SOME EFFECTS OF INDUCER ON SYNTHESIS AND UTILIZATION OF β-GALACTOSIDASE MESSENGER RNA IN ACTINOMYCIN-SENSITIVE ESCHERICHIA COLI

Loretta Leive

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, U.S. Public Health Service
Bethesda, Maryland

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Induction of β-galactosidase in Escherichia coli, according to currently available evidence, results in the accumulation of a specific unstable messenger RNA. Thus, RNA synthesis after inducer addition is necessary for enzyme production (Nakada and Magasanik, 1964), induced cells contain increased levels of RNA hybridizable with the lactose operon (Hayashi et al, 1963), and inducer removal is followed by exponential decay in ability to produce the enzyme (Kepes, 1963; Nakada and Magasanik, 1964). The mechanism by which inducer addition produces this increased amount of messenger RNA is incompletely understood.

The effect of inducer on various steps in enzyme production can be studied if RNA synthesis is halted. Actinomycin D is an inhibitor of RNA synthesis (Reich and Goldberg, 1964) but normally does not affect E. coli (Hurwitz et al, 1962); however, this organism can be made sensitive to actinomycin by treatment with ethylenediaminetetraacetate (Leive, 1965a). The present experiments use this technique to study the time course of production of β-galactosidase messenger RNA, and the effect of inducer on its utilization and destruction.

The results indicate that the synthesis of non-DNA bound β-galactosidase messenger RNA requires 2.5 minutes of the 4 minute period between induction and appearance of active enzyme. In addition, as in the case of histidase induction in Bacillus subtilis (Hartwell and Magasanik, 1964), the rate of destruction and utilization of β-galactosidase messenger RNA is

independent of the presence or absence of inducer.

Materials and Methods - E. coli AB 1105 (β-galactosidase-inducible, permease negative) was grown with aeration at 37° on the previously described minimal medium buffered with Tris-Cl, with 0.25% glycerol as the carbon source (Leive, 1965a). The incubation temperature was changed to 30° 1-2 hours prior to the beginning of the experiment. All subsequent incubations were at this temperature.

Cells were sensitized to actinomycin as described previously (Leive, 1965b): they were harvested at a density of 2-4 x 10^8 cells/ml, washed once at room temperature with 0.12 M Tris-Cl, pH 8, and resuspended in the same buffer. Ethylenediaminetetraacetate (EDTA) was added at the indicated concentration and the suspension incubated for 2 min at 30° with aeration. Growth medium (10 volumes) was then added to terminate the EDTA treatment, and incubation with aeration was continued for 5 - 6 minutes before the experiment was begun. Cells that have been treated in this manner will hereafter be referred to as sensitized cells.

Actinomycin D was the gift of Dr. A. Patchett of Merck, Sharp and Dohme and was used at a final concentration of 10 μg/ml.

β-Galactosidase was induced by addition of 5 x 10^{-4} M isopropylthiogalactoside (IPTG). Induction was terminated either by diluting the cells 70-fold into inducer-free medium (Kepes, 1963) or by filtering on a Millipore filter (0.45 mμ pore size) at room temperature, washing with 10 volumes of inducer-free medium, and resuspending in inducer-free medium at 30° (Nakada and Magasanik, 1964).

For the β-galactosidase assay, 1.2 ml portions of the cell suspension were shaken with approximately 0.05 ml toluene for 45 min at 37°, both to prepare the cells for the assay (Pardee et al, 1959) and to remove actinomycin, which is extracted into the toluene. The removal of actinomycin is necessary because it absorbs strongly at the same wavelength as the assay product, o-nitrophenol. The toluene was then removed, and aliquots of the cells were assayed for β-galactosidase according to the method of

Pardee et al (1959). Because the concentrations of enzyme were low, the incubation was for 15-17 hours; preliminary experiments indicated that the assay was linear for at least 22 hours.

