OSCILLATIONS OF THE RATE OF SYNTHESIS OF ß-GALACTOSIDASE IN ESCHERICHIA COLI ML 30 AND ML 308

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The synthesis of ß-galactosidase in Escherichia coli can be regarded as involved in a control system (Monod and Jacob,1961). This control system contains two closed feedback loops for induction (Burstein et al., 1965) and catabolic repression respectively (Magasanik, 1961). A simplified mathematical model was proposed (Knorre, 1965;1968) for ß-galactosidase synthesis based on the generally accepted characteristics of the operon model of Jacob and Monod (1961). Our model is defined by a system of nonlinear differential equations which considers induction, catabolic repression,active transport and exponentially growth. Analog computer techniques are used to elucidate the dynamical behavior of the system. One of the major features is the occurrence of oscillations in the variables involved in the control circuit: mRNA, enzymes and enzyme products. Many bacterial enzymes are synthesized periodically rather than continuously. The ß-galactosidase of E. coli is believed to be synthesized continuously (Masters et al., 1964; Cummings, 1965). The results reported in this communication show that the rate of ß-galactosidase synthesis in E. coli ML30 and ML308 oscillates after a step from glucose to lactose in exponentially growing cells for 4 periods.

MATERIALS AND METHODS

Strains and media. *E. coli* strains ML 30 (inducible), and ML 308 (constitutive) were employed. Bacteria were grown at 34° in a synthetic medium(pH = 6.8)of the following composition(g per l): 2.7 KH_2PO_4, 7.1 $Na_2HPO_4 \cdot 12H_2O$, 0.5 NH_4Cl, 5 NaCl, 1 Na_2SO_4, 0.02 $MgCl_2 \cdot 6H_2O$, 0.003 $FeCl_2$, 0.002 $MnCl_2$, and 2 glucose or lactose.

Growth conditions. The cultures were pregrown overnight on glucose, and 5 ml of the exponentially growing cells were used to inoculate 200 ml of synthetic medium with glucose. Growth was followed by optical density measurements at 470 nm for 4-5 generations of exponential growth so that an accurate evaluation of the growth rate could be made under steady-state conditions. The generation time did not vary significantly from 49 (45) min for ML 30 (ML 308) in these experiments. When the cultures had grown to a cell density of 5×10^7 bacteria per ml (optical density of 0.5, 1cm cuvette) 50 ml of the culture were withdrawn, the cells collected on a filter (Schott G5, 10cm diam.), washed twice with synthetic medium without carbon source,and resuspended in 200 ml of this same synthetic medium the carbon source of which was now lactose instead of glucose. These operations were completed within 2 min at 34°. After about 50 (5) min the generation time of 49 (45) min for ML 30 (ML 308) has been achieved again.

ß-Galactosidase assay. Samples of 4 ml removed at time intervalls of 6 min were measured for optical density (X) and protein synthesis was arrested by the addition of chloramphenicol. In the determination of ß-galactosidase greater precision (about 0.5%) was attained by adding 0.12 ml of a 1% solution of bovin serum albumin to the samples (Ben-Hamida and Schlessinger, 1965). The assay for ß-galactosidase was essentially according to Pardee *et al.* (1959). Three 1 ml portions of each sample were shaken with

0.05 ml toluene for 30 min at 37°. Then the ß-galactosidase was assayed by adding of 0.5 ml o-nitrophenyl-ß-D-galactoside (ONPG) and incubating for 5-60 min at 28 ±0.01°. The reaction was stopped by adding of 0.2 ml Na_2CO_3. The absorbancy was measured at 420 nm (0.5 cm cuvette) against a blank prepared identically except that ONPG was added after Na_2CO_3. Enzyme activities are expressed as $\Delta O.D._{420} \times 500/min$. Division by the bacterial density, taken as $O.D._{470} \times 100$, gives the specific activity(Y). This is a convenient scale, since the strain ML 30 (ML 308) shows steady-state activities with lactose (glucose) near 100 (200).

