CONTROL BY ENDOGENOUSLY SYNTHESIZED ARGININE OF THE FORMATION OF ORNITHINE TRANSCARBAMYLASE IN *ESCHERICHIA COLI*[1]

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Studies in recent years have revealed the widespread occurrence among microorganisms of feed-back inhibition of enzyme formation by end products of metabolic pathways; this phenomenon was termed repression by Vogel (1957). The function of repression in cellular metabolism was elucidated by Gorini and Maas (1957; 1958) in a study of the inhibition of ornithine transcarbamylase synthesis by arginine. When wild-type *Escherichia coli* strain W was grown in a minimal medium, a characteristic steady-state level of ornithine transcarbamylase activity was found. When arginine was added to the growth medium, synthesis of the enzyme was inhibited and a much lower level of activity resulted. On the other hand, when an arginine-requiring mutant of *E. coli* was grown in a chemostat (Novick and Szilard, 1950) with arginine limiting the growth rate, the synthesis of ornithine transcarbamylase took place at a much more rapid rate than in the wild-type growing in minimal medium. It was concluded that the increased rate of ornithine transcarbamylase synthesis in the chemostat was the result of a relative intracellular arginine shortage and that therefore in the wild-type strain growing on minimal medium, where no such shortage exists, the lower rate of enzyme formation must have been the result of repression by endogenously produced arginine.

In the experiments of Gorini and Maas with an arginine auxotroph in the chemostat the possibilities were not eliminated that the release of repression is contingent upon the fact that arginine was supplied exogenously, or upon the mutation itself. To examine these latter possibilities, two types of experiments were performed. In the first, ornithine transcarbamylase synthesis was measured in bradytrophs[4] ("leaky mutants"), strains whose growth rates are limited by their impaired ability to synthesize arginine; in the other, synthesis of the enzyme was measured in the wild-type under conditions of an increase in the rate of arginine utilization relative to its rate of production.

MATERIALS AND METHODS

Organisms. Strains used were *E. coli* W (ATCC 9637) and the following mutants: 160-37, an arginine auxotroph, blocked between N^{α}-acetylornithine and ornithine, isolated by Dr. B. D. Davis; R185-823, an arginine + pyrimidine auxotroph, blocked in carbamyl phosphate synthesis, isolated by Dr. R. Roepke; bradytrophs 160-A1, 160-A2, 160-A3, derived from 160-37; bradytroph R185-A, derived from R185-823. The bradytrophs were isolated from the above parent mutant strains after ultraviolet irradiation and selection in minimal medium.

Media. The minimal medium used was medium A of Davis and Mingioli (1950) with 0.5 per cent sodium lactate as carbon source. Medium AF (arginine-free) is minimal medium supplemented with a mixture of amino acids, purines, pyrimidines, and vitamins, the amino acids being taken in approximately the same proportions in which they occur in the protein of *E. coli* (Roberts et al., 1955). The composition of the enrichments in medium AF is shown in table 1. To prepare the corresponding solid media, Difco agar was added to a final concentration of 2 per cent.

Growth experiments. Growth experiments were

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[4] The word bradytroph is taken from the Greek roots, *bradys*, slow, and *trophe*, nourishment. It appears in *Stedman's Medical Dictionary* (18th edition) where it is defined as "characterized by sluggish metabolism."

TABLE 1

Enrichments for the arginine-free medium (medium AF)

Compound	Final Concn	Compound	Final Concn
	mg/liter		*mg/liter*
DL-Alanine	580	L-Tyrosine	60
L-Aspartic acid	60	L-Threonine	200
L-Cysteine	70	L-Valine	330
L-Glutamic acid	400	L-Asparagine	200
L-Glycine	160	Guanosine	20
L-Histidine	50	Adenosine	20
DL-Isoleucine	320	Uracil	20
L-Leucine	270	Pantothenic acid	1
L-Lysine	360	Riboflavin	1
L-Methionine	120	Thiamine	1
DL-Phenylalanine	560	Nicotinic acid	1
L-Proline	140	Biotin	1
DL-Serine	330	Pyridoxine	1
L-Tryptophan	55		

A 25-fold concentrated sterile stock solution was prepared in distilled water and diluted into minimal medium.

carried out in flasks with shaking at 37 C. Cell density was estimated turbidimetrically with a Beckman model DU spectrophotometer at 490 mμ. Cells were grown on the surface of agar plates unless otherwise indicated. They were harvested by centrifugation, washed in medium A by suspension and centrifugation, and resuspended in medium A for inoculation into growth flasks. Viable counts and dry weight determinations revealed that an optical density of 1.00 was equivalent to 0.26 mg dry weight per ml of bacteria and to 6×10^8 viable cells per ml. The quantity of bacteria contained in 1 ml of a culture in the exponential phase of growth whose optical density is 1.00 was taken as the unit of bacterial mass in these experiments. This quantity of bacteria shall be referred to as one OD unit.

Irradiation experiments. Ultraviolet irradiation was carried out on washed cells suspended in medium A at a cell density of about 10^{10} organisms per ml. Suspensions were irradiated for 90 sec at a distance of 45 cm from a 15-watt Hygeaire germicidal lamp.

