DIFFERENTIAL INHIBITION OF β-GALACTOSIDASE INDUCTION AND SYNTHESIS BY DEUTERIUM OXIDE*

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Evidence has recently been presented which suggests that exposure to deuterium oxide under certain conditions may result in structural modifications of DNA in vivo (Dicken et al., 1962; Henderson and Dinning, 1962 a, b). It was hypothesized that these changes may be the cause for the potent inhibition of DNA synthesis which has been observed (Gross and Harding, 1960; Wilson and Dinning, 1961; Dicken et al., 1962), as well as the blockage of cell differentiation which has been reported (Amarose and Czajka, 1962; Hughes et al., 1959). No effect of deuterium on the functioning of DNA in vivo has been reported.

For this reason, we have investigated the effect of inclusion of deuterium oxide in culture media on β-galactosidase formation in Escherichia coli. The induction and synthesis of this enzyme have been shown to be under direct genetic control (Monod et al., 1962), and to depend on the synthesis of a specific messenger RNA from a DNA primer (Eisenstadt et al., 1962).

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MATERIALS AND METHODS

For these studies we have utilized E. coli strain 112 and the basal salts medium of Mandelstam (1962) supplemented with either .1% glycerol or 2% lactose as a carbon source. During assay periods, cell suspensions were incubated in glass stoppered bottles (to prevent deuterium exchange with atmospheric water vapor) and shaken on a Dubnoff shaker maintained at 37°C. The β-galactosidase activity was estimated by a modification of the procedure of Wallenfels et al. (1959). Toluene treated cells (0.1 to 0.5 aliquots) were added to ONPG (1.5 μmol/ml. in M/5 sodium phosphate buffer, pH 7.0 at 28°C.) and the rate of formation of o-nitrophenol measured by 15 second readings at 405 mμ in a Beckman Spectrophotometer. The rate of growth was estimated by absorbance measurements at 600 mμ (one unit of absorbance corresponds to 540 μgm bacteria/ml. dry weight or ca. 480 μgm/ml. bacterial protein). TMG (methyl-β-D-thiogalactopyranoside) and ONPG (ortho-nitrophenyl-β-galactopyranoside) were obtained from Mann Chemical Company, New York.

RESULTS AND DISCUSSION

When cells from overnight stationary cultures were incubated in glycerol-TMG-shake cultures, it was found that the rate of β-galactosidase synthesis reached a peak after approximately 4 hours of incubation. When growth and enzyme activity measurements were made on 4 hour cultures in H_2O and D_2O, a strong inhibition of both growth and enzyme formation was noted in the presence of deuterium (Fig. 1). The formation of enzyme appeared to be even more sensitive to environmental deuterium than was the increase in turbidity, particularly at levels of D_2O greater than 80%. Experiments were then carried out to determine whether it was the induction of the enzyme or the synthesis of the enzyme after induction (or both) which was affected by environmental D_2O.

Fig. 1.
THE EFFECT OF D_2O ON GROWTH AND β-GALACTOSIDASE PRODCTN.

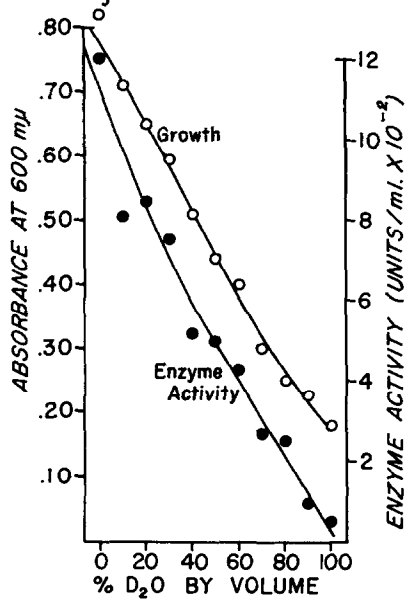

Fig. 1. E. coli 112 cells were grown in .1% glycerol medium for 18 hours and inoculated into fresh medium containing 5 x 10^{-4} M TMG and the indicated level of D_2O so as to give an initial absorbance of 0.05 at 600 mμ. The cultures were incubated 4 hours at 37°C. with shaking in glass stoppered bottles. The figures represent the average of two duplicate experiments.

