ULTRAVIOLET LIGHT INHIBITION OF GALACTOSIDE PERMEASE INDUCTION

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Pardee and Prestidge (1963) have stated that the inactivation by ultraviolet light of galactoside permease induction seems to be an all-or-none phenomenon in each damaged cell whereas the inactivation of β-galactosidase induction progressively increases in each bacterium with increasing ultraviolet dose. This suggestion is difficult to reconcile with the concept of a single galactoside operon, the structural genes of galactoside permease and β-galactosidase being part of this operon, controlled by a single operator as proposed by Jacob and Monod (1961) unless there is a high probability of a direct effect of the ultraviolet light on the structural gene of the permease but not on the β-galactosidase structural gene.

Cells in which the capacity for galactoside permease induction has been completely inhibited while the capacity for β-galactosidase induction has been only partially repressed should behave phenotypically as cryptic cells. Inducing such cells under conditions where the permease system is superfluous should result in a rate of β-galactosidase synthesis proportional to the non-repressed capacity of the cells for β-galactosidase induction.

The present experiments do not support the contention that the inactivation of galactoside permease by ultraviolet light is an all-or-none phenomenon.

MATERIALS AND METHODS

E. coli strains K12, wild type, and K12/37, a cryptic mutant, were used in all experiments. Strain K12 was originally secured from ATCC #10798 and has been maintained in this laboratory for several years. Strain K12/37 was isolated recently in this laboratory from the wild type. The culture medium employed was a synthetic one containing 0.2% $(NH_4)_2SO_4$, 1.4% K_2HPO_4, 0.1% $Na_3C_6H_5O_7 \cdot 2H_2O$, and 0.02% $MgSO_4 \cdot 7H_2O$ (Spizizen, 1958) with 0.5% glycerol as the carbon source. The bacteria were grown at 37°C with aeration by shaking or by vigorous stirring.

Bacteria in exponential growth were either harvested by millipore filtration and resuspended in medium at 0°C or simply rapidly chilled. Aliquots of the chilled suspension were irradiated and then used to inoculate fresh medium at 37°C containing inducer. The cell suspensions were irradiated with a Mineralite ultraviolet lamp having 90% of its output at 2537 Å and at a distance such that the average intensity was 130 μwatt cm^{-2}. The ultraviolet output was monitored by a Hanovia UV meter, Model AV-971.

Extracts for the assay of β-galactosidase were made by adding 1 ml of culture to 0.04 ml toluene and 4 ml cold 0.05 M sodium phosphate buffer (pH7) containing 3×10^{-4} M $MnSO_4$, 50 μg sodium desoxycholate, and 2×10^{-3} M mercaptoethanol and immediately emulsifying on a vortex mixer. Before assaying for β-galactosidase, the toluene was evaporated in a water bath at 37°C. β-galactosidase was determined by a modification of Lederberg's method (Lederberg, 1950) utilizing the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) at 25°C. Enzyme units are expressed as mμM of ONPG hydrolyzed per ml of culture per minute at the appropriate temperature. Galactoside permease was determined by the in vivo hydrolysis of ONPG (Herzenberg, 1959: Rickenberg, et al., 1956). The differ-

ential rate of enzyme synthesis is expressed as the enzyme units synthesized per 0.100 increase in optical density at 610 mμ.

Bacterial density was determined turbidimetrically at a wavelength of 610 mμ with a Beckman DU spectrophotometer. An $O.D._{610}$ of 0.100 under these conditions of culture is equivalent to a bacterial dry weight of 56 μg/ml or 43 albumin equivalents as determined by the method of Lowry, et al., (1951).

Thiomethyl-β-D-galactopyranoside (TMG) was used to induce synthesis of β-galactosidase. TMG and ONPG were obtained from the California Corporation for Biochemical Research.

RESULTS AND DISCUSSION

When the bacteria were exposed to ultraviolet light immediately before induction with TMG, the reduction in the overall rate of synthesis of both the galactoside permease and β-galactosidase by the cell population was essentially of the same order. Figure 1 presents the results of one experiment where the inhibition of the rate of permease synthesis, as measured by the in vivo hydrolysis of ONPG, was slightly more than that of the β-galactosidase. These data could reflect either a partial reduction in the capacity of each cell in the population to synthesize the two systems or a complete loss in capacity of a fraction of the cells to synthesize either one or both systems.

It is possible to make at least a partial distinction between these possibilities by comparing the rates of β-galactosidase synthesis with different inducer concentrations. With the cryptic strain under these cultural conditions but without being irradiated, the differential rate of β-galactosidase synthesis with 5×10^{-4} M TMG is only 4% of the maximum rate of the wild type