Evaluation of the specific rate of ß-galactosidase synthesis. The growth rate of bacteria is expressed by $\mu(t)\left[h^{-1}\right]$, defined by the equation $dX/dt = \mu(t)X$. The values $\mu(t)$ were evaluated by a digital computer from optical density data. The specific rate of enzyme synthesis is expressed by f(t), defined by the equation $dY/dt = f(t) - \mu(t)Y$. The values for the specific rate in FIG.2 were obtained by an approximation method ($\Delta t = 0.05$ h) from a digital computer. When the protein synthesis is arrested, f(t)=0, the specific activities Y satisfy the equation $Y_t = Y_0 e^{-\mu(t)}$. On the other hand, if the derivative dY/dt of the specific activity vanishes, the specific rate of synthesis approaches its steady-state value $f(t) = \mu Y$.

RESULTS

Changes of the specific ß-galactosidase activity after a step from glucose to lactose for E.coli ML 30 and ML 308 are shown in FIG. 1. It can be seen that about 3 h after the step both ML 30 and ML308 have achieved the same steady-state value. More clearly than the time course of the specific activity, the specific rate shows the periodic changes in the cells. The typical behavior of the specific rate of ß-galactosidase synthesis under

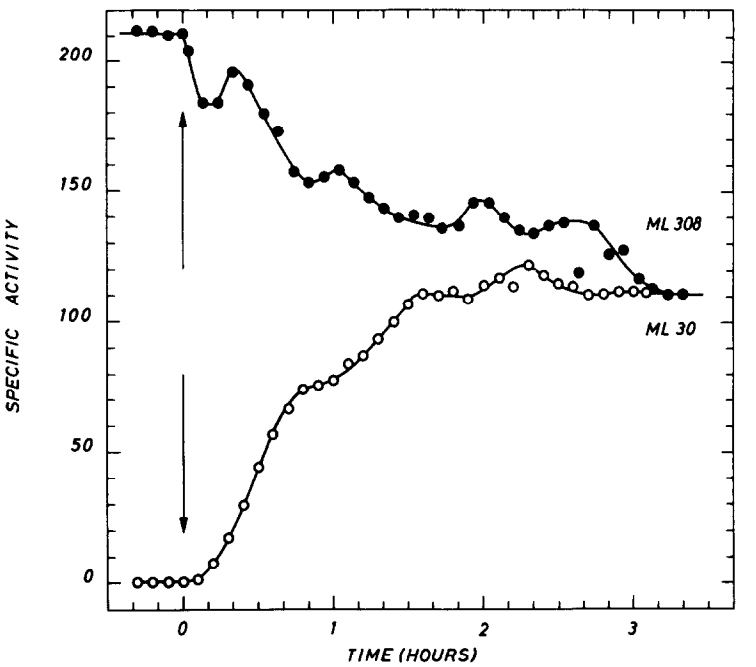

FIG. 1. Specific activity of ß-galactosidase in E. coli ML 30 and ML 308 after a step (arrows) from glucose to lactose.

these conditions shows FIG. 2. The specific rate of ML 30 periodically increases from 0.2(steady-state value on glucose) to 100 (steady-state value on lactose) whereas the rate of ML308 periodically decreases from 200 to 100. The period of oscillations is about 50 min. This is comparable with the generation time of ML30 and ML308, but there is a phase shift of 180° between their oscillations. It can be assumed that the rate of synthesis of ß-galactosidase is proportional to the concentration of corresponding mRNA. Consequently, in FIG. 2 the curves also show the changes in the level of mRNA.

The occurrence of damped oscillations in the rate of enzyme synthesis was found in a number of similar experiments for about 4 periods. Especially, comparable results were obtain with an Hfr strain (E. coli, 3000, thymine⁻ and thiamine⁻).

