Tryptophan synthetase, anthranilate synthetase, and DAHP synthetase activity in various strains*[a]

Strain[b]	Supplement	Specific activity		
		Tryptophan synthetase	Anthranilate synthetase	DAHP synthetase
WB746	0	0.27	0.0004	70
WB746	Excess tryptophan	0.21	<0.0004	65
168	Limiting tryptophan	5.5	0.15	
168	Excess tryptophan	0.20	<0.0004	
SB455	0	4.9	0.15	76
SB455	Excess tryptophan	6.1	0.14	70

[a] Each of the strains was grown, extracts prepared, and enzyme activities determined as described in Materials and Methods. Excess L-tryptophan was present at 50 μg/ml. Strain 168 was grown in a chemostat on 0.75 μg of L-tryptophan per ml growing with a generation time of 180 min. This same strain was grown in a batch culture on excess tryptophan. The level of <0.0004 is the lower limit of the assay.

[b] WB746, prototroph; 168, trp auxotroph; SB455, 5-methyl trp-resistant prototroph.

and in nonrepressible mutants. This suggests that the two enzymes may not be regulated coordinately, as they are in *Escherichia coli* (8). Although the same clustering of this pathway exists in *Salmonella* as in *E. coli*, regulation of these two enzyme activities is not coordinate (2).

Prephenate dehydrogenase. Tyrosine represses the synthesis of prephenate dehydrogenase. Enzyme synthesis in the wild-type strain (WB746) can be repressed threefold by growth in tyrosine-supplemented medium (Table 5). The enzyme in cells of SB167 is elevated and can be repressed fourfold when grown on tyrosine-supplemented medium. The mutant (SB455) genetically derepressed for enzymes of the tryptophan pathway has the same level of prephenate dehydrogenase as does the wild-type strain.

Prephenate dehydratase. The enzyme prephenate dehydratase exists in several aggregational states which differ in their catalytic properties (3). The interconversion of these forms in vivo seems to be mediated by phenylalanine. We routinely observed about a twofold increase in the specific activity of this enzyme in extracts prepared from cells grown in the presence of the three aromatic amino acids, but it is not clear whether this results from a difference in the level of enzyme or a change in its form (Table 6). Prephenate dehydratase from SB167 has approximately the same specific activity as prephenate dehydratase from WB746 (grown in the presence or absence of the three aromatic amino acids), and the mutant genetically derepressed for enzymes of tryptophan biosynthesis (SB455) has parental levels of prephenate dehydratase.

A fluorophenylalanine-resistant mutant (WB740) has been isolated which overproduces phenylalanine, as evidenced by its excretion into the medium. This strain has a genetically altered prephenate dehydratase enzyme which is activated

TABLE 5. *Prephenate dehydrogenase activity in various strains*[a]

Strain[b]	Supplement	Specific activity
WB746	0	20
WB746	Tyrosine	6.5
SB167	Shikimate	27
SB167	Shikimate + tyrosine	6.7
SB167	Shikimate + aro	7.0
SB455	0	21
SB455	Aro	5.0

[a] Each of the strains was grown on the minimal salts medium with the indicated supplements: shikimate, 50 μg/ml; tyrosine, 100 μg/ml; phenylalanine, 100 μg/ml; tryptophan, 20 μg/ml.

[b] WB746, prototroph; SB167, multiple aro auxotroph; SB455, 5-methyl tryptophan-resistant mutant.

TABLE 6. *Prephenate dehydratase activity of various strains*[a]

Strain	Supplement	Specific activity
WB746	0	1.0
WB746	Aro	2.1
SB167	Shikimate	0.78
SB167	Shikimate + aro	1.0
SB455	0	1.2
SB455	Shikimate + aro	2.3
WB740	0	8.2
WB740	Shikimate + aro	9.3

[a] Each strain was grown on the medium indicated, extracts were prepared, and the assay was performed as described in Materials and Methods. SB167 was supplemented with 50 μg of shikimate per ml. The aro supplements were present at a concentration of 100 μg of tyrosine and phenylalanine and 50 μg of tryptophan.

rather than inhibited by phenylalanine (3). Significantly, the level of DAHP synthetase in this phenylalanine-excreting mutant is not elevated, indicating that the level of DAHP synthetase in the wild-type strain is sufficient to synthesize more phenylalanine than it normally does. Presumably, any excess synthesis of phenylalanine in the wild-type strain is prevented by feedback control of prephenate dehydratase activity (3).

Regulatory mutants of tyrosine synthesis. We attempted to verify and also gain additional insight into the key role that tyrosine plays in the regulation of aromatic acid synthesis by analyzing mutants concerned with tyrosine synthesis.

Mutant SB443 possesses a defective prephenate dehydrogenase which limits its synthesis of tyrosine. DAHP synthetase and shikimate kinase activity are derepressed in this strain (Table 7).

