INDUCTION LAG AS A FUNCTION OF INDUCTION LEVEL[1]

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Received July 19, 1968

This paper presents evidence, and a supporting theoretical analysis, that the induction lag of the E. coli lac operon does not lengthen as the induction level is lowered. Our reinvestigation of this question was motivated by its theoretical importance and by the conflict in the literature discussed below.

Gilbert and Müller-Hill (1967) claimed recently, on the basis of a theoretical argument, that the induction lag of the lac operon should increase dramatically as the induction level is lowered. Their formula relates this increase to their measured repressor-operator affinity constant. Previously Boezi and Cowie (1961) measured the dependence of the lag on inducer level and found just such an increase. With the empirical formula of Boezi and Cowie, identical in form to their theoretical one, Gilbert and Müller-Hill obtained an independent determination of the affinity constant which agreed with their experimental value. Neither paper mentions the prior result of Pardee and Prestidge (1961) that no change in induction lag with inducer level is found. In 1966 Alpers and Tomkins published a study with the same negative result.

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[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.

Our more detailed data show no increase in induction lag with lowered induction level. Neither the time of first appearance of β-galactosidase in excess of basal nor the value of the intercept of the straight-line part of the induction curve with basal level is increased. We also criticize the theoretical analysis of Gilbert and Müller-Hill (1967). From our corrected version, we conclude that such an increase is inconsistent with the fact that the lac operon can be switched off about as fast as it can be switched on.

Experimental

These experiments were performed on the permeaseless *E. coli* strain 300 U ($i^+ o^+ z^+ y^-$), obtained from V. Moses, Chemical Biodynamics Laboratory, Lawrence Radiation Laboratory, Berkeley, California. Our induction procedure and β-galactosidase assay were essentially as described by Kepes and Beguin (1966).

Our data, which are values proportional to total β-galactosidase content of the culture as a function of time, were corrected for growth of the culture to give numbers proportional to the (average) output of a single lac operon present during the entire course of induction.

Results

The strain used seems to possess a typical lac operon. Induction is maximal at 10^{-3} M isopropyl β-D-thiogalactopyranoside (IPTG), half maximal at 2×10^{-4} M and roughly zero at 10^{-5} M; maximum induction is approximately 900 times higher than basal. Results vary considerably from day to day; induction at 10^{-5} M IPTG for 10 minutes gives β-galactosidase concentrations varying from 15% to 125% above basal.

In two experiments, 600 ml of exponentially growing culture was divided into six aliquots and kept growing with no break. These were

immediately induced to various levels, and following its induction each was sampled at one-minute intervals for 15 minutes. Plots of the results showed no significant variation of intercept with IPTG level (Figs. 1 and 2). Table I gives the intercept values.

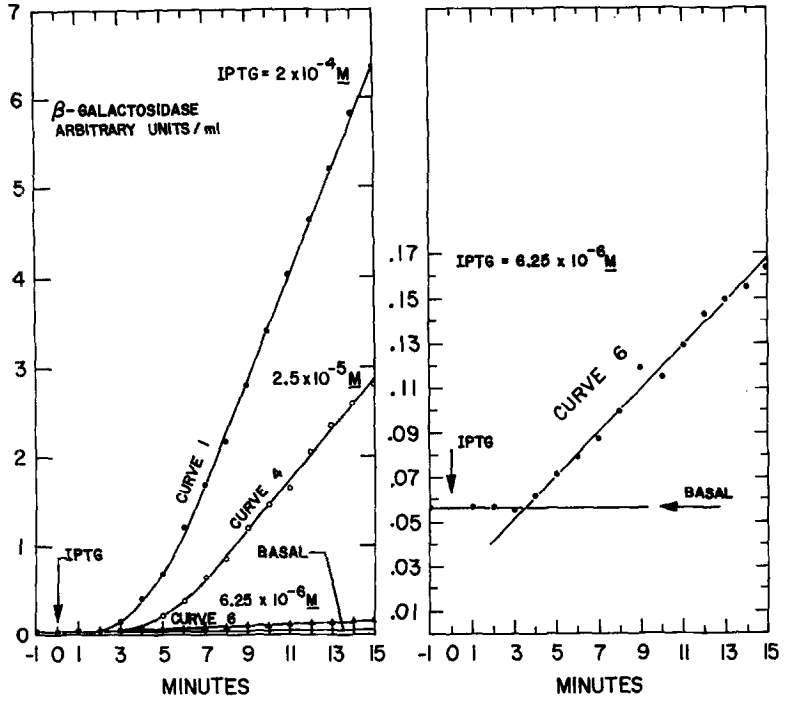

Figure 1. Plot of curves 1, 4, and 6 of Experiment 2 (freshly separated 100-ml cultures simultaneously induced with IPTG at zero time). The ordinate is proportional to the (averaged) output of a single copy of the lac operon.

Figure 2. An expanded scale plot of curve 6 of Figure 1.

