JOURNAL OF BACTERIOLOGY, Jan. 1983, p. 423–428
0021-9193/83/010423-06$02.00/0

Vol. 153, No. 1

Regulation of the Aromatic Pathway in the Cyanobacterium *Synechococcus* sp. strain PCC6301 (*Anacystis nidulans*)

GERALDINE C. HALL,† MARYANN B. FLICK, AND ROY A. JENSEN*

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

Received 26 July 1982/Accepted 5 October 1982

A pattern of allosteric control for aromatic biosynthesis in cyanobacteria relies upon early-pathway regulation as the major control point for the entire branched pathway. In *Synechococcus* sp. strain PCC6301 (*Anacystis nidulans*), two enzymes which form precursors for L-phenylalanine biosynthesis are subject to control by feedback inhibition. 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (first pathway enzyme) is feedback inhibited by L-tyrosine, whereas prephenate dehydratase (enzyme step 9) is feedback inhibited by L-phenylalanine and allosterically activated by L-tyrosine. Mutants lacking feedback inhibition of prephenate dehydratase excreted relatively modest quantities of L-phenylalanine. In contrast, mutants deregulated in allosteric control of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase excreted large quantities of L-phenylalanine (in addition to even greater quantities of L-tyrosine). Clearly, in the latter mutants, the elevated levels of prephenate must overwhelm the inhibition of prephenate dehydratase by L-phenylalanine, an effect assisted by increased intracellular L-tyrosine, an allosteric activator. The results show that early-pathway flow regulated in vivo by 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase is the dominating influence upon metabolite flow-through to L-phenylalanine. L-Tyrosine biosynthesis exemplifies such early-pathway control even more simply, since 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase is the sole regulatory enzyme subject to end-product control by L-tyrosine.

Mutants deficient in the regulation of biosynthetic pathways are a valuable aid in determining not only the enzymatic steps subject to regulation, but also the relative importance of each point of regulation, the general pattern of regulation, and the channeling of intermediates. Recently, two general patterns of regulation, the exo-oriented and the endo-oriented patterns, have been described for multibranched pathways (10). Endo-oriented organisms are characterized by regulatory patterns geared to the endogenous formation of initial-pathway substrates. Emphasis is placed on end-product regulation of the prime branch point that monitors the flow of the earliest-pathway metabolites. Exo-oriented organisms, exemplified by *Bacillus subtilis* and *Escherichia coli*, display regulatory patterns that maximize efficient response to the exogenous availability of pathway end products. Although early-pathway regulation remains important, emphasis is placed upon sensitive control at the peripheral branch points.

The autotrophic cyanobacteria exhibit endo-oriented control of the multibranched aromatic biosynthetic pathway. All cyanobacteria are likely to excrete L-tyrosine in response to early-pathway deregulation since 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase is the sole point of regulation along the 10-step sequence of L-tyrosine biosynthesis in most, if not all, cyanobacteria (5). In *Synechocystis* sp. strain PCC6902 (ATCC 29108), a unicellular cyanobacterium, deregulation of the first enzyme of the aromatic pathway, DAHP synthase, results in elevated metabolite flow-through which overcomes weak end-product inhibitions of anthranilate synthase and prephenate dehydratase at the peripheral branch points (7). Thus, all amino acid end products of the pathway (i.e., L-tyrosine, L-phenylalanine, and L-tryptophan) are overproduced and excreted. In a multicellular cyanobacterium, *Anabaena* sp. strain PCC7119 (ATCC 29151), a similar pattern has been seen, even though two isozymes of DAHP synthase were found (8). Deregulation of the major isozymic species of DAHP synthase results in excretion of all three amino acid end products.

For *Synechococcus* sp. strain PCC6301 we now report the results of studies of the control of

† Present address: Elmira College, Elmira, NY 14901.

L-phenylalanine biosynthesis, using a mutant approach, to evaluate the relative in vivo impact of two allosteric control points (Fig. 1). The early control point is at DAHP synthase, an enzyme subject to feedback inhibition by L-tyrosine. Prephenate dehydratase (the peripheral control point) is subject to feedback inhibition by L-phenylalanine and to allosteric activation by L-tyrosine. In well-known organisms such as *E. coli* or *B. subtilis*, only deregulation of prephenate dehydratase, not that of DAHP synthase, leads to excretion of L-phenylalanine. In this study we selected phenylalanine excretors from *Synechococcus* sp. strain 6301 to ascertain whether DAHP synthase desensitization might be found as one class of mutant.

