SHORT COMMUNICATIONS

**Absence of Repression in a Phenylalanine Auxotroph of
*Synechococcus cedrorum***

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INTRODUCTION

Repression and derepression of enzymes of amino acid biosynthesis in blue-green algae have been studied by using auxotrophic strains cultured with and without the required amino acid. Ingram *et al.* (1972) reported repression of the enzymes of tryptophan synthesis in *Agmenellum quadruplicatum*, while Delaney, Dickson & Carr (1973) observed no control of an enzyme of methionine biosynthesis in *Anacystis nidulans*. In addition, lack of repression was noted for arginine-synthetic enzymes in prototrophic cells of *A. nidulans* (Hood & Carr, 1971).

This paper presents evidence that three enzymes involved in phenylalanine biosynthesis in *Synechococcus cedrorum* are not subject to repression or derepression. A phenylalanine-requiring mutant strain previously shown to lack prephenate dehydratase activity (Kaney, 1973) was used. Enzyme assays showed no significant increase in the specific activities of chorismate mutase and phenylalanine aminotransferase from phenylalanine-starved cells over unstarved cells. Furthermore, no significant decreases in the specific activities of these enzymes or of prephenate dehydratase were observed when cultures of prototrophic cells were fed phenylalanine.

METHODS

Cultures. *Synechococcus cedrorum* (No. 1911) was obtained from the algal culture collection, Indiana University, Bloomington, Indiana, U.S.A. Stock cultures were maintained on solid BG-11 medium (Stanier, Kunisawa, Mandel & Cohen-Bazire, 1971). Liquid cultures were grown in BG-11 medium in 500 ml gas-washing bottles illuminated with incandescent bulbs and maintained at approximately 38 °C. Air was continuously bubbled through the cultures. L-Phenylalanine (0.1 mM) was added to the medium whenever necessary for growth of the auxotroph.

Chemicals. Prephenic acid (purity approx. 95 %) and chorismic acid (purity approx. 66 %), both from *Aerobacter aerogenes*, were obtained from Sigma.

Enzyme assays. The cells were harvested in mid-exponential phase by centrifugation at 8000 g for 20 min and were resuspended in 0.25 M-tris-hydroxymethylamino methane-HCl, pH 8.3, to give a concentration of approximately 10¹⁰ cells/ml. The algae were disrupted by means of a French pressure cell at 400 atm, and unbroken cells and debris were removed by centrifugation at 10 000 g for 10 min. The supernatant fraction (extract) was maintained at 0 °C and assays were performed within 2 h of cell rupture. Chorismate mutase and prephenate dehydratase were determined using the methods of Cotton & Gibson (1970). Phenylalanine

Table 1. *Specific activities of enzymes in extracts of cells cultured with and without phenylalanine*

Strain	Enzyme	Phenylalanine	
		Present	Absent
Prototroph	Chorismate mutase	101 $\pm$ 4.7	96 $\pm$ 2.9
	Prephenate dehydratase	8.7 $\pm$ 1.9	10.5 $\pm$ 3.3
	Phenylalanine aminotransferase	128 $\pm$ 40.7	114 $\pm$ 34.3
Auxotroph	Chorismate mutase	78.5 $\pm$ 5.1	74.2 $\pm$ 4.6
	Phenylalanine aminotransferase	123 $\pm$ 27	148 $\pm$ 36
	Alkaline phosphatase	1.5 $\pm$ 0.3	1.3 $\pm$ 0.5

Each value given represents the average of 4 assays and includes the standard deviation of the mean.

aminotransferase was determined by the method of Fujioka, Morino & Wada (1970). Appropriate dilutions of the extract were used in the assays to ensure that response was related linearly to enzyme concentration. Acid-insoluble material was obtained by mixing equal volumes of extract and cold 0.6 N-HClO₃ and incubating overnight at 4 °C. The resulting precipitate was removed by centrifugation at 8000 g for 10 min, dried in an oven at 300 °C for 30 min, and weighed. The specific activity of the enzymes was calculated as nmol product/min/mg cold HClO₃-precipitable material.

RESULTS AND DISCUSSION

Two types of experiments were performed to detect enzyme repression and derepression, involving (i) the starvation of auxotrophic cells for phenylalanine or (ii) the feeding of prototrophic cells with phenylalanine. In the first type, cultures of the auxotroph were grown and harvested. Half the culture was washed, resuspended in phenylalanine-free medium and allowed to continue growth until exhaustion of the phenylalanine supply. There was no further increase in the extinction of the culture after 4 h growth without phenylalanine. Assays for chorismate mutase and phenylalanine aminotransferase were performed on extracts from both halves of the culture to compare specific activities under the presumed repressed and derepressed conditions (Table 1). The specific activity of alkaline phosphatase was also examined in both extracts using the method of Bone (1971) as a control for protein degradation under conditions of starvations.

In the second type of experiments, a culture of prototrophic cells was grown to mid-exponential phase and phenylalanine added to half the culture at a concentration of 0.1 mM. The cells were allowed to grow in the presence of phenylalanine for 4 h and extracts of both halves of the culture were assayed for chorismate mutase, prephenate dehydratase and phenylalanine aminotransferase activities (Table 1). It should be noted that the phenylalanine auxotroph grew at the same rate as the prototroph; therefore, assimilation of exogenous phenylalanine must occur at a rate sufficient not to limit the size of the intracellular phenylalanine pool.

The data indicate that phenylalanine biosynthesis in *S. cedrorum* may be added to the lengthy list of metabolic pathways in blue-green algae which are apparently not subject to metabolic control by enzyme repression and derepression (Carr, 1973). As Carr (1973) pointed out, the conclusive evidence must come from studies using auxotrophic mutants, which obviate the unlikely possibility that endogenous pools of end-products in proto-

trophic cells might prevent the derepression of enzymes involved in their synthesis. This has now been accomplished with strains requiring tryptophan (Ingram *et al.* 1972), methionine (Delaney *et al.* 1973) and phenylalanine (this work); however, the first mentioned remains the sole example of enzyme repression in cyanophytes.

The absence of transcriptional-level control of enzymes in the blue-green algae, as in other photoautotrophic organisms, has been discussed by Carr (1973) as a possible basis for some of their metabolic peculiarities, such as their general failure to respond with increased growth rates to exogenously supplied nutrilites such as hexoses and amino acids. In view of the documented presence of enzyme repression in phenylalanine biosynthesis in other prokaryotes such as *Escherichia coli* and *Aerobacter aerogenes* (Gibson & Pittard, 1968), it appears that the general lack of such control mechanisms remains one of the most interesting metabolic features of the blue-green algae.

This work was supported by grant GM 17715 from the United States Public Health Service. We thank Dr R. L. Conner and Dr M. J. Koroly for valuable technical advice.

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