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Cross Pathway Regulation: Effect of Histidine on the Synthesis and Activity of Enzymes of Aromatic Acid Biosynthesis in *Bacillus subtilis*

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L-Histidine and, to a lesser degree, L-phenylalanine at concentrations of 10^{-4} M inhibit the growth of leaky mutants (bradytrophs) of *Bacillus subtilis* that are deficient in the synthesis of *p*-hydroxyphenylpyruvate, the first intermediate specific to tyrosine synthesis. The inhibition can be overcome by growth factor amounts of L-tyrosine and *p*-hydroxyphenylpyruvate. Histidine and phenylalanine are capable of inhibiting the synthesis of tyrosine in several ways, and the major physiological effect which results in growth inhibition has not been established. Both L-histidine and L-phenylalanine inhibit the activity of prephenate dehydrogenase at concentrations about 100-fold higher than the inhibitory concentration of L-tyrosine. Histidine also appears to repress the synthesis of prephenate dehydrogenase because a histidine bradytroph growing in histidine-supplemented medium has a twofold lower level of this enzyme than the same cells growing in unsupplemented medium. These same two amino acids also inhibit the growth of a bradytroph deficient in dehydroquinate synthetase, an early enzyme in the pathway of tyrosine, phenylalanine, and tryptophan synthesis. The inhibition is overcome by a combination of tyrosine and phenylalanine. Histidine-resistant derivatives of both the prephenate dehydrogenase and dehydroquinate synthetase-deficient strains, which simultaneoulsy have gained resistance to phenylalanine, have been isolated. Most of these resistant mutants synthesize additional tyrosine compared with the parent strain. One class of resistant mutants excretes tyrosine and has a number of enzymes of aromatic acid synthesis which are no longer repressible by any combination of the aromatic amino acids. Tyrosine inhibits the growth of histidine bradytrophs. Histidine, at growth factor levels, overcomes the inhibition.

The efficient control of a biosynthetic pathway would seem to demand that only the end products or intermediary metabolites of that reaction sequence exercise regulatory control. In unbranched pathways, control very often is mediated by the end metabolite through the regulation of activity of the first enzyme, as well as through the synthesis of enzymes of the pathway (1, 34). In branched pathways leading to several end products, bacteria have evolved a variety of sophisticated control mechanisms designed to insure that the level of synthesis and the activity of enzymes common to several metabolites respond to the level of all end metabolites (7). However, a number of cases have been studied in which a single metabolite dominates the regulation of the pathway to such a degree that its addition inhibits the growth of the culture. In some cases, the wild-type cell is inhibited (2, 14, 35). In many instances, mutants which have lost one of several isozymes specifically regulated by one end product may now be growth-inhibited by the addition of the remaining end product (28, 37). In each case, however, the derangement in enzyme regulation revealed by the growth inhibition can be reconciled with the recognized biochemical or steric relationships between the metabolites causing and those overcoming the inhibition.

In the course of a biochemical-genetic study of aromatic amino acid biosynthesis in *Bacillus subtilis*, a number of observations suggested a regulatory role for histidine, a biosynthetically

unrelated metabolite. The most compelling observation concerned a mutant which grew on minimal, but not on histidine-supplemented, medium. This inhibition was overcome by tyrosine (27). This report explores the properties of this mutant in more detail, and presents data which indicate that histidine regulates both the synthesis and the activity of key enzymes of tyrosine synthesis.

MATERIALS AND METHODS

Chemicals. All commercially available chemicals were of the highest purity obtainable. They were purchased from either Calbiochem, Los Angeles, Calif., or Sigma Chemical Co., St. Louis, Mo. Barium prephenate and chorismic acid were prepared by published procedures (10, 13, 16). The barium prephenate and the chorismic acid were assayed to be 80 and >99% pure, respectively, by the criteria previously described (19). Data that indicate concentrations of these compounds reflect this purity. R. Somerville of Purdue University donated the 3-fluorotyrosine.

Growth of cells. Cultures were grown as previously described (25), unless stated otherwise.

