KINETIC STUDIES OF β-GALACTOSIDASE INDUCTION

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ABSTRACT The kinetics of β-galactosidase induction in *E. coli* ML 3 have been studied. Following addition of inducer, the rate of enzyme synthesis accelerates from the uninduced to a steady-state rate. At saturating concentration of inducer the time constant (T_o) for this process is 2.5 to 3 minutes. With decreasing inducer concentration (I), increasing time constants are observed. $I/I+K'$ approximates $1/T_o$. The steady-state rate of β-galactosidase synthesis is approximated by I^2/I^2+K^2. K' and K have been estimated for IPTG and TMG. The kinetics of β-galactosidase production after the removal of inducer by dilution or after the addition of glucose have been investigated. A transition time of 2.5 to 3 minutes is observed before enzyme synthesis slows or stops. These results are consistent with the hypothesis that the enzyme-forming unit is unstable.

INTRODUCTION

The time course of enzyme synthesis following the addition or removal of an inducer provides one of the most important clues to the mechanism of induction. Analysis of induction kinetics has been used frequently to support one or another model of the process (Monod, 1958).

Enzyme synthesis, however, comes after a long and complex sequence of events, any one of which may be rate-limiting. Thus, in some of the early experiments the enzyme was needed to release energy for its own production. Accordingly the time course had predominantly exponential features. This complication can be avoided by the use of inducers which are not substrates, together with an energy supply which is not influenced by the enzyme. The heterogeneous response of the population to induction at less than saturating concentrations of inducer causes another distortion of the induction curve (Novick and Weiner, 1957). This can be eliminated by the use of cells, "cryptic mutants," which lack the concentrating system (Herzenberg, 1959; Cohn and Horibata, 1959*b*).

When these precautions are followed the rate of enzyme synthesis is rapidly

responsive to changes in the inducer concentration. In fact the response is so rapid that Herzenberg found "no trace of an autocalatytic tendency" (Herzenberg, 1959). This rapidity of the response was cited by Monod as evidence that enzyme induction involved activation of existing templates rather than the synthesis of new ones (Monod, 1958). However, the concept of existing (but not activated) stable enzyme forming units soon encountered serious difficulties in explaining the results of mating experiments (Pardee, Jacob, and Monod, 1959). In these experiments enzyme synthesis achieved its optimal rate within a few minutes after the introduction of DNA from genetically competent cells into genetically incompetent recipients.

The evidence for immediate induction was derived from experiments in which samples were taken at 10 to 20 minute intervals. Accordingly it seemed desirable to repeat these experiments using the better time resolution which can be obtained simply by taking more frequent samples. The results show that a short but readily measurable time interval is, in fact, required before the cells reach their final rate of synthesis. The data support the concept of unstable enzyme-forming units which are rapidly created upon the addition of the inducer.

After these experiments were completed, a paper by Pardee and Prestidge appeared which described a similar series of experiments (Pardee and Prestidge, 1961). Our results are in general agreement with theirs, and provide additional data.

METHODS

Strain. *E. coli* ML 3, a galactoside permease–negative strain of *E. coli* ML 30, was used throughout these experiments.

Medium and Growth Conditions. All experiments were performed with exponentially growing cells vigorously aerated and maintained at 37°C. C medium of the following composition was the only medium employed: 2 gm NH_4Cl, 6 gm Na_2HPO_4, 3 gm KH_2PO_4, 3 gm NaCl, 0.01 gm Mg as $MgCl_2$, 0.026 gm S as Na_2SO_4, 10.0 ml 10 per cent maltose, and 900 ml distilled H_2O. Cell density was measured by absorption at 650 mμ using the Beckman DU spectrophotometer. One mg wet weight of cells per ml gives an absorption of 0.400.

Enzyme Assay and Induction of β-Galactosidase. β-galactosidase was determined by the rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside (ONPG). A unit of enzyme is defined as producing 0.1 μM of *o*-nitrophenol per minute at pH 7.2. The thiogalactosides, isopropyl-thio-β-D-galactoside (IPTG) and methyl-thio-β-D galactoside (TMG), both gratuitous inducers, were used to induce the synthesis of β-galactosidase. The inducer was added to exponentially growing cultures at the desired time and concentration. Periodically samples were removed and added to tubes containing 2 drops of toluene. The tubes were then shaken at 37°C for 30 minutes prior to enzymic assay. Under the conditions employed, no correction for the inhibitory effect of the inducers on the enzyme activity was necessary. The IPTG and the TMG used were gifts of Dr. M. Cohn.