Enzyme measurement. Ornithine transcarbamylase was estimated according to the method of Jones, Spector, and Lipmann (1955), in suspensions of toluenized cells. One unit of enzyme activity is the amount of enzyme which synthesized 1 μmole of citrulline in 1 hr at 37 C; the lower limit of sensitivity of the method in these experiments was 0.1 unit.

RESULTS

Enzyme formation in bradytrophs. As seen in figure 1, a bradytroph derived from mutant 160-37 grows faster in minimal medium supplemented with arginine (curve 3) than without arginine (curves 1 and 2). Thus its growth rate on minimal medium is limited by its decreased capacity to synthesize arginine. The growth rate of the bradytroph in the presence of arginine is the same as that of the wild-type, with or without arginine.

The kinetics of formation of ornithine transcarbamylase during growth in minimal medium is illustrated in figure 2. In this experiment strain 160-A1 was grown in the presence of arginine to prevent ornithine transcarbamylase formation and then transferred to minimal medium (figure 1, curve 1). Enzyme measurements were carried out at various times during

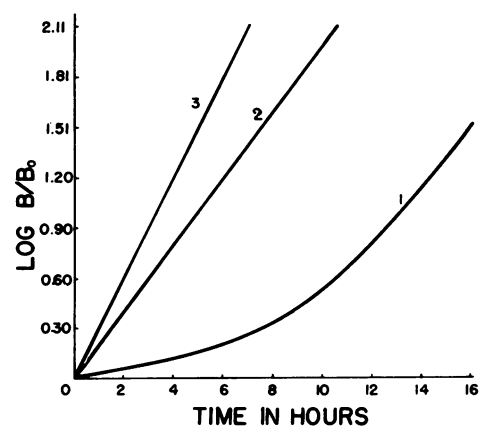

Figure 1. Growth of arginine bradytroph 160-A1. Cells were grown first in minimal medium supplemented with L-arginine, 100 μg per ml, for the experiments represented by *curves 1* and *3*; unsupplemented for the experiment represented by *curve 2*. Subsequent growth was in minimal medium, supplemented with L-arginine, 100 μg per ml for flask 3, unsupplemented for flasks 1 and 2. B_0 is bacterial mass at times zero, B at any time. The initial optical densities were approximately the same for the three flasks.

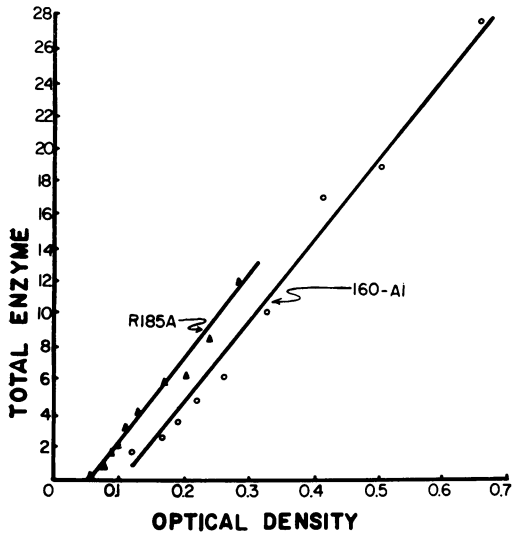

Figure 2. Kinetics of ornithine transcarbamylase formation. Strains 160-A1 and R185-A were grown first in the presence of L-arginine, 200 μg per ml, washed, and transferred to minimal medium, supplemented for strain R185-A with uracil, 100 μg per ml. Total Enzyme refers to the number of units of ornithine transcarbamylase per ml of culture and is plotted as a function of total bacterial mass, represented by optical density.

subsequent growth. As seen in figure 2, in which increase in total enzyme is plotted as a function of increase in total mass (Monod, Pappenheimer, and Cohen-Bazire, 1952), there is a linear relationship between the two variables. The rate of enzyme formation is as great as that previously observed with an arginine auxotroph under conditions of arginine-limited growth in the chemostat (50 units per mg dry weight per generation) and is 25 times as great as that in the wild-type growing in minimal medium (Gorini and Maas, 1957). Similar results were obtained with two other bradytrophs, 160-A2 and 160-A3. Under these conditions of arginine limitation, therefore, the same release of repression is observed as in the chemostat experiment.

It should be noted that under the conditions of the above experiment the growth rate of the bradytrophic strain following growth in the presence of arginine was not constant (figure 1, curve 1). However, despite the accelerating growth, the increase in enzyme was proportional to the increase in total mass. The kinetics of enzyme formation during accelerating growth has been studied in detail and will be reported separately.