MATERIALS AND METHODS

Growth of cultures. The unicellular cyanobacterium *Synechococcus* sp. strain PCC6301 (ATCC 27144) (synonymous with *Anacystis nidulans* TX20 [14]) and all derivative mutants were routinely maintained under fluorescent lights at room temperature on Cg10 medium (16) solidified with 1.4% (wt/vol) Difco agar which was sterilized separately.

Growth rate determinations and amino acid excretion patterns were determined by cultivation in 50 ml of Cg10 medium in tubes (25 by 200 mm) incubated in a 34°C water bath under fluorescent lights and aerated with a 2% (vol/vol) CO_2-air mixture. For extract preparation the cells were grown in 250-ml volumes in 500-ml flasks. Turbidities were monitored at 600 nm with a Bausch & Lomb Spectronic 20 colorimeter.

Mutant selection. Spontaneous analog-resistant mutants were obtained by spreading a lawn of 10^6 to 10^8 cells on Cg10 medium and then placing 0.2 to 0.5 mg of 3-fluorophenylalanine or 3-methylphenylalanine in the center of the plate. Resistant colonies arose after 5 to 7 days of incubation at 34°C and were then purified by several rounds of single-colony isolation. Resistant mutants were checked for phenotypic stability and for excretion of aromatic amino acids as previously described (6).

Extract preparation. Cells were harvested in the mid- to late exponential phase of growth (optical density at 600 nm = 3 to 5) and washed three times with 50 mM potassium phosphate buffer (pH 7.5). Cell pellets were stored at −40°C. After thawing, the cells were suspended at 4°C in buffer containing 1 mM dithiothreitol. Cells were disrupted with six 30-s bursts of ultrasound energy with a Lab-Line Ultratip sonicator at 70 mV and then centrifuged at 150,000 × *g* for 1 h in a Beckman preparative ultracentrifuge. The resulting supernatant was passed through a Sephadex G-25 column equilibrated with extract buffer. The resulting preparation is termed crude extract.

Analytical procedures. Excreted aromatic amino acids were measured as previously described (6). Protein concentrations were determined by the method of Bradford (1). All enzymatic reactions were carried out at 37°C. DAHP synthase was assayed in crude extracts by the method of Srinivasan and Sprinson (15) as modified by Jensen and Nester (11). Reaction mixtures contained the following in 50 mM potassium phosphate buffer (pH 7.5): 2 mM each erythrose-4-phosphate and phosphoenolpyruvate, 0.2 mM $CoSO_4$, 0.25 to 0.50 mM dithiothreitol, and 500 to 900 μg of extract protein.

Before being assayed, prephenate dehydratase was separated from interfering aminotransferase activities by ion-exchange chromatography on a DEAE-cellulose (Whatman DE-52) column (11 by 44 mm) equilibrated with 50 mM potassium phosphate (pH 7.5) containing 1 mM dithiothreitol and 40 μM L-tyrosine. An amount of extract containing 1.0 to 10 mg of protein was applied to the column. The column was then washed with 5 ml of the buffer described above followed by 5 ml of buffer containing 0.2 M KCl and