RESULTS

General Characteristics of Induction Kinetics. The addition of IPTG or TMG to an exponentially growing culture of *E. coli* ML 3 results in the synthesis of β-galactosidase at an accelerated rate. In Fig. 1 the time course of β-galactosidase induction at a saturating concentration of IPTG is given. The response to the inducer is rapid so that an increase in enzymic activity may be detected within 15 to 30 seconds.

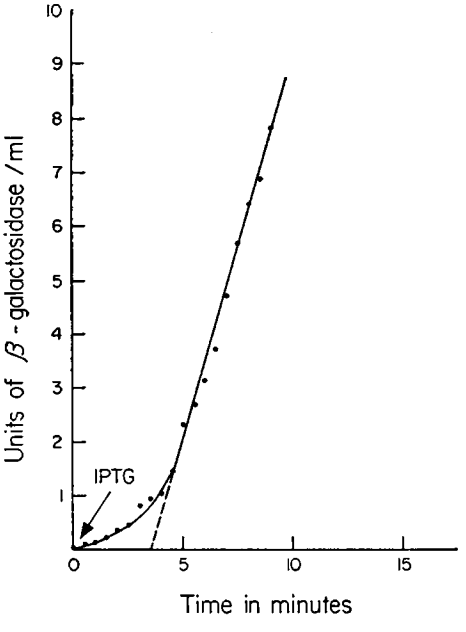

FIGURE 1 Time course of β-galactosidase induction for *E. coli* ML 3.5 × 10^{-4} M IPTG employed as inducer.

The time constant (T_c) of the transition to the maximum rate of enzyme synthesis from the uninduced level may be obtained by extrapolating the linear portion of the curve to the abscissa (time axis). For the experiment shown in Fig. 1 the time is 3.5 minutes. Slight variations in this time are observed from experiment to experiment with the average being 3.0 minutes. *Times of less than 2.5 minutes have never been observed even when IPTG at a concentration of 10^{-1} M was employed.*

Similar induction kinetics have been observed for ML 30 and 15 TAU⁻, two galactoside permease–positive strains of *E. coli,* using saturating concentrations of inducer.

Effect of Inducer Concentration on Induction Kinetics. Both the steady-state rate of enzyme synthesis after induction and the time required to reach this steady-state rate are dependent on inducer concentration. In Fig. 2 β-galactosidase induction for several concentrations of IPTG is given. As the inducer concentration is decreased from a saturating concentration of inducer the steady-state rate of

enzyme synthesis decreases. The time required to reach this rate, on the other hand, increases with decreasing inducer concentration. In Fig. 2 the quantity of enzyme is plotted against bacterial mass. This procedure is convenient to determine accurately the steady-state rates of enzyme synthesis especially in those cases in which measurements must be made over several generations.

The time constant, T_c, may be obtained from such a plot by extrapolating the linear portion of the curve to the basal enzyme level and converting the corre-

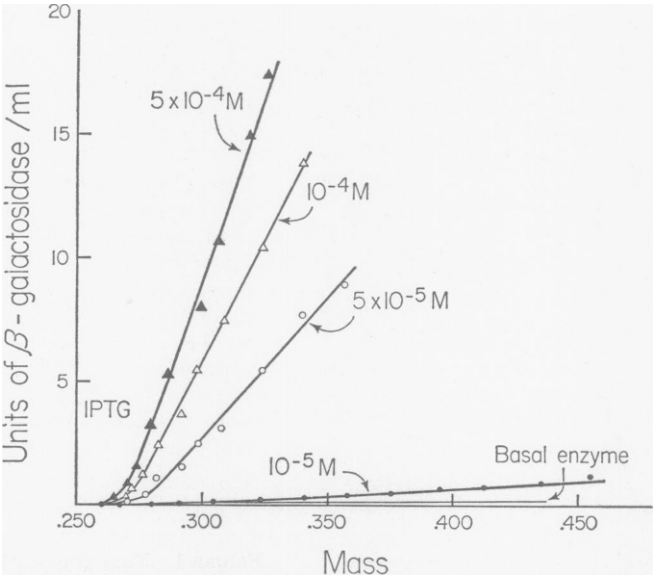

FIGURE 2 Induction of β-galactosidase at several concentrations of IPTG. Basal enzyme is the uninduced level. Bacterial mass is given as the absorption at 650 mμ. 1 mg wet weight of cells per ml corresponds to an absorption of 0.400.