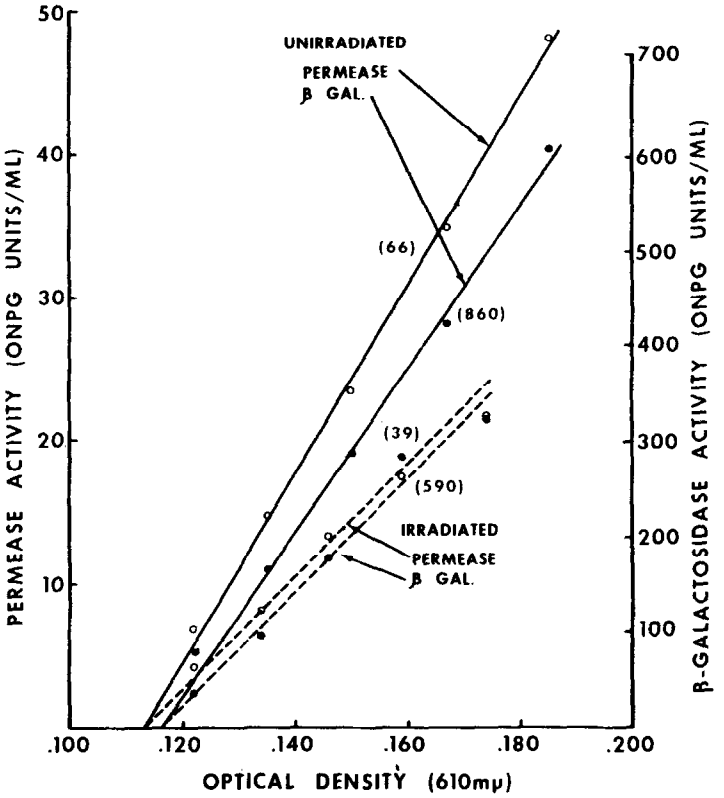

Fig. 1. Differential rate of synthesis of galactoside permease (o-o) and β-galactosidase (•-•) in E. coli K12. Solid lines represent unirradiated aliquots and dashed lines represent aliquots irradiated for 100 seconds. Figures in parentheses are values of differential rate of synthesis. Analysis of enzyme activity carried out at 35°C.

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whereas with 10^{-2} M TMG it is about 80%, indicating that with the high concentration of inducer the permease system is essentially superfluous for β-galactosidase induction. If a fraction of the irradiated cells lacks the capacity for permease induction, these cells should synthesize very little β-galactosidase when induced with $5x10^{-4}$ M TMG but when induced with 10^{-2} M TMG should synthesize the enzyme at a rate commensurate with the residual capacity for β-galactosidase induction and therefore should contribute additional enzyme synthesis in proportion to their relative number.

In Fig. 2 it can be seen that the differential rate of β-galactosidase synthesis is essentially the same at both inducer concentrations and at all doses of ultraviolet light irradiation tested. Therefore, if the ultraviolet light irradiation results in an all-or-none effect on induction it must affect simultaneously the induction of both permease and β-galactosidase. But Pardee and Prestidge (1963) have shown that the inhibition of β-galactosidase synthesis is not an all-or-none phenomenon. Consequently both β-galactosidase and permease production must be progressively repressed to the same extent in each bacterium with increasing ultraviolet dose.

The criterion on which Pardee and Prestidge based their conclusion that lowered permease production in the irradiated cells is an all-or-none phenomenon

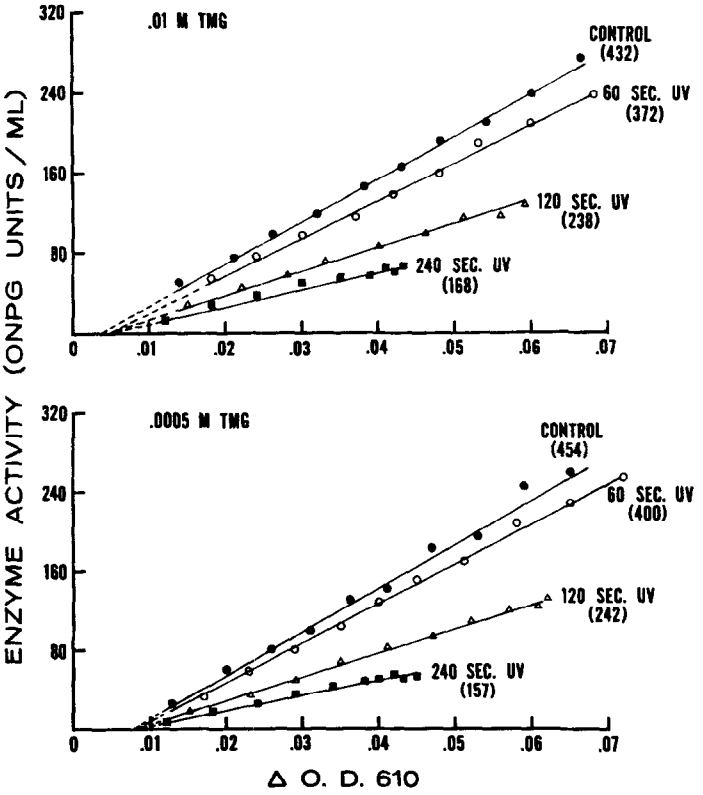

Fig. 2. Effect of inducer concentration on induction of β-galactosidase synthesis in E. coli K12. Analysis for β-galactosidase activity carried out at 25°C. Figures in parentheses represent measured differential rate of enzyme synthesis.

was the failure of these cells to increase their β-galactosidase forming ability in a low concentration of inducer following increased preinduction periods at a high inducer concentration. According to Kepes (1963), β-galactosidase messenger RNA and presumably permease messenger RNA production starts immediately upon addition of inducer and proceeds at a constant rate. Therefore, in the wild type, even a brief exposure to a saturating concentration of inducer may initiate sufficient permease production that increasing the period of exposure to high levels of inducer would not significantly affect enzyme synthesis after the inducer concentration has been lowered.

The only effect of the high inducer concentration evident from the data in Fig. 2 is to reduce the lag period of induction, essentially eliminating the autocatalytic phase of the induction process seen at lower inducer concentrations. Although the ultraviolet light increases the lag period of induction on a time scale, it can be seen from Fig. 2 that on a differential basis there is no apparent increase in lag phase. The increase in lag time is compensated by the decrease in growth rate.

These results suggest that the effect of ultraviolet light on the lag time of induction is not specific to the induction process itself and therefore does not represent catabolite repression, but is merely a reflection of an effect on protein synthesis in general. However, the reduction in the rate of β-galactosidase synthesis is undoubtedly in large part due to catabolite repression (Bowne and Rogers, 1962).

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