Enzyme assays. The DAHP (3-deoxy-D-arabino-heptulosonic acid 7-phosphate) synthetase assay was performed according to the procedure of Jensen and Nester (17), a modification of the procedure of Srinivasan and Sprinson (33). Extracts were prepared by lysing cells with lysozyme according to the procedure of Nester and Jensen (25). The crude lysate was either dialyzed against 1,000 volumes of 0.05 M potassium phosphate buffer (*p*H 6.8) or passed through a column containing G-25 Sephadex, using this same buffer for elution.

Shikimate kinase. The extract was prepared and assayed according to the procedure of Nester, Lorence, and Nasser (26) with two modifications; the level of adenosine triphosphate (ATP) was raised to 5 μmoles per ml, and that of shikimate to 200 nmoles per ml. Crude, undialyzed extracts were used because of the extreme instability of the two molecular species of shikimate kinase (26).

Prephenate dehydrogenase. The assay was performed as described by Nester and Jensen (25), with extracts prepared by lysing cells with lysozyme at 37 C. The reaction product was measured as a Millon-positive material (31). The crude lysate was passed through a G-25 Sephadex column employing as the buffer 0.1 M tris(hydroxymethyl)aminomethane (Tris; *p*H 8.1), 6×10^{-3} M mercaptoethanol, 2×10^{-3} M $MgCl_2$, and 10^{-4} M ethylenediaminetetraacetic acid (tetra sodium; 6).

Except for DAHP synthetase, all assays were performed on the same day the extract was prepared.

Protein assay. Protein concentration was determined by the method of Lowry et al. (20), using crystalline bovine plasma albumin as a standard.

Growth curves. Culture (10 ml) was grown in a 125-ml flask fitted with a side-arm test tube. The relative absorbance of a culture was determined by measuring the turbidity in a Klett-Summerson colorimeter with the no. 66 red filter. Appropriate corrections were made to establish linearity between Klett reading and turbidity.

Bacterial strains. The strains that were used are described in Table 1 (3).

RESULTS

Inhibition of SB443 by histidine and phenylalanine. The data in Fig. 1 show that histidine inhibits the growth of mutant strain SB443. This strain grows in unsupplemented glucose-salts medium (32) with a generation time of 70 min, whereas the parent strain has a generation time of 66 min. Inhibition becomes apparent 30 min after the addition of histidine, and is overcome by growth factor amounts of tyrosine and its immediate precursor, *p*-hydroxyphenylpyruvate, added simultaneously with the histidine. If tyrosine is added after inhibition by histidine has taken hold, 30 min elapse before growth resumes at the normal rate. The level of tyrosine required to overcome the inhibition is independent of the histidine added, at least within certain limits.

Further experiments (*unpublished data*) showed that (i) the kinetics of inhibition are essentially identical between concentrations of 0.05 and 0.5 mM histidine, whereas, at lower concentrations, inhibition is reduced; (ii) cells are inhibited at 45, 37, and 30 C in liquid and on agar plates; and (iii) D-histidine has no effect. Except for tyrosine, no amino acid, purine, pyrimidine or a

TABLE 1. *Strains of Bacillus subtilis*

Strain	Relevant information
SB133	A phenylalanine auxotroph; lacks prephenate dehydratase
SB168	Tryptophan-requiring auxotroph, originating from the collection of Burkholder and Giles (3), as reported by Spizizen (31)
WB746	A spontaneous revertant of 168; prototroph
SB167	A derivative of 168; a multiple aromatic acid auxotroph; grows on shikimate
SB443	A tyrosine bradytroph, inhibited by histidine and phenylalanine; UV-induced mutant of WB746
WB550	A histidine bradytroph
WB698b	A histidine-resistant derivative of SB443; derepressed for enzymes of aromatic acid synthesis
WB2055	A derivative of WB698b which contains the wild-type prephenate dehydrogenase locus, but the regulatory gene lesion of WB698b
WB2442	A histidine-resistant derivative of SB167