sponding change in bacterial mass to time. When the steady-state rate of β-galactosidase synthesis is fast compared to the basal rate, extrapolation to either the abscissa or the basal enzyme level gives approximately the same constant for induction. However, when the rate is slow, extrapolation must be made to the basal enzyme level rather than to the abscissa to avoid considerable error in determining the time constant.

In Fig. 3 the effects of various IPTG concentrations on the time constant and on the steady-state rate of β-galactosidase synthesis are summarized on a log-log plot. This is a convenient procedure for determining the relationship between these two parameters and the inducer concentration. In each case the experimental curves were compared with theoretical curves describing various functions of I, the inducer concentration.

A function of I that fits the experimental data on the effect of IPTG concentration

on the reciprocal of the time constant is $I/I + K'$. From the slope of the curve K' may be shown to be 5×10^{-5} moles per liter. A fit for the data showing the effect of IPTG concentration on the steady-state rate of enzyme synthesis is given by the function $I^2/I^2 + K^2$ where K is 1×10^{-4} moles per liter.

In Table I, the effects of TMG concentration on the rate of enzyme synthesis and on the time constant are summarized. A log-log plot of these data shows that at high TMG concentration the rate of β-galactosidase is approximated by $I^2/I^2 +$

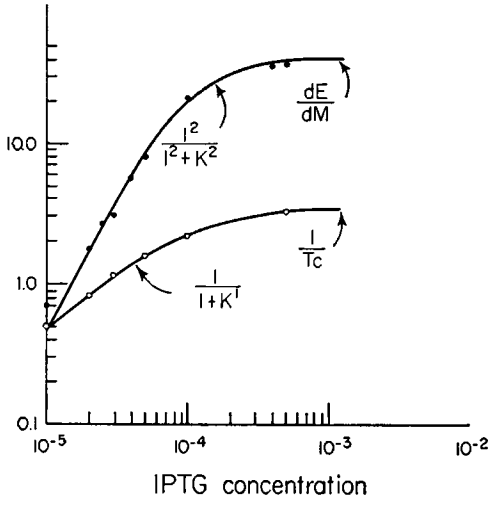

FIGURE 3 Log-log plot of the effect of various IPTG concentrations on the steady-state rate of β-galactosidase synthesis and on the time constant. Experimental data presented as circles. Solid circles describe effect on rate of synthesis. The corresponding line is theoretical curve of $I^2/I^2 + K^2$. Open circles describe the effect of inducer concentration on $1/T_c$ with the corresponding line that of $I/I + K'$. IPTG concentration is moles per liter.

K^2 where K equals 3.5×10^{-3} moles per liter but deviates from the theoretical curve at low inducer concentration. The reciprocal of the time constant fits the equation $I/I + K'$ where K' is 3×10^{-4} moles per liter.

Kinetics of Enzyme Production after Removal of Inducer. The synthesis

TABLE I

THE EFFECT OF TMG CONCENTRATION ON THE STEADY-STATE RATE OF β-GALACTOSIDASE SYNTHESIS AND ON THE TIME CONSTANT OF INDUCTION

TMG	dE/dM*	Tc min.
1×10^{-3} M	39.9	3
2×10^{-3} M	11.7	3
1×10^{-3} M	5.1	4
1×10^{-4} M	0.34	12
5×10^{-5} M	0.16	22

* dE/dM = steady-state rate of β-galactosidase synthesis, given as the change in the number of enzyme units per increase of 250 μg wet weight of cells.

of β-galactosidase at induced rates stops after the removal of the inducer. However, the cessation of enzyme production is not immediate, as previously reported (Cohn, 1957); there is a short transition period after the removal of inducer before enzyme synthesis at induced rates ceases. The transition is conveniently studied using a culture of *E. coli* ML 3 in which the inducer concentration is suddenly decreased to a low level by dilution. In Fig. 4 the time course of enzyme production following dilution of the IPTG concentration from 3×10^{-5} M to 1×10^{-5} M is given. This dilution causes the rate of enzyme production to decrease by a factor of 4. A transition period of approximately 3 minutes is observed before the new rate of synthesis is established. There is no preinduction effect (Monod, 1956). The rate