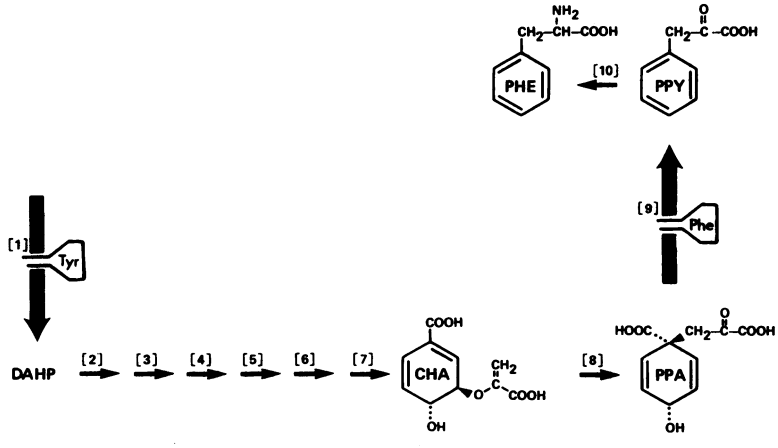

FIG. 1. Biosynthetic pathway for L-phenylalanine and its regulation. Heavy arrows indicate enzyme control points. Enzyme 1, the early-control point, is DAHP synthase and is feedback inhibited by L-tyrosine (Tyr). Enzyme 9, the late-control point, is prephenate dehydratase and is feedback inhibited by L-phenylalanine (Phe). Prephenate dehydratase is also activated by L-tyrosine. The branch to L-tryptophan (not shown) diverges from chorismate (CHA), and the branch to L-tyrosine (not shown) diverges from prephenate (PPA). Other abbreviations: PPY, phenylpyruvate; E-4-P, erythrose-4-phosphate; PEP, phosphoenolpyruvate.

then 5 ml of buffer containing 0.6 M KCl. Fractions (1.0 ml) were collected. Prephenate dehydratase activity was determined by measuring the formation of phenylpyruvate as previously described (12). Aminotransferase activity was determined by measuring the phenylpyruvate formed when α-ketoisocaproate and L-phenylalanine were present as substrates at 1 mM each. It was not necessary to add pyridoxal 5'-phosphate to the reaction mixtures.

Biochemicals. Prephenate was isolated from culture supernatants of *Salmonella typhimurium* (3). All other biochemicals were obtained from Sigma Chemical Co.

RESULTS

Enzyme targets of regulation by L-phenylalanine and L-tyrosine. In *Synechococcus* sp. strain PCC6301 prephenate dehydratase is feedback inhibited by L-phenylalanine, whereas DAHP synthase is feedback inhibited by L-tyrosine (Table 1). L-Tyrosine is also an allosteric activator of prephenate dehydratase. *Synechococcus* sp. strain PCC6301 fits the general pattern of most cyanobacteria in having an unregulated arogenate dehydrogenase, a regulated prephenate dehydratase, and a regulated DAHP synthase (5). Although this strain has been one of the most widely used cyanobacteria for physiological studies, its tyrosine-regulated DAHP synthase is not typical of most named *Synechococcus* species; most strains possess a phenylalanine-sensitive enzyme instead (5). Inhibition of prephenate dehydratase by L-phenylalanine was more than offset by the presence of an equimolar concentration of the allosteric activator L-tyrosine (Table 1).

TABLE 1. Effects of aromatic pathway amino acids and their analogs on activities of DAHP synthase and prephenate dehydratase in *Synechococcus* sp. strain PCC6301

Enzyme	Addition[a]	Relative activity (%)[b]
DAHP synthase	None	100
	L-Tyrosine	27
	L-Phenylalanine	97
Prephenate dehydratase[c]	None[d]	100
	L-Phenylalanine	37
	L-Tyrosine	303
	L-Phenylalanine + L-tyrosine	126
	3-Fluorophenylalanine	26
	3-Fluorophenylalanine + L-tyrosine	30
	3-Methylphenylalanine	22
	3-Methylphenyl alanine + L-tyrosine	30

[a] For DAHP synthase assays all additions were made to give final concentrations of 0.5 mM; for prephenate dehydratase assays the additions were at 1.0 mM, except for 3-fluorophenylalanine (0.2 mM).

[b] A relative activity of 100% corresponds to a specific activity of 2.90 or 8.35 nmol of product formed per min per mg of extract protein for DAHP synthase or prephenate dehydratase, respectively.

[c] Prephenate dehydratase was partially purified to eliminate contaminating aminotransferase activities.

[d] The partially purified enzyme preparations contained L-tyrosine as a stabilizing agent. All assays, therefore, contained 10 μM L-tyrosine in addition to any other effector molecules listed.