For reasons which are considered in another publication (Nester, *in preparation*), histidine inhibits the growth of SB443. Single-step histidine-resistant mutants can be isolated. A high proportion of these resistant strains are genetically derepressed for DAHP synthetase, shikimate kinase, and chorismate mutase activities. If the mutation responsible for the histidine resistance is introduced into a prototroph with a wild-type prephenate dehydrogenase gene (WB698b), then prephenate dehydrogenase activity in the prototroph (WB2055) is also derepressed (Table 7). Thus, a single-step mutation can abolish control of the early enzymes as well as of prephenate dehydrogenase, an enzyme shown to be under the control of tyrosine.

Fluorotyrosine-resistant mutants. 3-Fluorotyrosine is a potent inhibitor of the growth of wild-type *B. subtilis*, and serves as a false feedback inhibitor of prephenate dehydrogenase (Fig. 3). A resistant strain (WB2508) has been isolated which is insensitive to the feedback effects of tyrosine as well as fluorotyrosine. The locus conferring resistance maps very close to or

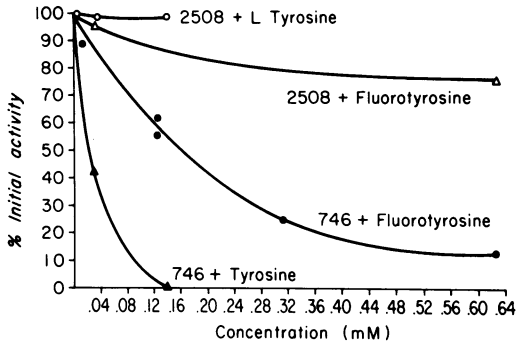

FIG. 3. *Effect of L-tyrosine and 3-fluorotyrosine on prephenate dehydrogenase activity in wild-type (WB746) and fluorotyrosine-resistant strain (WB2508). Tyrosine and fluorotyrosine, in the final concentrations indicated, were added to an incubation mixture for the assay of prephenate dehydrogenase, using crude extracts of the indicated strains. Enzyme activity was determined by the procedure described in Materials and Methods.*

TABLE 7. *Enzyme activity of strains mutant in tyrosine synthesis*[a]

Strain	Supplement	Specific activity						
		Prephenate dehydrogenase	DAHP synthetase	Dehydroquinase	Shikimate kinase	Chorismate mutase	Prephenate dehydratase	Anthranilate synthetase
WB746	0	20	70	4.3	562	3.2	1.0	<0.0004
WB746	+Tyrosine	6.5	19	8.9	140	1.2	2.1	
SB443	0	<1	120		700		1.0	
SB443	Tyrosine	<1	30		150		1.8	
WB698b (WB2055)	0	39	300	4.0	1,800	7.7	1.4	0.004
WB698b (WB2055)	Tyrosine	37	280		1,832	5.0	1.8	<0.0004
WB2508	0	11.7	44					
WB2508	Tyrosine	8.6	31					
WB2508-WB698b	0	40	260					
WB2508-WB698b	Tyrosine	45	230					

[a] Each of the strains was grown with supplements indicated, the extracts were prepared, and enzymes were assayed as described in Materials and Methods. Tyrosine was present at a concentration of 100 μg/ml. The activity of prephenate dehydrogenase was measured in strain WB2055 because of the low activity in WB698b. The other enzyme activities are identical in WB698b and WB2055.

[b] WB746, prototroph; SB443, bradytroph deficient in prephenate dehydrogenase; WB698b, histidine-resistant derivative of SB443; WB2055, prototroph with the regulatory gene of WB698b; WB2508, fluorotyrosine resistant of WB746.

within the structural gene for prephenate dehydrogenase activity (Nester, *unpublished data*). Because feedback regulation in the terminal branch is lost in the fluorotyrosine-resistant mutant, tyrosine would be expected to be overproduced. Thus, DAHP synthetase as well as prephenate dehydrogenase activity are maximally repressed in the mutant growing in minimal medium (Table 7). The lowered activity of prephenate dehydrogenase is not a direct consequence of the mutation to fluorotyrosine resistance, since the introduction of the regulatory locus of WB698b into this strain results in a derepressed level of both enzyme activities.

DISCUSSION

The two salient features of repression control of aromatic amino acid synthesis in *B. subtilis* are: (i) that the synthesis of enzymes prior to prephenate is primarily under the control of tyrosine, and (ii) that each of the terminal branches, except perhaps for that concerned with phenylalanine synthesis, is under the repression control of the amino acid which it synthesizes.