Results and Discussion - It was first necessary to determine whether the course of induction of β-galactosidase is normal in sensitized cells. When IPTG was added to such cultures, β-galactosidase production began 4 minutes later; within 1 minute the maximal rate of synthesis was achieved. These kinetics are very similar to those described for induction in non-sensitized cells of other strains under various growth conditions (e.g., Pardee and Prestidge, 1961). This similarity supports the assumption that the events following induction in sensitized cells resemble those occurring in non-sensitized cells. In addition, it had been previously determined that sensitized cells synthesize RNA and protein at a virtually normal rate (Leive, 1965b), and can support the multiplication of phage T4 (Korn, Protass and Leive, 1965). It is therefore reasonable to assume that control mechanisms and protein synthesis are normal in such cells. Sensitized cells were then used in the following studies:

(1) Does inducer affect the destruction or utilization of messenger RNA?

The addition of actinomycin to a sensitized culture of E. coli results in an immediate cessation of RNA synthesis (Leive, 1965b) and, if the culture has been induced for β-galactosidase, the ability to make this enzyme declines (Leive, 1965a). To determine whether the rate of this decay is affected by inducer, an induced sensitized culture was filtered, washed, and resuspended in actinomycin-containing medium with and without IPTG. Figure 1 shows that both the half-life of the decay in enzyme production, and the final amount of enzyme produced, is independent of the presence or absence of inducer. In this experiment, the half-life of decay was 2.4 minutes. In other experiments it was 1.8-2.4 minutes.

It has been inferred, from previous work (Nakada and Magasanik, 1964; Kepes, 1963), that the loss of β-galactosidase-synthesizing capacity, when inducer is removed, reflects destruction of specific messenger RNA.

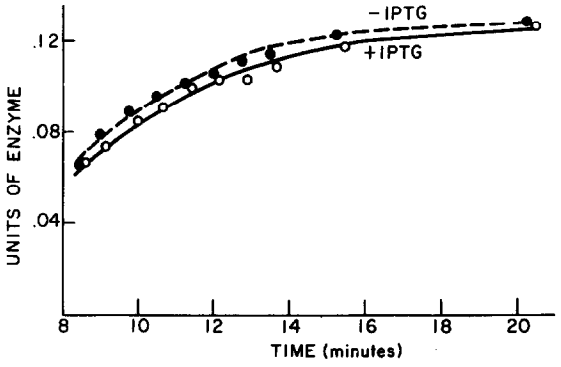

Fig. 1. Decline in synthesis of β-galactosidase in actinomycin-treated cells in the presence and absence of inducer. Cells were sensitized as described in the text at a concentration of 1 x 10^9 cells/ml using 2 x 10^{-4} M EDTA. The sensitized cells (20 ml; 1 x 10^8 cells/ml) were induced at 0 time and inducer removed by filtration at 5.5 minutes. The washed cells were resuspended in 3 ml of medium and 1 ml aliquots added to 10 ml of warm actinomycin-containing medium with or without inducer. These manipulations were complete by 7.5 minutes. At various times thereafter, 1 ml samples were removed to chilled tubes containing toluene. The samples were shaken at 37° and duplicate 0.4 ml samples assayed for β-galactosidase as described in the text. The final concentration of cells after filtration and resuspension was approximately 5 x 10^7 cells/ml. The units of enzyme given are per 0.4 ml of culture.

If this inference is correct, the present results indicate that inducer does not extend the half-life of β-galactosidase specific messenger RNA, and also does not accelerate its utilization in protein synthesis. The same conclusions were reached by Hartwell and Magasanik (1964) on the basis of studies of histidase induction in B. subtilis. Results such as these are consistent with the hypothesis of Jacob and Monod (1961) that inducer stimulates formation of specific messenger RNA. They do not distinguish whether inducer directly catalyzes initiation of new messenger RNA molecules, as implicit in the "operator" model (Jacob and Monod, 1961) or whether it causes release of messenger RNA molecules from the DNA, thus permitting further new messenger RNA synthesis (Ames and Hartman, 1963; Stent, 1964).

(2) How long does it take to produce an actinomycin-insensitive messenger RNA molecule?

As discussed above, current evidence, including the present experiments, favors the following two postulates: (a) inducer in some manner stimulates formation of specific messenger RNA (b) actinomycin prevents this formation but does not affect action of preformed messenger RNA. If these postulates are correct, one may predict that the addition of actinomycin after inducer has catalyzed the initiation of a messenger RNA molecule, but before the molecule is complete and released from the DNA, should prevent formation of functional messenger RNA and thus prevent subsequent formation of active enzyme. This prediction enables an experimental determination of the length of time required to synthesize and release a messenger RNA molecule.