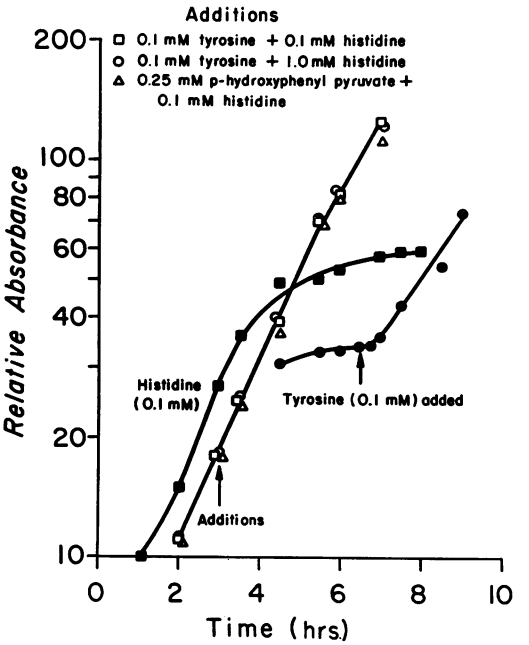

FIG. 1. *Growth patterns of SB443. SB443 was grown in minimal medium in four flasks. At 3 hr, appropriate additions were made to each flask. The cells in the histidine-containing flask were diluted twofold at 4 hr and, at the indicated time, tyrosine was added.*

number of cations (Ca^{++}, Mg^{++}, Mn^{++}, Cu^{++}, Fe^{++}; all 10^{-3} M) reverse the inhibition. Other amino acids were tested for their ability to inhibit SB443 as well as the parent strain, WB746. Serine and threonine inhibit the growth of WB746 both in liquid and on agar. These inhibitions have not been studied further. On agar, 0.05 mM L-phenylalanine inhibits the growth of SB443, but it has no effect on the parent strain even at concentrations 10-fold higher (Table 2). Inhibition by phenylalanine (0.1 mM final concentration) is relatively modest compared to that of histidine, because its addition does not result in any obvious decrease in the growth rate of the culture observed over several generations. However, if phenylalanine is added to a culture of 10^3 cells per ml after incubation for 24 hr, the cell mass is 10-fold less (measured by turbidity in the Klett colorimeter) than in a culture grown without phenylalanine. These data suggest that inhibition by phenylalanine must be amplified by many cell generations before it becomes apparent. Tyrosine also overcomes this inhibition (Table 2).

Enzyme defect in SB443. Our initial studies were aimed at identifying the mutant enzyme involved in the inhibition. Since *p*-hydroxyphenylpyruvate and tyrosine overcome the amino acid inhibitions (Fig. 1), the most likely candidate was prephenate dehydrogenase. The data in Table 3 indicate that SB443 has negligible prephenate dehydrogenase activity in vitro. In crude extracts, approximately 1% of wild-type activity is detectable and this represents the lower limits of this assay. Passing extracts through Sephadex G-25, assaying whole cells treated with toluene or cetyl trimethyl ammonium bromide (10 μg per ml), and plasmolysing with sucrose (15) did not increase the activity significantly. The addition of an extract of SB443 to a wild-type extract did not decrease the prephenate dehydrogenase activity of the latter, suggesting that SB443 contains no inhibitor. The presence of histidine and tyrosine either in the growth medium or in the lysing buffer did not increase the activity. Because the growth rates of this mutant and the parent are essentially the same, it is likely that prephenate dehydrogenase of SB443 is active in vivo but very labile to any treatment that disrupts the integrity of the cell.

Although tyrosine added to an SB443 culture does not decrease the generation time significantly, measured in a short-term experiment, tyrosine does slightly stimulate growth of colonies on agar (Table 2). This result suggests that even in the absence of exogenous histidine or phenylalanine, SB443 is not synthesizing enough tyrosine for growth at its maximum rate. However, it is quite conceivable that internal histidine or phenylalanine, or both, are inhibiting growth.

