Effect of Temperature on the Differential Rate of Synthesis of Proteins of the Lactose Operon in E. coli*

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In order to obtain high concentrations of thiogalactoside transacetylase as a preliminary to purification of the enzyme from E. coli, the effect of growth conditions on the relative quantity of enzyme has been studied. As a matter of interest, assays for β-galactosidase were also carried out, because the synthesis of both enzymes is subject to the same genetic control affecting the lactose operon as a unit (Jacob and Monod, 1961). Data are presented here showing an unequal effect of temperature during growth on the relative rate of synthesis of each of these proteins.

A culture of a constitutive strain (ML 308) of E. coli in the exponential phase of growth on medium 63 (Herzenberg, 1959)and 0.4 per cent succinate was divided into two portions. One was continued at 37° and the other placed at 30° and growth was continued with shaking in air. Aliquots were removed at the times shown in Figure 1, the cells were harvested by centrifugation, suspended in a small volume of 0.05 M potassium phosphate buffer, pH 7.2, and treated for five minutes at 10° in the 10 kc Raytheon sonic oscillator. The broken cell suspensions were used directly for enzyme assays; β-galactosidase was measured by hydrolysis of o-nitrophenyl galactoside (Horiuchi et al., 1962) and thiogalactoside transacetylase was determined with alkaline hydroxylamine (Zabin et al., 1962). Protein was determined by the Lowry method (Lowry et al., 1951).

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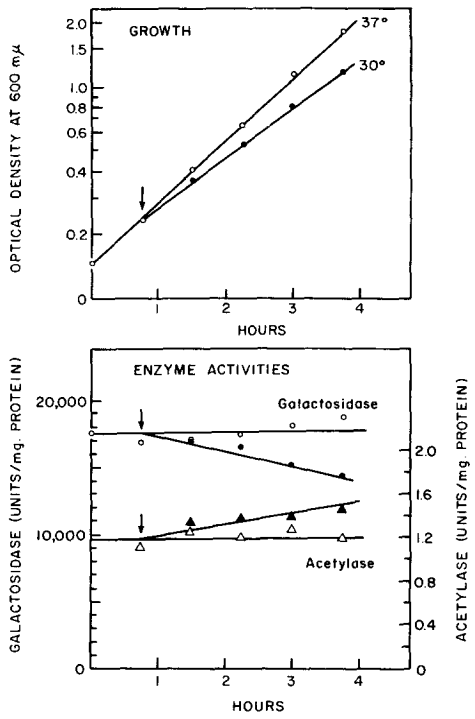

Figure 1. Change in growth rate and enzyme activities in E. coli ML 308 when temperature was changed from 37° to 30°. At the time indicated by the arrow, a culture growing at 37° was divided and one portion continued at 37° while the other was placed at 30°. -O- and -Δ- represent 37°; -●- and -▲- represent 30°.

It may be seen that in cells transferred to the lower temperature, the relative rate of synthesis of β-galactosidase became less, but that of thiogalactoside transacetylase was increased. Thus, the formation of each of the two coordinately expressed enzymes (Jacob and Monod, 1961) is affected in an opposite manner by change in temperature. As expected, the levels of these enzymes in cells maintained at 37° stayed constant.

Support for the belief that the measurements of enzyme activities are equivalent to measurements of enzyme concentrations and are not due to inhibition or activation at each temperature was obtained from the following experiments. Cells were grown at 37°, harvested, suspended in fresh medium without succinate,

and one half held at 37° and the other at 30° for one hour without shaking. At the end of this time, the cells were disrupted and assayed for each of the enzymes in the usual way. No differences were observed between the two halves, nor were any differences detected when another culture grown at 30° was divided and tested in the same manner. Also, when a broken cell preparation obtained from a culture grown at 37° was mixed with one derived from a culture grown at 30°, the activities of the mixture were identical to the sum of each.

Table I

Relative Quantities of β-Galactosidase and Thiogalactoside Transacetylase as Per Cent of Total Protein in ML 308

Temperature during growth	β-Galactosidase %	Thiogalactoside Transacetylase %	Ratio β-Gal: acet
37°	5	0.15	33:1
30°	3	0.2	15:1

Cells in the exponential phase of growth at 37° or at 30° on succinate-mineral medium were harvested and treated for 5 minutes in the sonic oscillator. Doubling times were about 1 hour. Enzyme activities were determined under standard conditions on the complete sonicate. Per cent of enzyme was calculated by comparison of the specific activities obtained to those for the pure enzymes, using the Lowry method for protein determinations.

Table I shows the average of results of a number of experiments comparing the relative amounts of the two enzymes in cultures grown at 37° and at 30°. The quantity of thiogalactoside transacetylase was calculated by comparison of specific activities of the enzyme in cell sonicates to that of the crystalline

material (Zabin, 1963). The same calculations were carried out for β-galactosidase purified and crystallized in this laboratory. The enzyme content in cells grown at 37° were found to be in agreement with values previously reported (Cohn, 1957). It may be seen that the ratio of the two enzymes in cells grown at the usual temperature was about 33:1, and that this decreased to 15:1 when cultures were grown at 30°. The change in ratio was by a factor of about 2. Similar results were obtained with an inducible strain (ML 30) in the presence of 5×10^{-4}M isopropyl-β-D-thiogalactoside as inducer.

According to the Jacob-Monod model, the site of control for expression of genetic information in an operon resides at the stage of transcription, i.e., the formation of m-RNA. The differential effect of temperature recorded here is most easily interpreted as an effect at the translation stage, where m-RNA is utilised, and therefore indicates the existence of modifying influences at this level on the biosynthesis of protein.

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