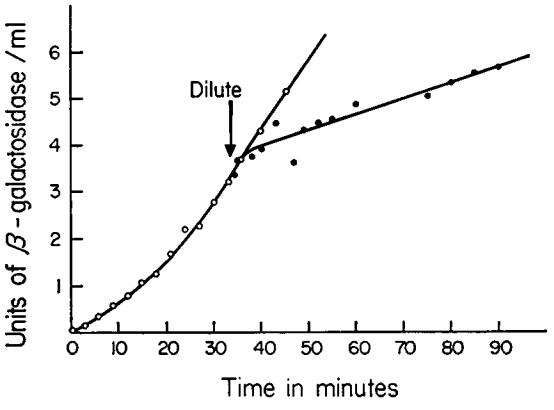

FIGURE 4 The kinetics of β-galactosidase production after dilution of the inducer concentration. Induction was initiated by the addition of 3×10^{-5} M IPTG at T = 0. After 34 minutes, an aliquot of the culture was diluted threefold. Solid circles are measures of the enzyme production in the diluted culture. Values have been multiplied by 3 for presentation on this graph.

established in the presence of 1×10^{-5} M IPTG after dilution is the same as that observed when the induction is initiated by the addition of that concentration of inducer to an uninduced culture of cells.

The time during which the rate of enzyme synthesis deaccelerates from one rate to another is independent of the time required to reach the faster rate from a lower one, thus suggesting different mechanisms for the two processes. For example, a transition time of approximately 3 minutes is observed when the concentration is diluted from 3×10^{-5} M to 1×10^{-5} M (Fig. 4). However, a period of about 8 minutes is required to achieve the faster rate when the IPTG concentration is raised from 1×10^{-5} M to 3×10^{-5} M.

The Glucose Effect. The stock of *E. coli* ML 3 used in these experiments is "glucose-insensitive," *i.e.*, galactosidase may be induced to the maximal level in a culture growing exponentially on glucose. The kinetics of induction, however, are altered by the presence of glucose.

If 10^{-2} M glucose is added simultaneously with 5×10^{-4} M IPTG, an effect on the time constant of induction but not on the final steady state rate of enzyme production, is observed (Fig. 5). The time constant for induction is lengthened from 3 minutes to approximately 13 minutes by the addition of glucose. By decreasing the inducer concentration from 5×10^{-4} M to 5×10^{-5} M, an effect of glucose not only on the time constant but also on the steady-state rate may be observed.

If glucose is added after the induced synthesis of β-galactosidase has been initiated, enzyme production stops or slows for a short time and then returns to the maximal rate. In one of the experiments shown in Fig. 5, 10^{-2} M glucose was

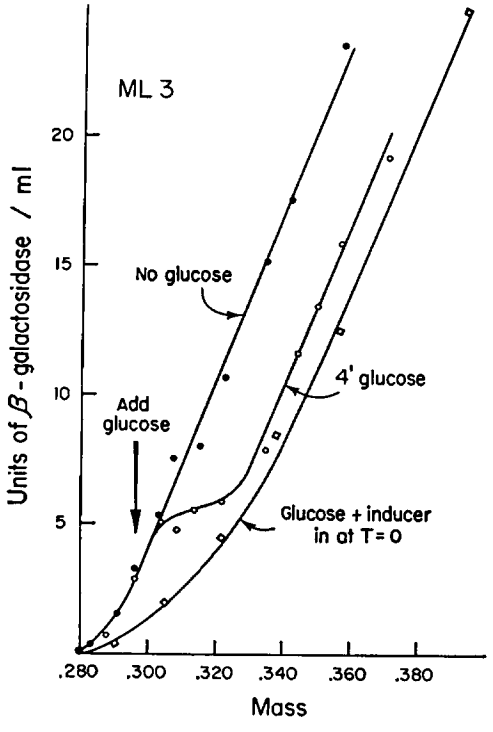

FIGURE 5 The effect of glucose on β-galactosidase induction. The induction was initiated by the addition of 5×10^{-4} M IPTG at T = 0 (mass = 0.280). 10^{-2} M glucose was added simultaneously with inducer (□) or 4 minutes after the inducer (○). No glucose was added in the control (●) Bacterial mass is given as absorption at 650 mμ. 1 mg wet weight per ml corresponds to an absorption of 0.400.

added 4 minutes after the addition of 5×10^{-4} M IPTG. The rate of enzyme production fell, after the addition of the glucose, to one-eighth the maximal rate with a transition time of about 2.5 minutes. Six minutes later the maximal rate was reestablished.

