SHORT COMMUNICATION

Regulation of Anthranilate Synthase in *Escherichia coli* Growing in Glucose-limited Chemostats

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Strains of *Escherichia coli* isogenic except for the *trpR* locus were grown in glucose-limited chemostats. Anthranilate synthase was assayed spectrofluorimetrically to measure *trp* expression. The specific activity was about ten times greater in the *trpR*[−] strain than in the *trpR*⁺ strain. In glucose-limited chemostats, the specific activity of anthranilate synthase was independent of growth rate. Both strains produced two to three times more anthranilate synthase in chemostats than in batch culture. The addition of 19 amino acids (not including tryptophan) increased *trp* expression of anthranilate synthase fivefold in the *trpR*⁺ strain.

INTRODUCTION

The regulation of gene expression in response to growth rate has been termed 'metabolic regulation' (Harvey, 1970). The specific activity in *Escherichia coli* of enzymes such as glutamate dehydrogenase and glutamate-oxaloacetate transaminase (Harvey, 1970) as well as superoxide dismutase (Hassan & Fridovitch, 1977) decreases as cells grow more slowly in glucose-limited chemostats. The mechanisms controlling this mode of regulation are not known. Rose & Yanofsky (1972) observed metabolic regulation of the *trp* operon in *trpR*[−] strains lacking a functional repressor. Both *trp* mRNA and the specific activity of tryptophan synthase were found to be 1/6 to 1/7 as great when cells grew with generation times of 400 min compared with 70 min. In these experiments cells were grown in batch culture with the generation time determined by the nature of the carbon source. In nutrient-limited chemostats the growth rate of the culture is independent of the composition of the culture medium. One might be able to capitalize on this to investigate metabolic regulation of *trp* expression as a function of the growth rate unaffected by changes in the growth medium. As described in the present paper, we have found that the specific activity of anthranilate synthase (EC 4.1.3.27), the first enzyme of the *trp* operon, is invariant with respect to the growth rate of the culture in cells growing in a glucose-limited chemostat. Furthermore, the results suggest that it is inappropriate to compare the physiological situation with respect to tryptophan regulation in cells growing in batch culture with cells growing in glucose-limited chemostats.

METHODS

Organisms. The strains used were derivatives of *Escherichia coli* K12. Strains RM309 and RM310 contained the *trpR*[−] and *trpR*⁺ alleles, respectively, and were obtained from Dr R. Mosteller of the University of Southern California, School of Medicine. These strains are isogenic in a *cysB*, *tna* background.

Growth condition. Cultures were grown in SBG minimal salts medium supplemented with 0.1 mM-cysteine (Botsford & DeMoss, 1971). All supplements except the carbon source, glucose, were dissolved in SBG and added to chemostat reservoirs. Glucose was dissolved in water then added to the reservoir to give a concentration of 10 mM. Amino acids, including cysteine, were added at twice the concentrations recommended by Neidhardt *et al.* (1974), i.e. the concentrations used were: alanine, 1.6 mM; arginine, 10.4 mM; asparagine, 0.8 mM; aspartate, 0.8 mM; cysteine, 0.2 mM; glutamate, 1.2 mM; glutamine, 1.2 mM; glycine, 1.6 mM; histidine, 0.4 mM; isoleucine, 0.8 mM; leucine, 1.6 mM; lysine, 0.8 mM; methionine, 0.4 mM; phenylalanine, 0.8 mM; proline, 0.8 mM; serine, 20 mM; threonine, 0.8 mM; tyrosine, 0.4 mM; valine, 1.2 mM.

Chemostats were constructed as described by Koch (1971). The working volume was maintained at approximately 200 ml. The initial volume of medium in the reservoir was 2 l. The temperature was maintained at 37 °C by a circulating water bath. Cultures were inoculated at least 4 h before the vessels were completely filled, and were not harvested until at least six generations had occurred, to ensure that the cultures were in steady state (Harman & Botsford, 1979). The flow rates and resulting growth rates were calculated from the measured volumes in the culture vessels and the amount of medium left in the reservoir.

Anthranilate synthase assay. The cells were harvested in a refrigerated centrifuge, washed twice with 0.85% (w/v) NaCl and once with 0.1 M-Tris/HCl pH 7.8. An extract was prepared by sonication, and cell debris was removed by centrifugation at 12 100 g for 15 min. The extract was then assayed spectrofluorimetrically by the technique of Ito & Crawford (1965), using an Aminco-Bowman spectrofluorimeter equipped with thermal spacers. Assays were done at 37 °C.