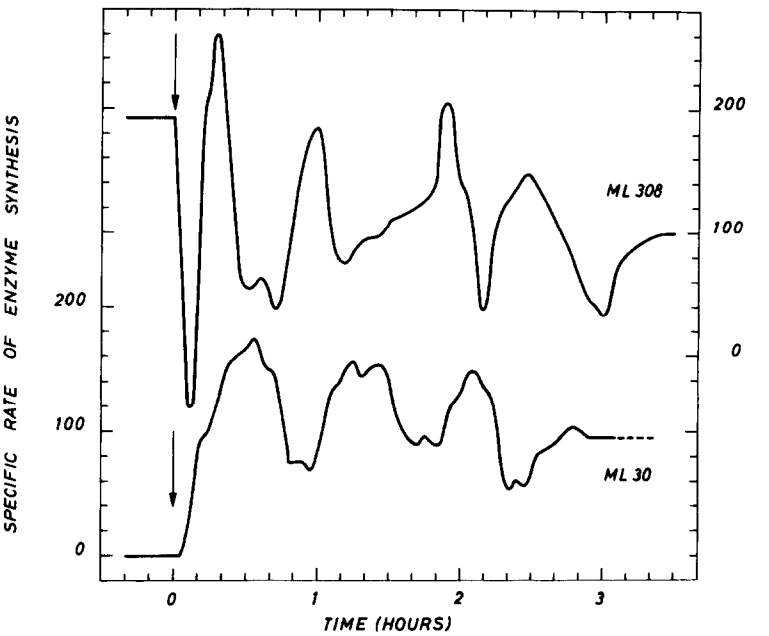

FIG. 2. Oscillations of the specific rate of ß-galactosidase synthesis in E. coli ML 30 and ML 308 evaluated from specific activity data and growth rate with a digital computer. The ordinate on the right refers to ML 308.

DISCUSSION

The results presented reinforce earlier findings (Knorre and Bergter, 1966). It may be assumed that the oscillations of the ß-galactosidase synthesis are intimately connected with the interaction of induction and catabolic repression. An extensive investigation of such oscillatory behavior in cellular control systems was made by Goodwin (1963) and Knorre (1967).

Masters et al. (1964) and Cummings (1965) have reported that ß-galactosidase was synthesized continuously in synchronously dividing cultures of E.coli. Since in both cases ß-galactosidase was induced by a gratuituous inducer (IPTG) it should be emphasized that the oscillatory behavior is only detectable when there is a feedback signal by the enzyme products. Furthermore, in our experiments the cultures were grown asynchronously, but

the step from glucose to lactose provides a synchrony of the control systems in the single cells at least for some periods so that oscillations can be detected. A synchrony of cell division after the step was not found by means of light scattering measurements. Thus, a gene dosage effect can be excluded.

The experiments show also that the steady-state response of the ß-galactosidase control system to a step from glucose to lactose in both ML 30 and ML 308 is identically. According to Palmer and Moses(1968) the i-gene product interacts with the catabolite co-repressor in such a way that its affinity for the operator is increased. If we assume this hypothesis the observed steady-state behavior suggests the tentative explanation that the strain ML 308 produces a repressor which is active only together with the catabolite corepressor.

Our finding of oscillations in asynchronously growing cultures shows, that such periodic behavior results from the feedback system regulating enzyme synthesis and that they seem to be to some extent autonomously.

REFERENCES

Ben-Hamida, F. and Schlessinger, D. (1965) J.Bacteriol. 90,1611.
Burstein, C., Cohn, M., Kepes, A. and Monod, J. (1965) Biochim. Biophys. Acta 95, 634.
Cummings, D. J. (1965) Biochim. Biophys. Acta 85, 341.
Goodwin, B. C.(1963)Temporal Organisation in Cells, Acad. Press.
Jacob, F. and Monod, J. (1961) Cold Spring Harbor Symp. Quant. Biol., 26, 193.
Knorre, W. A. (1965) Vortrag auf der Tagung der Biophysikali - schen Gesellschaft der DDR 1965.
Knorre, W. A. and Bergter, F. (1966) Monatsber. der Deutschen Akademie der Wiss. zu Berlin, 8, 127.
Knorre, W. A. (1967) Theoret. u.experim. Untersuchungen zur Biophysik von Regulationsprozessen,Dissertation Leipzig
Knorre, W. A. (1968) studia biophysica, 6, 1.
Magasanik, B. (1961) Cold Spring Harbor Symp. Quant.Biol.,26.249
Masters, M., Kuempel, P. L. and Pardee, A. B. (1964) Biochem. Biophys. Res. Comm. 15, 38.
Monod, J. and Jacob, F. (1961) Cold Spring Harbor Symp. Quant. Biol. 26, 389.
Palmer, J. and Moses, V. (1968) Biochem. J. 106, 339.
Pardee, A. B., Jacob, F. and Monod, J. (1959) J.Mol.Biol. 1,165.