The slowing down of β-galactosidase synthesis after the addition of glucose is not due to a general effect on the culture. Growth as measured by absorption continues at the same rate and the incorporation of radiosulfur into proteins is unaffected. Also the reestablishment of the maximal rate of synthesis is not due to depletion of the glucose. It is present in a concentration high enough to provide growth for several generations. The time required for the enzyme production to

slow to the new rate is the same as that observed after the removal of inducer by dilution (Fig. 4).

DISCUSSION

The time course of induction at any particular concentration of inducer measures two quantities of theoretical interest; the final steady-state rate of enzyme synthesis (S) and the time constant of the transition to the new rate (T_c). In the mutant ML 3 the rate does not depend on the past history of the cells.

When the rates are plotted as a function of the inducer concentration a saturation curve results (Fig. 3). For the inducer IPTG,

$$S \sim I^2/I^2 + K^2$$

This relationship indicates that reactions requiring two molecules of inducer determine the rate of enzyme synthesis.

The cause of the delay in enzyme synthesis is not immediately obvious. Képès has studied the time required for the accumulation of inducers in various strains of *E. coli* (Képès, 1960). With ML 3 blocked by chloramphenicol the time constant for the accumulation of TMG at 26° was ~ 6 minutes. In his experiments it was necessary to wash the cells so that his results pertain only to that fraction of the TMG which was not readily removed by washing. Pardee and Prestidge, on the other hand, have carried out numerous experiments which indicate that the time required for the entry of the inducer does not limit the rate of enzyme induction (Pardee and Prestidge, 1961).

In the experiments reported here it is found that the time constant T_c varies with the concentration of the inducer as shown in Fig. 3:

$$\frac{1}{T_c} \sim \frac{I}{I + K'}$$

Such a relationship would not be expected if the delay in reaching the final rate of enzyme synthesis were due to the time required for the inducer to penetrate the cell. If this were the case no delay should be observed at high or saturating concentrations of inducer. Furthermore, higher concentrations of inducer should be required to give minimal delays than are required to give maximal rates of synthesis. In our experiments concentrations of inducer which give minimal delays are not sufficient to produce maximal rates of synthesis. It is concluded, in agreement with Pardee and Prestidge, that the entry of the inducer is not a rate-limiting factor.

When the steady-state rate of enzyme synthesis is reduced by dilution of the inducer or by the addition of glucose, the time constant of the transition to the lower rate is 2.5 to 3 minutes. This time constant is the same as the time constant for induction at saturating concentrations of inducer. For these transitions the time constant should be determined by the time constant for the decay of the enzyme-

forming unit. This is in agreement with Pardee and Prestidge that the enzyme-forming units are unstable. These results indicate that the enzyme-forming units decay with a time constant of 2.5 to 3 minutes.

The rate of enzyme synthesis and the time constant for induction show quite different dependences on the kind of inducer employed (whether TMG or IPTG) and on the concentration of these inducers. It therefore seems necessary to envisage that induction involves two different reactions involving the inducer molecules. One of these reactions influences the rate of synthesis and the other affects only the time constant.

A further indication of the existence of two separate reactions is found in the temporary inhibition caused by glucose. The repression is of brief duration and quite unlike the permanent repression usually reported (Cohn and Horibata, 1959*a*) Képès observed that glucose caused a reduction in the level of intracellular TMG (Képès, 1960). However, he found no indication that the original level was reestablished. Thus his findings might pertain more to the situation in which the repression was permanent. Alternatively the addition of glucose might cause the temporary accumulation of a repressive derivative, but such a product would not be expected to be completely removed after a short time interval even though an ample supply of glucose remained. It seems more likely that the glucose affected the reaction controlling the time constant without effect on the reactions controlling the final rate.

Dr. Boezi is a postdoctoral fellow of the National Science Foundation, 1960-1962.
Received for publication, July 26, 1961.

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