Identification of effective antimetabolite analogs of L-phenylalanine. Since both 3-fluorophenylalanine and 3-methylphenylalanine were potent inhibitors of growth, their in vitro effects on DAHP synthase and prephenate dehydratase were examined. Neither analog inhibited DAHP synthase activity. In contrast, the analogs were at least as inhibitory to prephenate dehydratase as was L-phenylalanine (Table 1). Analog inhibition was not significantly compensated for by L-tyrosine activation, even though L-tyrosine was able to offset L-phenylalanine inhibition very effectively.

If the analogs inhibit growth by inhibiting prephenate dehydratase and hence L-phenylalanine synthesis, then L-phenylalanine should reverse growth inhibition. Data showing specific reversal of 3-fluorophenylalanine growth inhibition were indeed found. For example, 0.27 mM 3-fluorophenylalanine prevented growth altogether, whereas the additional presence of 0.55 mM L-phenylalanine allowed a doubling time of 6.9 h (compared with 6.3 h for uninhibited controls). An initial concentration of 0.05 mM L-phenylalanine yielded a doubling time of 18.6 h. L-Tyrosine or other amino acids did not reverse growth inhibition by either analog. Since permeases seem to be absent in cyanobacteria (6), a trivial explanation of reversal at the transport level can probably be excluded.

Regulatory mutants. Spontaneous, analog-resistant mutants were selected. All such mutants seem to be regulatory mutants, in agreement with the apparent lack of permeases. These mutants fell into two classes (Table 2). One class excreted L-phenylalanine but not L-tyrosine into the culture medium, a phenotype to be expected of mutants deregulated in prephenate dehydratase. The second class excreted both L-tyrosine and L-phenylalanine, a phenotype to be expected of mutants deregulated in DAHP synthase. The three mutants in the latter group differed from one another in the ratio of L-tyrosine to L-phenylalanine excreted.

Prephenate dehydratase mutants. In organisms capable of prephenate transamination, assays for prephenate dehydratase are complicated

TABLE 2. Excretion of L-tyrosine and L-phenylalanine by analog-resistant mutants

Strain	Concn (μM) in culture filtrate[a]	
	L-Tyrosine	L-Phenylalanine
Wild type	<3	10
Mutant 3FP8	<3	40
Mutant 3FP13	<3	30
Mutant 3FP9	260	25
Mutant 3MP1	550	130
Mutant 3FP6	490	290

[a] Cultures were harvested in the mid- to late exponential phase of growth (optical density at 500 nm = 3.0 to 4.0).

when amino acids are tested as allosteric effectors. Aromatic amino acids are usually very effective amino donors in combination with prephenate. The use of a small DE-52 column as described above proved to be a rapid and effective method for separating prephenate dehydratase activity from the aminotransferase activities that interfere with the reaction assay when aromatic amino acids are added (Fig. 2). The separation could be easily achieved in less than 20 min with little, if any, loss of enzyme activity. This procedure resulted in an 11-fold purification of prephenate dehydratase.

Mutants 3FP8 and 3FP13, which excrete only L-phenylalanine, possessed prephenate dehydratase activities that were no longer sensitive to feedback inhibition by L-phenylalanine (Fig. 3). However, the enzyme activity was still subject to tyrosine activation.

Double-reciprocal plots of wild-type prephenate dehydratase activity (Fig. 4) showed mixed

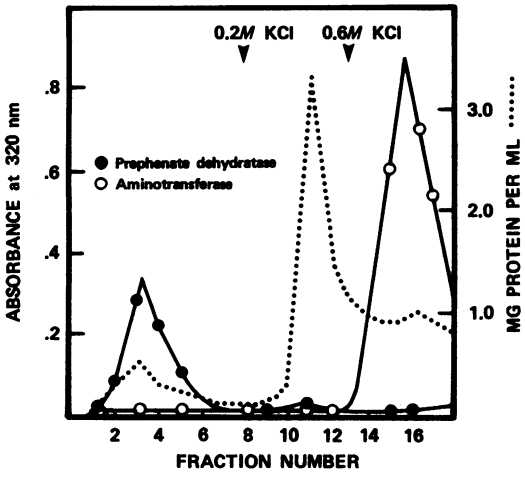

FIG. 2. Separation of prephenate dehydratase from aromatic aminotransferase activity on DEAE-cellulose. The details of extract preparation, gradient elution, and enzyme assay are given in the text.