TABLE 2. *Colony size of SB443 on supplemented agar medium*[a]

Supplementation	Colony size
	mm
None	1.3 ± 0.2
L-Histidine	No colonies observed
L-Phenylalanine	No colonies observed
L-Tyrosine	1.6 ± 0.3
L-Phenylalanine + L-tyrosine	2.2 ± 0.3
L-Histidine + L-tyrosine	1.8 ± 0.3

[a] Cells were plated on a minimal salts-glucose medium (32) at a dilution yielding approximately 20 colonies per plate. The amino acids were present in concentrations of 0.1 mM. The plates were incubated at 30 C and the colony sizes were measured after 60 hr. The numbers represent the average of 10 colonies. When tyrosine was added after 60 hr to the plates supplemented with histidine and phenylalanine, approximately 20 colonies appeared on each plate after incubation. SB443 is a histidine-inhibited strain.

TABLE 3. *Specific activity of prephenate dehydrogenase in histidine-inhibited and wild-type strains*[a]

Strain	Supplementation	Specific activity
SB443	None	<0.2
WB746	None	20
WB746	Tyrosine	6.5
SB167	None	27
SB167	Tyrosine	9.7

[a] The strains were grown on minimal salts-glucose medium with the indicated supplements; the extracts were prepared, and the assays were performed as described. SB167 was grown on 0.28 mM shikimate in both instances. Tyrosine was added at a concentration of 0.5 mM. The specific activity is defined as the nanomoles of Millon-positive product formed per minute per milligram of protein.

Genetic studies support the enzymological data of Table 3. The locus conferring sensitivity to histidine is very closely linked to 17 mutations in a structural gene specifying prephenate dehydrogenase (27). Thus far, all mutants that have an absolute, single requirement for tyrosine map at this site. Two additional nonidentical histidine-inhibited and phenylalanine-inhibited mutants have also been studied. Each has approximately 1% of the wild-type level of prephenate dehydrogenase in vitro, and both mutations map in this same region of the linkage map.

Growth inhibition of other aromatic amino acid bradytrophs. The data presented suggest that a single mutation results in a defective prephenate dehydrogenase and a sensitivity to growth inhibition by histidine and phenylalanine. We next determined whether either the specific mutation in SB443 or the limitation in tyrosine synthesis is crucial for inhibition. We looked at the histidine and phenylalanine sensitivity on agar of a strain blocked in the synthesis of DAHP synthetase, dehydroquinate synthetase, or dehydroquinase, and at several partial revertants of several strains blocked in prephenate dehydrogenase. Whether histidine inhibits depends on the specific mutant locus involved. Bradytrophs deficient in DAHP synthetase or dehydroquinase are not inhibited by histidine and phenylalanine. A leaky mutant blocked in dehydroquinate synthetase (SB167) is inhibited by histidine and phenylalanine. A combination of tyrosine and phenylalanine overcomes the inhibition. All of the partial revertants of mutants blocked in prephenate dehydrogenase are inhibited by histidine and phenylalanine, and tyrosine overcomes both inhibitions. It is apparent that a number of different mutants will be inhibited by histidine and phenylalanine if they are limited in the synthesis of certain enzymes of aromatic amino acid biosynthesis. Such mutants synthesize a limited supply of one or more of these amino acids.

Histidine repression of prephenate dehydrogenase. A reasonable explanation for the inhibitory effect of histidine on SB443 is that histidine further limits the synthesis of tyrosine in a strain already deficient in tyrosine synthesis. This limitation could conceivably result from the inhibition of prephenate dehydrogenase activity and the repression of synthesis of this enzyme, or from both. The 30-min lag (Fig. 1) between histidine addition and growth inhibition might suggest that enzyme synthesis is being curtailed. One would expect that any significant inhibition of this enzyme would result in an immediate effect on growth because the supply of tyrosine is already growth-limiting. Therefore, we studied the regulation of synthesis of prephenate dehydrogenase (Table 3) as well as two earlier enzymes of aromatic acid synthesis, DAHP synthetase and shikimate kinase (Table 4). We have shown previously that the rate of synthesis of all these enzymes is primarily under the control of tyrosine (*unpublished data*). The observations made on SB443 and SB167 are consistent with these conclusions. SB443, limited in tyrosine synthesis, is derepressed for both DAHP synthetase and shikimate kinase activities. SB167 is also deficient in tyrosine synthesis because its generation time on shikimate alone is 84 min and on shikimate plus tyrosine, 66 min. All three enzymes are derepressed in SB167 grown on shikimate, and they are markedly repressed in the strain grown on shikimate plus tyrosine. If this strain is grown on shikimate plus either phenylalanine or tryptophan, these enzymes are not repressed (*unpublished data*).