The level of DAHP synthetase in the wild-type cell growing in minimal medium is apparently higher than is actually required for the rate of synthesis of the three aromatic amino acids. Thus, regulatory mutants which synthesize excess tryptophan, phenylalanine, and tyrosine can accommodate this additional synthesis without increasing the level of DAHP synthetase. Indeed, excess tyrosine is produced by a regulatory mutant which has a level of DAHP synthetase almost twofold lower than that of the parent. This level is adequate to provide the rate of synthesis of the aromatic metabolites, since this strain grows at about the same rate as the parent strain. Since the level of DAHP synthetase as well as prephenate dehydrogenase can be repressed in wild-type cells by tyrosine, the cell is apparently limiting its synthesis of tyrosine. This control is probably exerted by the inhibition of prephenate dehydrogenase activity by a level of tyrosine insufficient to repress DAHP synthetase or prephenate dehydrogenase activity. Accordingly, cells deficient in this control (WB2508) produce a level of tyrosine which will repress both of the enzyme activities. In the wild-type cell, the tyrosine is poised at a level sufficient for maximal protein synthesis, but low enough to cause only partial repression of several enzyme activities.

Do tyrosine and phenylalanine merely control the synthesis of the real corepressor, prephenate or chorismate (or both)? Since both of these intermediates are feedback effectors of DAHP synthetase and shikimate kinase activity (Nasser and Nester, *submitted for publication*), it is attractive to consider the possibility that one or both of these compounds might be the real corepressors of the early enzymes. In theory, this scheme provides efficient physiological control, since the concentration of the intermediates reflects the concentration of all three aromatic amino acids. Several observations tend to mitigate against this possibility. The triply blocked mutant, WB1000, would be expected to accumulate chorismate and prephenate, especially under those conditions in which enzyme activity is maximal, i.e., under tyrosine limitation. One would predict that low levels of DAHP synthetase would be observed under all nutritional regimens in this strain. Presumptive evidence that prephenate does indeed accumulate in this mutant is found in the fact that the strain is quite leaky on a solid medium containing tyrosine and tryptophan, presumably because the accumulated prephenate is converted nonenzymatically to phenylalanine (6, 12). Mutants which require tyrosine, phenylalanine, tryptophan, and *p*-aminobenzoate, and therefore would *not* be expected to accumulate chorismate and prephenate, have low levels of DAHP synthetase activity when grown with excess tyrosine. The fact that DAHP synthetase, shikimate kinase, chorismate mutase, and prephenate dehydrogenase have at least one regulatory element in common is consistent with the notion that the same corepressor is involved in repression control of all of these enzymes. Since the corepressor of prephenate dehydrogenase is almost certainly tyrosine (or a derivative), this corepressor is likely involved in the repression of the early enzymes. Although the regulation of prephenate dehydrogenase and DAHP synthetase share a common element, the regulation of synthesis is markedly different with certain nutritional supplementations. The addition of histidine decreases the rate of synthesis of prephenate dehydrogenase, but increases the rate of DAHP synthetase synthesis (Nester, *in preparation*).

The reason for tyrosine domination of the control of synthesis of the early enzymes is not clear. It may be that a cell growing in unsupplemented medium fulfills its requirements for tryptophan and phenylalanine before fulfilling its requirement for tyrosine. The level of tyrosine would then reflect the levels of tryptophan and phenylalanine, since a cell would contain high levels of tyrosine after the tryptophan and phenylalanine requirements had been fulfilled. In *Salmonella* (7) and *E. coli* (5), chorismate is utilized to satisfy the tryptophan requirement first. The fact that leaky mutants in *B. subtilis* blocked prior to chorismate synthesis require

only phenylalanine and tyrosine suggests that the same situation prevails. However, it is not clear that the phenylalanine requirement is fulfilled before tyrosine. No apparent enzymological reason has been uncovered to suggest this. Thus, the K_m of prephenate dehydratase and prephenate dehydrogenase for prephenate are not markedly different (Nester, *in preparation*). The concentrations of phenylalanine and tyrosine, respectively, required for inhibition of the two enzymes are of the same order of magnitude. The V_{max} of prephenate dehydrogenase is higher than that of prephenate dehydratase (Nester, *unpublished data*). The amount of tyrosine and phenylalanine in cell protein is approximately the same (25). In the presence of exogenously added tyrosine, the tyrosine requirement would certainly be satisfied first. Exogenous tyrosine does not repress sufficiently to starve the cell for tryptophan and phenylalanine. This is not surprising, since excess phenylalanine is required for maximal repression. Further, the efficient feedback and repression control of the terminal branches serves to shunt any chorismate and prephenate to the branch synthesizing the limiting amino acid.

The regulation of the early enzymes is unique in that both tyrosine and phenylalanine are required for maximal repression, but tyrosine by itself represses very markedly. Our data are not precise enough to state with certainty whether phenylalanine per se also represses.

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