When induced cells were grown in a H_2O and in a D_2O medium, the rate of enzyme formation per unit of cell mass was found to be essentially the same for the first three hours of incubation (Fig. 2). When TMG was added to non-induced cells suspended in 90% D_2O, a definite inhibition in the rate of enzyme formation per absorbance unit of cells was noted (Fig. 3). An increased inhibition of enzyme induction was noted in the case of cells pre-incubated for 2 hours in 90% D_2O medium before the addition of inducer. Thus it is the induction process which is affected by deuterium rather than the process or enzyme synthesis.

These results suggest certain common features between the capacity for induction of β-galactosidase synthesis and DNA replication leading to cell division, since both are affected very rapidly in cells transferred into

Fig. 2.
THE EFFECT OF D_2O ON β-GALACTOSIDASE SYNTHESIS IN PRE-INDUCED CELLS

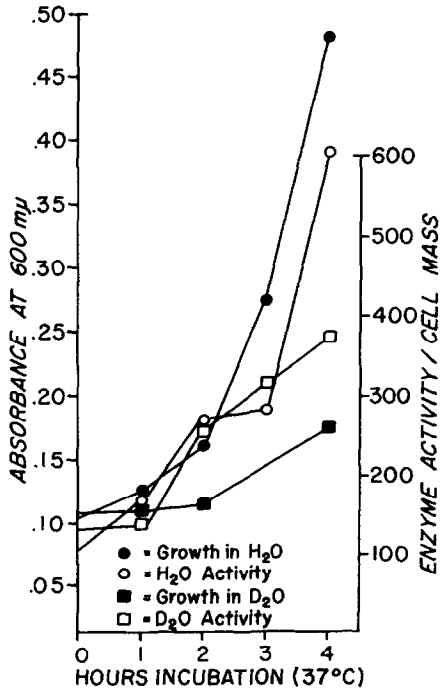

Fig. 2. E. coli 112 cells were grown in 2% lactose medium for 18 hours, washed and suspended in 1% glycerol medium containing 5×10^{-4} M TMG. After being equilibrated in this medium for two hours, this suspension ($A_{600\ m\mu} = 1$) was diluted 1:10 with glycerol-TMG medium in H_2O or in D_2O and incubated with shaking for the indicated time intervals.

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a deuterium environment. Yet it has been shown that β-galactosidase induction is not dependent on DNA synthesis, but on the state of DNA in vivo (Roberts, 1960).

The hypothesis of Platt (1962) that changes in the folding of DNA chains occur during induction periods and result in specific nucleotide sequences being made available for reaction with transcription mechanisms provides an explanation for these findings; changes in the structure of DNA would be required both for replication and induction of enzyme synthesis. Evidence for the alteration of the ordered helix-random coil equilibrium in DNA

Fig. 3.
THE EFFECT OF D_2O ON
β-GALACTOSIDASE INDUCTION

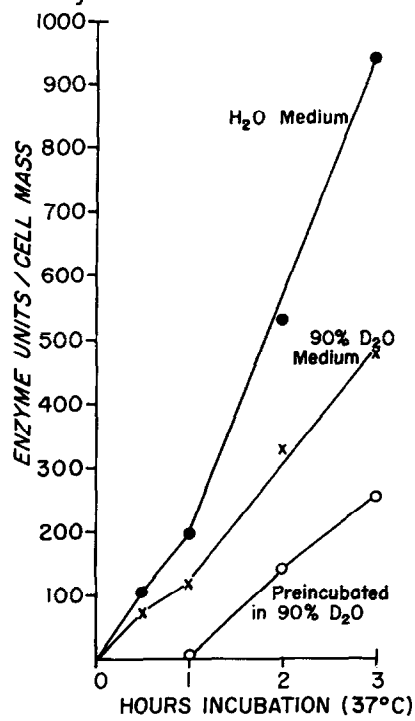

Fig. 3. E. coli 112 cells were grown in shake cultures of 1% glycerol medium in H_2O or in 90% D_2O for 2 hours ($A_{600\ m\mu}$ = 1). An aliquot of the cells preincubated in H_2O medium was diluted 1:10 with glycerol TMG medium in H_2O and in D_2O. An aliquot of the cells preincubated in 90% D_2O was diluted 1:10 with glycerol-TMG medium in 90% D_2O.

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structure in vivo by D_2O have been presented (Dicken et al., 1962; Henderson and Dinning, 1962 a, b).

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