We then determined whether histidine exercised any control over the synthesis of DAHP synthetase and prephenate dehydrogenase. In order to maximize any effect, we grew a histidine bradytroph (WB550) in the presence and in the absence of histidine and tyrosine. This strain grows about 20% slower in the absence of histidine. The results indicate that the level of prephenate dehydrogenase is lowered twofold when cells are grown in histidine-supplemented medium, and threefold in tyrosine-supplemented medium. The reason the combination of these two amino acids represses prephenate dehydrogenase less effectively than does either singly is not clear. With the decrease in the level of prephenate dehydrogenase, the level of DAHP synthetase increases simultaneously in the histidine-grown cells. This is precisely what we would expect if histidine were inhibiting the synthesis of tyrosine by affecting prephenate dehy-

TABLE 4. *DAHP synthetase and shikimate kinase activity in strains grown in the presence and in the absence of aromatic supplements*[a]

Strain	Supplementation	DAHP synthetase	Shikimate kinase
WB746	None	134	562
WB746	Aro[b]	50	
WB746	Tyrosine	59	281
SB443	None	285	1,537
SB443	Aro	65	
SB443	Tyrosine	60	334
SB167	Shikimate	200	708
SB167	Tyrosine	67	210
SB167	Aro	60	

[a] The strains were grown, extracts were prepared, and the enzymes were assayed as described. SB167 was grown with 0.28 mM shikimate in all cases. L-Tyrosine, L-phenylalanine, and L-tryptophan were added at a final concentration of 0.5 mM, 0.5 mM, and 0.10 mM, respectively, where indicated. Specific activity of DAHP synthetase is expressed as nanomoles of DAHP formed per minute per milligram of protein. Specific activity of shikimate kinase is expressed as counts per minute of shikimate phosphate formed per minute per milligram of protein. WB746 is a wild-type strain; SB443 is a histidine-inhibited strain; SB167 is an aromatic acid bradytroph.

[b] Aromatic amino acids and vitamins.

TABLE 5. *DAHP synthetase and prephenate dehydrogenase activity in WB550*[a]

Supplementation	DAHP synthetase	Prephenate deyhdrogenase
None	120	26.5
Histidine	200	13.7
Tyrosine		8.2
Tyrosine + histidine		16.5

[a] The strain was grown on minimal salts-glucose medium; the extracts were prepared and the assays were performed as described. The amino acids indicated were added in the growth medium at a final concentration of 0.5 mM. WB550 is a histidine bradytroph.

drogenase. It seems unlikely that histidine is derepressing the synthesis of DAHP synthetase by driving tyrosine out of the cell. If this were true, we would expect the level of prephenate dehydrogenase to increase also. However, this explanation cannot be ruled out completely because it is conceivable that the level of tyrosine required for repression of prephenate dehydrogenase is less than that required for DAHP synthetase.

Effect of histidine and phenylalanine on the activity of prephenate dehydrogenase. Previous work (25) has shown that phenylalanine inhibits the activity of prephenate dehydrogenase. We were interested in determining the effect of histidine, because any inhibition of this activity in SB443 could very likely contribute to growth inhibition. SB133, a phenylalanine auxotroph, was employed in this analysis because the kinetics of the reaction are not complicated by the conversion of prephenate to phenylpyruvate. Figure 2 illustrates the kinetics of inhibition by L-histidine, as well as L-phenylalanine and L-tyrosine. The D isomers had no effect.