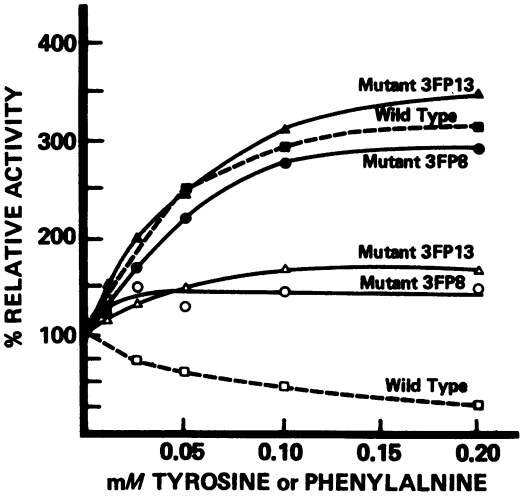

FIG. 3. Desensitization of prephenate dehydratase to phenylalanine inhibition in mutants of *Synechococcus* sp. strain PCC6301. Partially purified prephenate dehydratase, free of aminotransferase activity, was used for all determinations. The closed symbols denote the presence of L-tyrosine; the open symbols denote the presence of L-phenylalanine.

kinetics of allosteric activation by L-tyrosine, with the parameters of affinity and velocity both being altered. Reliable kinetic data could not be obtained in the presence of L-phenylalanine alone because activities were too low in the absence of at least low concentrations of L-tyrosine. A similar effect of L-tyrosine upon prephenate dehydratase has been found in several species of coryneform bacteria (4). However, when L-phenylalanine was added in combination with L-tyrosine, L-phenylalanine also gave mixed kinetics (Fig. 4). The non-linearity of the

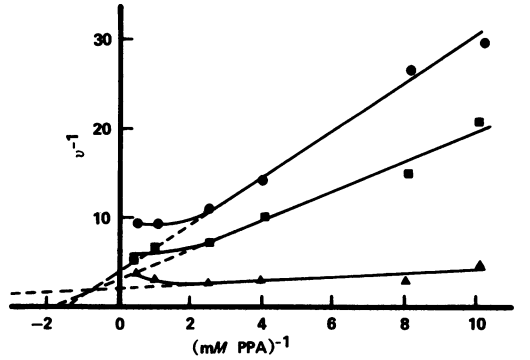

FIG. 4. Reaction kinetics of partially purified prephenate dehydratase activity of *Synechococcus* sp. strain PCC6301. Symbols: ●, no addition; ▲, 0.5 mM L-tyrosine; ■, 0.5 mM each L-tyrosine and L-phenylalanine. v^{-1} values are expressed as absorbance of phenylpyruvate at 320 nm. PPA, Prephenate.

Lineweaver-Burk plots at high substrate concentrations is probably the result of substrate inhibition. The alternative possibility of product inhibition was ruled out by the finding that the addition of 10 μM phenylpyruvate was not inhibitory. It was calculated that the concentrations of phenylpyruvate in reaction mixtures were less than 5 μM.

DAHP synthase mutants. The second group of analog-resistant mutants (3FP6, 3FP9, and 3MP1) were all identical to the wild type with respect to specific activities and allosteric sensitivities of prephenate dehydratase. They all lacked the normal sensitivity of DAHP synthase to inhibition by L-tyrosine (Table 3). However, these mutants consistently showed characteristic differences in the total end-product excretion and in the ratio of L-tyrosine to L-phenylalanine (Table 2). The DAHP synthase specific activities also varied in the mutants (Table 3). A proportional relationship was seen such that as the specific activity of a given feedback-insensitive DAHP synthase increased, more total end product was made (of which relatively more was L-phenylalanine). Thus, mutant 3FP9, which had the lowest specific activity, excreted mainly L-tyrosine and relatively little L-phenylalanine. Note that the L-phenylalanine excreted was still on the order of magnitude of amounts produced by prephenate dehydratase mutants. Mutant 3FP6 (which had the highest specific activity) excreted almost twice as much L-tyrosine as did mutant 3FP9, but also excreted more than 10 times as much L-phenylalanine as did mutant 3FP9.