Another example of release of repression of ornithine transcarbamylase formation during arginine-limited growth is provided by strain R185-A. This strain grows almost as fast in minimal medium as it does with arginine. The addition of uracil to minimal medium results in inhibition of growth, converting the mode of growth from exponential to linear. This inhibition is reversed by the addition of arginine or citrulline, but not ornithine. Although the mechanism of the uracil inhibition has not been analyzed in detail, these observations are consistent with the interpretation that uracil enhances the partial block in carbamyl phosphate formation, possibly by further inhibiting the production of the enzyme involved in the mutation. Regardless of the nature of the interpretation, when this strain is grown in the presence of uracil, its growth rate is limited by the endogenous supply of arginine. Under these conditions, ornithine transcarbamylase is formed at the same rapid rate as in bradytroph 160-A1 (figure 2). In the absence of uracil, on the other hand, no clear-cut release of repression could be demonstrated in strain R185-A.

Enzyme synthesis in the wild-type. In medium AF, wild-type cells grow 2.5 times faster than they do in minimal medium and therefore must utilize arginine for protein synthesis correspondingly faster. Addition of arginine to medium AF does not further accelerate the growth rate. Yet in AF medium there is a 6-fold increase in the rate of ornithine transcarbamylase synthesis as compared to minimal medium (figure 3), suggesting a decrease in the intracellular arginine level. This supposition was supported by the finding that if citrulline was added to AF medium, the enzyme was formed at a slower rate (figure 3). It has been shown that citrulline must be converted to arginine to repress ornithine transcarbamylase formation (Maas, 1956).

A clue to the mechanism responsible for the decreased endogenous supply of arginine in medium AF came from the observation that the addition of ornithine (in contrast to citrulline) did not result in a lowering of the rate of ornithine transcarbamylase formation (figure 3). This finding suggested that the arginine shortage during growth in medium AF was the result of a limitation in the cells' capacity to convert ornithine to citrulline. In this conversion, ornithine is

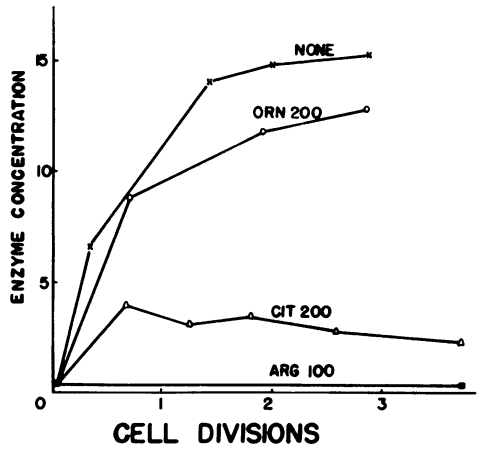

Figure 3. Formation of ornithine transcarbamylase in the wild-type strain during growth in medium AF. Cells were grown first in medium AF supplemented with L-arginine, 100 μg per ml. After washing they were inoculated into flasks containing medium AF and the indicated supplements. *ORN* = DL-ornithine; *CIT* = DL-citrulline, *ARG* = L-arginine. The numbers refer to concentrations in μg per ml. Enzyme concentrations are given in units of ornithine transcarbamylase activity per OD unit of bacterial mass. Growth rates were identical in the four flasks.

coupled with carbamyl phosphate to form citrulline. Since ornithine transcarbamylase, and one of its substrates, ornithine, were present in excess during growth in medium AF, it may be inferred that the formation of the other substrate, carbamyl phosphate, was rate-limiting. This conclusion is supported by the observation that during arginine-limited growth the rate of formation of carbamyl phosphate synthase was not increased (Gorini and Maas, 1958).

DISCUSSION

In the present paper several different situations have been described in mutant strains and in the wild-type in which the rate of formation of ornithine transcarbamylase was increased in response to a limitation in the availability of endogenously produced arginine. Thus it is clear that release of repression, previously observed with an arginine auxotroph in the chemostat was not conditioned by the necessity of supplying arginine exogenously or by the presence of a genetic lesion.

It is generally assumed that the rate of formation of a repressible enzyme is determined by the intracellular concentration of a repressor. In the case of arginine biosynthesis, the concentration of the repressor has been assumed to depend on the intracellular level of arginine. Direct determinations of intracellular arginine levels have thus far been possible only when an exogenous supply of arginine has been provided. (Schwartz, Maas, and Simon, 1959). This level was found to be quite high, about 1 mg per g of wet weight of bacteria. In the absence of exogenous arginine we have found the level of arginine to be at least 100-fold lower than in the presence of arginine. With this low level, our methods have not been precise enough to make an accurate determination of the intracellular arginine concentration and thus to establish a correlation of this concentration with the state of repression.

SUMMARY

The control of ornithine transcarbamylase synthesis in *Escherichia coli* by endogenously produced arginine has been analyzed in bradytrophs (partially blocked mutants) and in a wild-type strain grown in an enriched but arginine-free medium.

In arginine bradytrophs, in which the growth rate is limited by the rate of arginine formation, ornithine transcarbamylase is formed 25 times faster than in the wild-type in which the growth rate is not limited by the rate of arginine formation.

In the wild-type a limitation in the supply of endogenously formed arginine can be achieved by growth in the arginine-free medium. Under these conditions ornithine transcarbamylase is formed six times faster than during growth in minimal medium.

These results lend further support to the previously proposed hypothesis that the intracellular level of arginine regulates the rate of formation of enzymes involved in its own biosynthesis.

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