Separation of binding sites for histidine and tyrosine. Our initial experiments on the inhibition of prephenate dehydrogenase by histidine were plagued by inconsistency. On the other hand, inhibition by tyrosine and phenylalanine was quite reproducible, suggesting that the enzyme was being selectively desensitized to histidine inhibition. The results in Table 6 demonstrate that tyrosine, histidine, and phenylalanine inhibit the enzyme activity in extracts prepared at 37 C by lysozyme lysis. In extracts prepared at 44 C, the enzyme is insensitive to histidine inhibition, although tyrosine and phenylalanine inhibit the activity as well as they do in extracts prepared at 37 C. These data suggest that the histidine binding site is distinct from the tyrosine and phenylalanine sites. Since the specific activity of the enzyme in both preparations was the same, the histidine regulatory site is obviously distinct from the catalytic site. This latter observation falls into the pattern observed for a great number of enzymes subject to allosteric control (7). In a

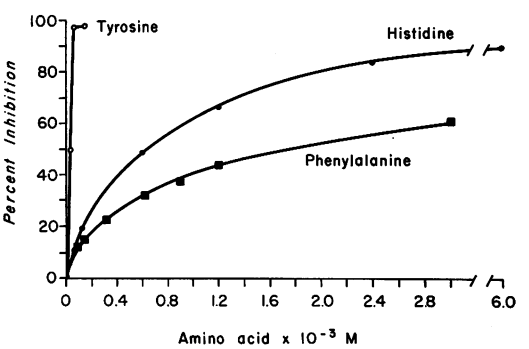

FIG. 2. *Inhibition of prephenate dehydrogenase by histidine. SB133 (phe⁻) was grown in minimal medium supplemented with 0.1 mM l-phenylalanine. Extracts were prepared and processed as described. The histidine was added to the enzyme at 0 C prior to the addition of prephenate. The reaction vessel contained 0.5 μmoles of barium prephenate per ml. Per cent inhibition equals* $(V_o - V_i)/V_o$.

TABLE 6. *Desensitization of PPA dehydrogenase to histidine inhibition*[a]

Temp of extract prepn	Inhibitor		
	Tyrosine	Phenylalanine	Histidine
C	%	%	%
37	51	72	40
44	51	62	1

[a] Extracts of SB133 were prepared by lysozyme lysis at either 37 C or 44 C for 30 min and then assayed in the presence of 0.026 mM L-tyrosine, 6.1 mM L-phenylalanine, and 3.2 mM L-histidine. The specific activities of the two extracts were the same, 19.2. SB133 is a phenylalanine auxotroph.

limited number of experiments, it has not been possible to separate the allosteric sites of tyrosine and phenylalanine regulation. We have isolated a mutant of WB746 that is resistant to fluorotyrosine, an analogue of tyrosine. This strain is resistant to inhibition by all three amino acids.

Effect of histidine on tyrosine utilization. That natural amino acids might inhibit growth because of misincorporation into cell protein is suggested by the results of Kindler and Ben-Gurion (18). To study this possibility, we determined whether histidine would inhibit the growth of a tightly blocked tyrosine auxotroph growing on limiting levels of tyrosine. The strain was grown in a chemostat on 0.02 mmoles of L-tyrosine per ml, with a generation time of 60 min. With excess tyrosine and under comparable conditions, the strain has a generation time of 45 min. After four generations, 0.4 mmoles of L-histidine per ml was added directly to the growth chamber, and the inflow reservoir and the cell density of the growth chamber were monitored at intervals. The cell density did not change after an additional six generations. The fact that histidine did not cause a washout indicates that it did not increase the generation time beyond 60 min. Similar studies in which the generation time of the cells was set at 120 min led to the same results. These data suggest that inhibition results solely from a deficiency in tyrosine synthesis and not in its subsequent utilization.