DISCUSSION

Synechococcus sp. strain PCC6301 possesses two enzyme targets of feedback inhibition to govern the control of L-phenylalanine and L-tyrosine biosynthesis. Since arogenate dehydrogenase is unregulated in this species (5), as in most cyanobacteria, regulation of DAHP synthase is crucial for control of L-tyrosine biosynthesis. On the other hand, prephenate dehydratase represents a peripheral control point governing L-phenylalanine biosynthesis that is characteristic of organisms such as *B. subtilis*.

A comparison of two classes of mutants isolated for their phenotype of resistance to growth inhibition by phenylalanine analogs was very dramatic. The loss of feedback inhibition of DAHP synthase results in much more overproduction of L-phenylalanine than does the loss of feedback inhibition of prephenate dehydratase. Relaxation of early control results in more carbon flow-through to L-phenylalanine than does relaxation of late control. Clearly, in DAHP synthase-deregulated mutants the increased substrate made available to prephenate dehydratase is effective in overwhelming feedback inhibition. The substantial overproduction of L-tyrosine in DAHP synthase-deregulated mutants would also tend to negate feedback inhibition of prephenate dehydratase by promoting an offsetting allosteric activation. Since prephenate dehydratase-deregulated mutants excrete only modest levels of L-phenylalanine, they must have access to limited substrate because of continued restraint imposed upon DAHP synthase by L-tyrosine.

Thus, when DAHP synthase is desensitized to feedback inhibition, early control by L-tyrosine is lost, and the late control of L-phenylalanine is largely overwhelmed. The preferential flow of intermediates into the L-tyrosine branch and the progressive overwhelming by substrate of prephenate dehydratase regulation in vivo is illustrated by the comparison of end-product production in desensitized DAHP synthase mutants which differ in specific activity. The molecular basis for pleiotropic alteration of allostery and

TABLE 3. Feedback densensitization of DAHP synthase in regulatory mutants of *Synechococcus* sp. strain PCC6301

Strain	Addition[a]	ΔA_{549}[b]	% Inhibition	Sp act[c] of DAHP synthase
Wild type	None	1.280		
	L-Tyrosine	0.205	84	1.6
Mutant 3FP9	None	0.912		
	L-Tyrosine	0.822	10	1.2
Mutant 3MP1	None	1.251		
	L-Tyrosine	1.120	10	3.1
Mutant 3FP6	None	1.468		
	L-Tyrosine	1.378	6	3.3

[a] When present, the final concentration of L-tyrosine was 2 mM.
[b] ΔA_{549}, Change in absorbance at 549 nm.
[c] Expressed as nanomoles of product formed per minute per milligram of protein.

specific activity is not known, but we have observed similar pleiotropic mutants in *Synechocystis* sp. strain 29108 (7).

Prephenate dehydratase in *Synechococcus* sp. strain PCC6301 is reminiscent of anthranilate synthase in *Anabaena* sp. strain PCC7119 (9). In each case, end-product overproduction is influenced less by regulation existing in the terminal branch than by increased availability of substrate. In each case, loss of early-pathway regulation increases carbon flow and provides sufficient elevation of substrate levels to these terminal branch enzymes to overwhelm their abilities to restrain excess flow-through.

An interesting example of endo-oriented focus at DAHP synthase has been found in *Anabaena* sp. strain PCC7119 (ATCC 29151) (9). In this case, DAHP synthase deregulation was accomplished physiologically with a bradytrophic mutant grown in the absence of L-tyrosine and L-phenylalanine, which are required for wild-type growth rates due to a partial loss of chorismate mutase activity. The lack of in vivo regulation of two regulatory isozymes of DAHP synthase (8) owing to the limiting formation of L-tyrosine and L-phenylalanine during growth resulted in L-tryptophan excretion. Increased endogenous chorismate overcame competitive inhibition of anthranilate synthase by L-tryptophan.

Riccardi et al. (13) have isolated analog-resistant mutants of the cyanobacterium *Spirulina platensis* which excrete L-phenylalanine. It would be of interest to determine the site of deregulation and to look for tyrosine excretion, especially since *Spirulina* species belong to section III of the grouping of Stanier (14), whereas *Synechococcus* and *Synechocystis* species belong to section I, and *Anabaena* species belong to section IV.