Protein concentrations were determined in the crude cell-free extracts colorimetrically using the Lowry technique, with bovine albumin as the standard.

RESULTS AND DISCUSSION

The results presented in Table 1 show that the specific activity of anthranilate synthase was dependent on *trp* repressor function even though cells were grown in medium without tryptophan. In glucose-limited chemostats, the specific activity was 8.6-fold higher in the *trpR*⁻ strain than in the otherwise isogenic *trpR*⁺ strain. A 15.3-fold difference was observed in batch culture.

Table 1. *Specific activities of anthranilate synthase in trpR*⁺ and *trpR*⁻ strains of *Escherichia coli* grown in batch culture and in glucose-limited chemostat culture

Growth condition	Strain	Medium	Growth rate, μ (h ⁻¹)	Anthranilate synthase activity [nmol min ⁻¹ (mg protein) ⁻¹]
Batch	<i>trpR</i> ⁺	Minimal	0.57	0.4
	<i>trpR</i> ⁺	19 amino acids	0.85	6.5
	<i>trpR</i> ⁻	Minimal	0.52	6.7
	<i>trpR</i> ⁻	19 amino acids	0.87	4.4
Chemostat	<i>trpR</i> ⁺	Minimal	0.37	1.4
			0.33	1.5
			0.32	0.9
			0.25	1.8
			0.24	1.4
			0.23	1.6
			0.16	1.8
			0.14	1.4
			0.13	1.4
			0.32	7.5
	<i>trpR</i> ⁻	Minimal	0.40	11.1
			0.33	14.0
			0.31	8.0
			0.26	10.6
			0.24	17.0
			0.23	14.0
			0.20	13.4

The mean $\pm$ standard deviation for the specific activity of anthranilate synthase in the *trpR*⁺ strain growing in minimal medium in chemostats was 1.5 ± 0.3 ; for the *trpR*⁻ strain growing in similar conditions, this value was 12.6 ± 2.9 .

One might expect that as cells grow faster in minimal media their demand for amino acids would cause an increase in *trp* expression. In chemostats, cells did respond to changes in growth rate by regulating the rate of synthesis of the enzyme to maintain a constant specific activity. This regulation was independent of *trpR* function. It has been proposed that this mode of regulation is mediated, in part, by attenuation of transcription (Oxender *et al.*, 1979) and responds to the intracellular pool of amino acids. When the *trpR*⁺ strain was grown with a supplement of 19 amino acids (not including tryptophan), the specific activity of anthranilate synthase was increased 5-fold. An amino acid analysis of the spent medium showed that some, but not all of the amino acids in the supplement decreased dramatically in concentration indicating that these amino acids were being catabolized. Since the wild-type strain could not use cysteine as a carbon and energy source when grown in batch culture it seemed that cysteine was not being catabolized. This stimulation of synthesis of anthranilate synthase was also noted in batch culture (Table 1). An enhancement of *trp* expression by the addition of amino acids was also observed by Stubbs & Stubbs (1971).

Activities of anthranilate synthase in cells grown in glucose-limited chemostats were invariant with respect to growth rate. In batch culture, at least in media without supplemental amino acids, expression of *trp* does change in response to changes in growth rate (Rose & Yanofsky, 1972; Shimizu, 1980; R. W. Shimizu & J. L. Botsford, unpublished results). Furthermore, the specific activity of the enzyme when wild-type cells were grown in glucose-limited chemostats was 3.3 times higher than in batch culture with glucose as the carbon source. It is apparent that the physiological situation in cells grown in glucose-limited chemostats is not comparable to the situation in batch culture, at least with respect to the metabolic regulation of anthranilate synthase. Similar results have been observed in the expression of hexokinase (Harvey, 1970). Koch (1971) has proposed that transport limits growth when cells grow in carbon-energy limited chemostats. Herbert & Kornberg (1976) showed that the rate of substrate catabolism is the same as the rate of substrate transport when cells are grown in carbon-limited chemostats. Our data and those of Harvey (1970) with hexokinase suggest that cells growing in carbon-limited chemostats overproduce both catabolic and anabolic enzymes to capitalize on any sudden influx of carbon source. If this is the case, the bacterium seems to be trading optimal efficiency, by allocating resources for the synthesis of excess enzymes rather than for the production of new cells, for the ability to respond quickly and capitalize on improved growth conditions.

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