Biochemical and genetic analysis of histidine-resistant mutants. Histidine-resistant mutants of SB443 and SB167 were readily obtained by isolating the colonies that arose on histidine-supplemented medium. Most proved to be phenylalanine-resistant also. A large number of the histidine-resistant derivatives of SB443 were surrounded by halos of background cells on histidine-supplemented media. The presumptive excretion of tyrosine by such resistant clones was verified by the observation that they would also feed authentic tyrosine auxotrophs. Enzymatically, the most distinguishing feature of these resistant strains is that they are derepressed for all of the early enzymes of aromatic acid synthesis that we have studied, DAHP synthetase, shikimate kinase (Table 7), and chorismate mutase (19); they cannot be repressed by supplementation with any combination of the aromatic amino acids. The succeeding paper (5) indicates that these strains are also derepressed for enzymes of histidine biosynthesis. These derivatives of SB443 still have a very low level of measurable prephenate dehydrogenase activity; therefore, to measure this activity, it was necessary to transfer the regulatory locus into a strain with a wild-type gene for prephenate dehydrogenase. Prephenate dehydrogenase activity is not repressible in such a strain (WB2055). Anthranilate synthetase and tryptophan synthetase activities remained repressible. The gene responsible for the nonrepressible enzyme activity is genetically unlinked to the structural gene for prephenate dehydrogenase, as measured by deoxyribonucleic acid (DNA) transformation.

A histidine-resistant derivative of SB167 (WB2442) gave essentially the same pattern of enzyme repression as did WB2055 (Table 7). All of the enzymes of aromatic acid synthesis studied were nonrepressible by any combination of the aromatic amino acids. We have analyzed a total of 35 histidine-resistant, tyrosine-excreting derivatives of SB443. Every mutant was nonrepressible for these enzymes of aromatic acid synthesis, as well as enzymes of histidine biosynthesis (5).

Derivatives of SB443 selected for resistance to analogues of tyrosine or phenylalanine are also resistant to histidine and phenylalanine. Strains resistant to *p*-aminophenylalanine, 3-amino-tyrosine, and 3-fluorotyrosine are no longer inhibited by histidine and phenylalanine. We have not determined the biochemical basis for resistance to the first two analogues, but 3-fluorotyrosine-resistant strains are feedback-resistant to tyrosine. All three resistant types apparently are producing more tyrosine than the parental SB443. This conclusion is based on the observation that the level of DAHP synthetase is significantly lower in each of the analogue-resistant strains grown on unsupplemented medium than it is in the sensitive SB443 parent. Because tyrosine overcomes the inhibition at growth factor concentrations, it is not surprising that any strain which by some mechanism achieves the ability to produce additional tyrosine simultaneously becomes resistant to histidine and phenylalanine. Resistant mutants have also been isolated which have reverted at the original mu-

TABLE 7. *Enzyme activity of histidine-resistant strains and their derivatives*[a]

Strain	Supplementation	DAHP synthetase	Shikimate kinase	Prephenate dehydrogenase
WB698b	None	272	1,835	0.1
	Aro[b]	296	1,844	0.1
WB2055	None	300	1,800	39
	Aro + histidine	280	1,832	37
	Tyrosine	280		35
WB2442	None	248		42
	Aro	246		40
	Tyrosine	270		40

[a] The strains were grown, extracts were prepared, and the enzymes were assayed as described. All supplements were present at 0.5 mM except tryptophan, which was present at 0.1 mM (final concentration). WB698b is a histidine-resistant derivative of SB443. WB2055, a derivative of WB698b, contains the wild-type prephenate dehydrogenase locus, but the regulatory gene mutation of 698b. WB2442 is a histidine-resistant derivative of SB167.

[b] Aromatic amino acids and vitamins.

tant site and have regained the wild-type level of prephenate dehydrogenase.

Inhibition of histidine bradytrophs by tyrosine. Tyrosine inhibits the growth of strains that have a partial requirement for histidine. Thus far, we have studied partial revertants of mutants blocked in the first enzyme of histidine biosynthesis, as well as mutants deficient in the cyclase enzyme. The inhibition is overcome by growth factor levels of histidine. Tyrosine-resistant mutants have been isolated and many are derepressed for enzymes of histidine as well as aromatic acid synthesis (5).

DISCUSSION

In 1961, Monod and Jacob (22) theorized that cross-regulation could serve a useful function and, therefore, would not be an unreasonable control pattern to find. A number of previous reports suggested that certain amino acids do modulate the activity of enzymes of unrelated pathways (29, 36). However, whether these effects demonstrated in vitro play any physiological role is as yet problematical. Carsiotis and Lacy (4) presented evidence that in *Neurospora* cross-regulation may operate in repression control. They reported that levels of certain enzymes of tryptophan synthesis were elevated two- to fourfold in histidine auxotrophs grown on limiting histidine. Excess histidine, but not tryptophan, repressed the synthesis of these enzymes.