In comparing the regulation of the aromatic pathway among the cyanobacteria studied thus far, a general pattern seems to be emerging. It is very common to find that a single end product is the major or sole feedback inhibitor of DAHP synthase in cyanobacteria (5) and in many other microbes as well (2). In such cases, the product that is the primary effector of the first enzyme, DAHP synthase, is also the preferred end product of carbon flow and, thus, the major excreted product in deregulated mutants. For example, in *Synechocystis* sp. strain PCC6902 (ATCC 29108), DAHP synthase is feedback inhibited solely by L-phenylalanine, and DAHP synthase-deregulated mutants excrete L-phenylalanine in quantities 6 to 12 times that of L-tyrosine (7). The high excretion of L-phenylalanine by these mutants dramatically underscores the dominating influence of DAHP synthase regulation on the pathway since prephenate dehydratase in these mutants is still feedback inhibited by L-phenylalanine.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service research grant AM-19447 from the National Institutes of Health and in part by funds from the Center for Somatic-Cell Genetics and Biochemistry.

We gratefully acknowledge the help of Terry Guarnieri with the typing of the manuscript and the help of Carol Percik with the illustrative art work.

LITERATURE CITED

1. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
2. **Byng, G. S., J. F. Kane, and R. A. Jensen.** 1982. Diversity in the routing and regulation of complex biochemical pathways as indicators of microbial relatedness. Crit. Rev. Microbiol. **9**:227–252.
3. **Dayan, J., and D. B. Sprinson.** 1970. Preparation of prephenic acid. Methods Enzymol. **17A**:559–561.
4. **Fazel, A. M., and R. A. Jensen.** 1980. Regulation of prephenate dehydratase in coryneform species of bacteria by L-phenylalanine and by remote effectors. Arch. Biochem. Biophys. **200**:165–176.
5. **Hall, G. C., M. B. Flick, R. L. Gherna, and R. A. Jensen.** 1982. Biochemical diversity for biosynthesis of aromatic amino acids among the cyanobacteria. J. Bacteriol. **149**:65–78.
6. **Hall, G., M. B. Flick, and R. A. Jensen.** 1980. Approach to recognition of regulatory mutants of cyanobacteria. J. Bacteriol. **143**:981–988.
7. **Hall, G. C., and R. A. Jensen.** 1980. Enzymological basis for growth inhibition by L-phenylalanine in the cyanobacterium *Synechocystis* sp. 29108. J. Bacteriol. **144**:1034–1042.
8. **Hall, G. C., and R. A. Jensen.** 1981. Regulatory isozymes of 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase in the cyanobacterium *Anabaena* sp. strain ATCC 29151. J. Bacteriol. **148**:361–364.
9. **Hall, G. C., and R. A. Jensen.** 1981. Isolation of cyanobacterial auxotrophs by direct selection of regulatory-mutant phenotypes. Curr. Microbiol. **6**:189–194.
10. **Jensen, R. A., and G. C. Hall.** 1982. Endo-oriented control of pyramidally arranged metabolic branchpoints. Trends Biochem. Sci. **7**:177–180.
11. **Jensen, R. A., and E. W. Nester.** 1966. Regulatory enzymes of aromatic amino acid biosynthesis in *Bacillus subtilis*. I. Purification and properties of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase. J. Biol. Chem. **241**:3365–3372.
12. **Patel, N., D. L. Pierson, and R. A. Jensen.** 1977. Dual enzymatic routes to L-tyrosine and L-phenylalanine via pretyrosine in *Pseudomonas aeruginosa*. J. Biol. Chem. **252**:5839–5846.
13. **Riccardi, G., S. Sora, and O. Ciferri.** 1981. Production of amino acids by analog-resistant mutants of the cyanobacterium *Spirulina platensis*. J. Bacteriol. **147**:1002–1007.
14. **Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier.** 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. **111**:1–61.
15. **Srinivasan, P. R., and D. B. Sprinson.** 1959. 2-Keto-3-deoxy-D-*arabo*-heptonic acid 7-phosphate synthetase. J. Biol. Chem. **234**:716–722.
16. **van Baalen, C.** 1967. Further observations on growth of single cells of coccoid blue-green algae. J. Phycol. **3**:154–157.