For this experiment, induction was stopped by diluting the culture (Kepes, 1963) since this manipulation is more rapid than filtration and washing. Under these conditions Kepes (1963) found that the final yield of enzyme was proportional to the time before dilution, indicating that the inducer-catalyzed event occurs with no lag after inducer addition. This result is confirmed for sensitized cells under the present experimental conditions in Figure 2, curve (A). Inducer was added to a culture at time zero, aliquots were diluted 70-fold into inducer-free medium at the times indicated and were incubated 30 minutes longer to allow complete expression of their enzyme-forming capacity. As in the experiments of Kepes (1963), enzyme formation was proportional to time of exposure to inducer, the level of enzyme extrapolating to basal enzyme level at zero time.

A very different result was obtained if the medium used for diluting the induced culture contained actinomycin (Figure 2 curve (B)). Under such conditions cells exposed to inducer for up to 2.5 minutes are incapable of producing enzyme. Thus the inducer-catalyzed event is completely sensitive to actinomycin for 2.5 minutes after inducer addition. This result indicates that, of the 4 minute lag before enzyme production in this strain under these conditions, 2.5 minutes are required for synthesis of non-DNA bound β-galactosidase messenger RNA.

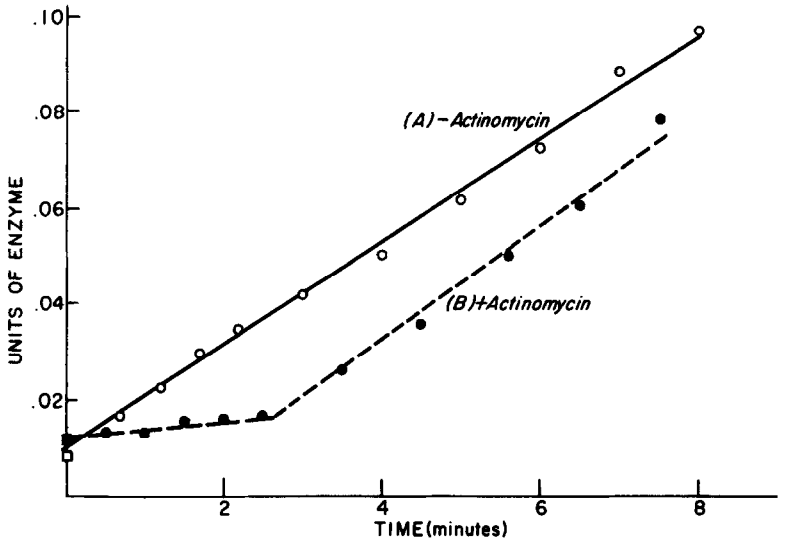

Fig. 2. Effect of actinomycin on the amount of β-galactosidase synthesized as a function of time of exposure to inducer. Cells were sensitized as described in the text at a concentration of 1 x 10^{10} cells/ml using 5 x 10^{-4}M EDTA. The sensitized cells (4 ml; 1 x 10^{9} cells/ml) were induced at 0 time. Just before addition of inducer and at the indicated times thereafter samples were diluted 70-fold into warm, inducer-free medium with or without actinomycin. The diluted samples were then allowed to incubate with shaking for an additional 30 minutes to allow them to produce their full complement of induced β-galactosidase. Duplicate 1.2 ml portions were shaken with toluene and 1 ml of each assayed as described in the text. The units of enzyme given are per 1 ml aliquot. The squares indicate the enzyme level in cells diluted prior to induction; i.e., basal enzyme level. Closed square and circles: diluted into medium + actinomycin. Open square and circles: diluted into medium - actinomycin.

Summary - The initial events in β-galactosidase induction were studied in Escherichia coli made sensitive to actinomycin by brief treatment with ethylenediaminetetraacetate. Experiments performed in the presence and absence of actinomycin or inducer lead to the following conclusions: (a) The synthesis of specific, non-DNA bound messenger RNA requires 2.5 minutes of the 4 minute interval between inducer addition and enzyme production. (b) The rate of destruction and the rate of utilization of this messenger RNA are independent of the presence or absence of inducer.

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