The potential for the formation of a biosynthetic enzyme in *Escherichia coli**

Studies on the formation of inducible enzymes, such as β-galactosidase, have shown that upon addition of an inducer to an exponentially growing, uninduced culture, enzyme synthesis starts immediately at a constant rate per cell, and hence the enzyme concentration per cell comes close to the theoretical limit only after several generations (*e.g.* 0.9375 of theoretical limit after 4 cell divisions)[1]. The increase in enzyme is described by the following equation (1): $\% E_{max} = 100\ (1 - e^{-\alpha t})$ where E_{max} is the maximum final level of enzyme per cell and α is the rate constant of the equation for exponential growth, $dN/dt = \alpha N$ (N = number of bacteria per unit volume).

It seemed reasonable to expect that formation of other enzymes should follow a similar pattern. The possibility of studying the kinetics of formation of a biosynthetic enzyme which does not require an added inducer arose through the observation that in certain cases the end-product of a biosynthetic sequence inhibits the synthesis of enzymes involved in the formation of this product. This negative feed-back effect has been described for certain amino acids[2,3,4], purines[5], and pyrimidines[6]. We have recently found[7] another example: inhibition by arginine of the synthesis of ornithine transcarbamylase, which catalyzes the conversion of ornithine to citrulline. When cells of the W strain of *Escherichia coli*, harvested from minimal medium, are grown in the presence of arginine (20 μg/ml), the formation of this enzyme is suppressed and continued growth yields cells with approximately 1/100 the initial concentration of the enzyme. When such cells, after washing, are transferred to a growth medium without arginine, the enzyme

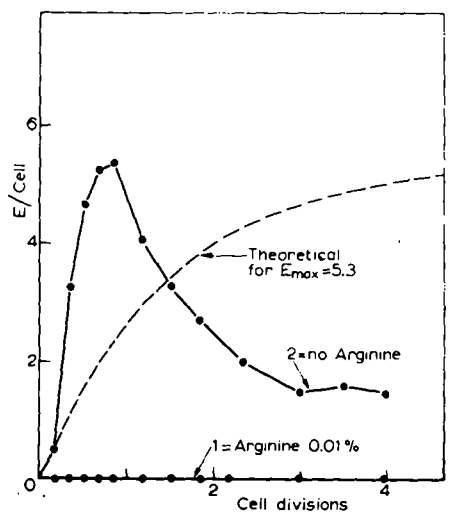

Fig. 1. Enzyme synthesis in flask cultures of the wild type. Washed cells of *E. coli* strain W, taken from exponentially growing cultures maintained on arginine were inoculated into minimal medium A[9] + lactate 0.5%, supplemented as indicated. Incubation was at 37° C with shaking. Enzyme activity was determined in toluenized cells according to the method of JONES *et al.*[10]. One enzyme unit = amount of enzyme which synthesizes 1 μmole of citrulline/h; lower limit of method = 0.01 enzyme units. *E*/cell = units of enzyme per mg of dry weight bacteria. Cell division time was 60 min.

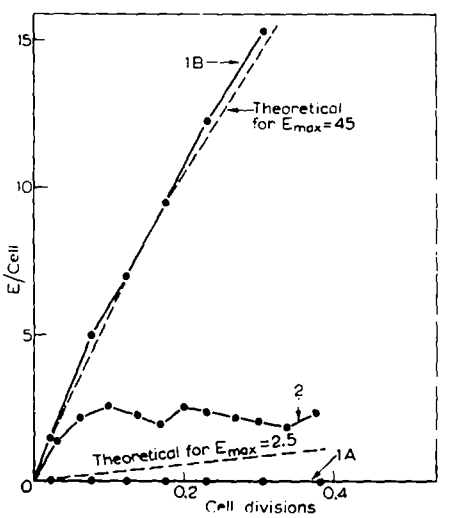

Fig. 2. Enzyme synthesis in the chemostat. The minimal medium and the method for the enzyme determinations were the same as described in the legend to Fig. 1. The histidine⁻ arginine⁻ auxotroph was grown with (μg/ml): histidine 1 and arginine 6 (curve 1A); histidine 10 and arginine 5 (curve 1B). The histidine⁻ auxotroph was grown with histidine 1 (curve 2). The level of growth with histidine limiting at 1 μg/ml was the same as that with arginine limiting at 5 μg/ml. Inocula were washed cells taken from cultures growing exponentially in excess of arginine. Cell division time was 460 min. Theoretical curves were calculated from the constant *E*/cell values, reached for both curves 1B and 2 after 4 cell divisions.

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is not formed at a constant rate at all (Fig. 1, theoretical) but is produced with an initial burst, following which the concentration per cell declines sharply and then remains fairly constant (Fig. 1, curve 2).

An explanation for this kinetic picture is suggested by the fact that added arginine inhibits the synthesis of this enzyme. If endogenous arginine should exert a similar effect, the initial burst of enzyme synthesis would occur when endogenous arginine is low. The rapid formation of ornithine transcarbamylase (and of other enzymes in the same pathway) would then lead to rapid synthesis of arginine resulting in an accumulation of the latter that in turn would slow down enzyme synthesis until a steady state is reached.

In order to test this hypothesis it is necessary to control the intracellular arginine concentration. Such control can be achieved in the chemostat, an instrument which permits one to maintain a culture in a steady state of growth whose rate is limited by the supply of a required metabolite[8].

Two mutants were chosen for chemostat experiments: one requiring only histidine, and a double auxotroph requiring histidine and arginine. The block in arginine synthesis was located between acetylornithine and ornithine. For all experiments, the cells were pregrown in the presence of arginine to produce a low level of ornithine transcarbamylase.

For the first experiment, cells of the double auxotroph were transferred to a medium with limiting arginine and an excess of histidine. Under these conditions the internal concentration of arginine should be low. It is seen (Curve 1B of Fig. 2) that the enzyme is formed at the same initial high rate as in the wild type (Fig. 1); but in the chemostat experiment the enzyme continues to be formed at this high rate until its concentration per cell reaches a plateau 25 times as high as the steady state level in the wild type. As shown in Fig. 2, the observed curve for enzyme synthesis approximates closely the theoretical curve for constant rate kinetics, calculated from the final level of enzyme. It therefore appears that the maintenance of an unusually low concentration of internal arginine preserves a constant and very high rate of enzyme synthesis.

The other two experiments are controls. In the experiment described by curve 1A, cells of the double auxotroph are grown with limiting histidine and are supplied with an arginine concentration of which they use all but 1 μg/ml. It can be seen that this slight excess of arginine completely suppresses formation of the enzyme. Finally, in the experiment described by curve 2, cells of the histidine auxotroph were grown with limiting histidine, their arginine being formed endogenously. Synthesis of the enzyme exhibited the same rate characteristics as were observed for the wild-type flask culture, with an initial burst and a subsequent slower rate. These controls show that the continued rapid formation of the enzyme seen in curve 1B was not due to any factor that limits growth, but specifically to arginine.

It can be concluded that in the wild type the level of internal arginine or of a derivative of arginine does indeed control the rate of formation of ornithine transcarbamylase. Such a mechanism was already suggested by the previously mentioned studies on end-product control of enzyme formation. In addition, the present study shows that wild-type cells have a potential for synthesizing a level of this enzyme greatly in excess of (25 times) that reached in actuality*. A possible use for this latent capacity is that it helps the cell to resume growth without a marked delay after arginine in its surroundings becomes exhausted.

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* YATES AND PARDEE[6] have recently provided independent evidence for a latent capacity of *E. coli* to increase the levels of enzymes involved in pyrimidine biosynthesis.

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