Although our knowledge of control of aromatic acid synthesis is still incomplete, it is difficult to rationalize any involvement of histidine in the regulation of this pathway. The two biosynthetic pathways do not share any metabolites, nor is there any evidence which suggests that an intermediate of one pathway is the precursor of a metabolite of the other. Furthermore, it seems unlikely that the same enzyme would function catalytically in both pathways. Nevertheless, the fact that histidine exercises repression as well as feedback control suggests that the effect is not fortuitous. The close physical relationship between genes controlling enzymes of histidine and aromatic amino acid synthesis further suggests a relationship between the two pathways (27).

From the data presented, it is not possible to pinpoint the principal target of histidine action on strain SB443. Perhaps the most reasonable explanation, considering all of the data, is that histidine inhibits growth as a consequence of repression and inhibition of prephenate dehydrogenase. If histidine were inhibiting only the enzyme, inhibition should be manifest immediately, not only after a 30-min lag. Further, since phenylalanine at comparable concentrations inhibits this enzyme, phenylalanine should inhibit growth as well as histidine. It does not. We have no evidence which indicates that phenylalanine represses prephenate dehydrogenase. Not all aromatic amino acid bradytrophs are inhibited by histidine. The inhibition appears to be locus-specific. Leaky mutants (bradytrophs) deficient in DAHP synthetase and dehydroquinase are not inhibited, in contrast to dehydroquinate synthetase and prephenate dehydrogenase bradytrophs. The inhibition of dehydroquinate synthetase, but not of prephenate dehydrogenase bradytrophs, is overcome by shikimate. We suggest that histidine inhibits by repressing the synthesis of an amino acid already in limited supply. Inhibition occurs only if synthesis of the limiting enzyme is repressed by histidine. DAHP synthetase is not

repressible (Table 4), nor does dehydroquinase appear to be repressible (*unpublished data*). Our data do not definitely rule out the possibility that histidine may partially inhibit by driving a growth-limiting quantity of tyrosine out of the cell. It has been shown that in *Salmonella* one permease actively transports both tyrosine and histidine into the cell (11). The fact that the inhibition is gene-specific mitigates against this as a likely possibility. However, because we have not quantitated the levels of tyrosine which are synthesized by each of these bradytrophs, we cannot state with absolute certainty that the level of tyrosine is not critical to inhibition.

Perhaps more interesting than a complete understanding of the inhibition in physiological terms is the explanation at a molecular basis for the regulatory relationship between histidine and tyrosine. We are now determining whether tyrosine inhibits the first enzyme of histidine biosynthesis. It is conceivable that this enzyme and prephenate dehydrogenase share a postulated common regulatory subunit (12). This consideration was prompted by the observation of Patte et al. (30) that two enzymes of threonine biosynthesis possess the same regulatory subunit. We will defer the discussion of cross-repression (5). Suffice it to say here that the isolation of single-step mutants derepressed for both pathways is perhaps the strongest evidence that the regulation of both pathways is intimately related in vivo.

It seems reasonable to expect that cross-regulation in vivo will only be observed in mutants or in organisms already made deficient in the synthesis of a metabolite. Prototrophs often can compensate for any deficiency in an end metabolite and quickly overcome any inhibition of enzyme activity (23). Further, since the prototrophic enzymes are often maximally repressed, additional repression is not possible. In either case, inhibitory effects in vivo are either masked or quickly overcome. This, of course, does not imply that the effect is insignificant in the regulation of synthesis of the metabolites in the prototroph. In contrast, a bradytroph is already compensating for its deficiency in metabolite synthesis by derepressing the synthesis of the appropriate enzymes. Therefore, any additional stress cannot be overcome. Other investigators have also observed the inhibition of bradytrophs by normal metabolites (8, 9, 21, 24).

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