Figure 3 shows an induction at 2×10^{-5} M IPTG which was sampled every 10 seconds for 10 minutes and every 30 seconds thereafter out to 15 minutes. Enzyme above basal first appears at 3.0 minutes and the intercept is at 4 minutes. The phenomenological curve of Boezi and Cowie would put this number at more than 10 minutes.

Table I

Intercept Time Versus IPTG Level from Two Experiments Each with 6 Simultaneous Inductions of Exponentially Growing Cultures Divided Just before Induction.

	Experiment 1		Experiment 2	
	IPTG (M)	Intercept time (min)	IPTG (M)	Intercept time (min)
1	5×10^{-4}	3.2 ± 0.2	2×10^{-4}	4.0 ± 0.2
2	2.5×10^{-4}	2.9 ± 0.1	1×10^{-4}	3.8 ± 0.1
3	1.25×10^{-4}	3.1 ± 0.1	5×10^{-5}	5.1 ± 0.2
4	6.25×10^{-5}	3.4 ± 0.2	2.5×10^{-5}	4.8 ± 0.1
5	3.13×10^{-5}	3.9 ± 0.2	1.25×10^{-5}	3.5 ± 0.3
6	1.56×10^{-5}	3.3 ± 0.3	6.25×10^{-6}	3.6 ± 0.2

Theoretical

Gilbert and Müller-Hill (1966) determined that the lac repressor-operator equilibrium binding constant is $1\text{-}2 \times 10^{-11}$ M, corresponding to 15-16 Kcal/mole binding energy and implying a disassociation rate of 10^{-3} to 10^{-4} sec^{-1}. It is known, especially from the pulsed induction experiments of Kepes (1963, 1967), that the lac operon can be switched on in less than 20 seconds; hence it is clear that the inducer must bind to the repressor while it is still on the operator and that it must substantially lower the repressor-operator binding energy.

To consider the simplest model of this system we assume (a) that the repressor-inducer interaction ($R \leftrightarrow I$) involves a single site and is in equilibrium: $RI/R = K \cdot I \equiv \iota$; (b) that the rate of association of R to the

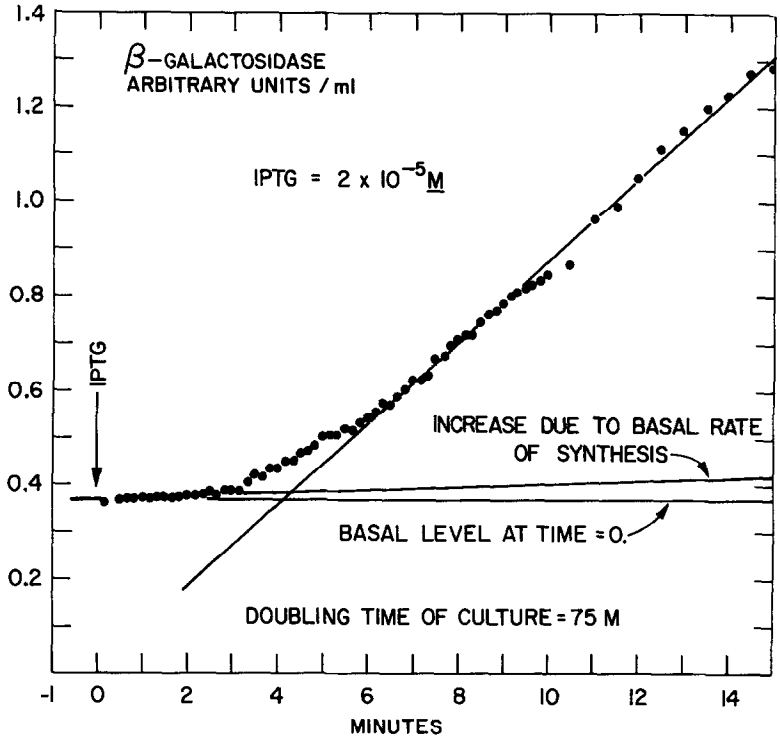

Figure 3. A 100-ml culture was induced with 2×10^{-5} M IPTG at zero time; 0.5-ml samples were withdrawn and stopped with chloramphenicol at 10-second intervals for 10 minutes and at 30-second intervals thereafter to 15 minutes. The ordinate is proportional to the (averaged) production of a single lac operon. Note the basal level at zero time and the production due to the basal rate.

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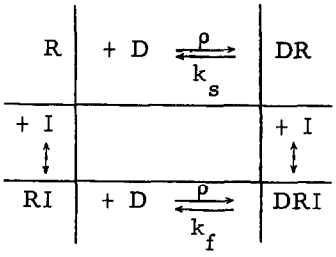
operator D ($R + D \xrightarrow{\rho} DR$) is unaffected by the binding of R to I ($RI + D \xrightarrow{\rho} DRI$), because it depends on diffusion and steric factors and is independent of the binding energy; (c) that the binding of I to R when R is complexed to D is also in equilibrium: $DRI/DR = K_D \cdot I \equiv \iota_D$. (It is in this last assumption that we do not follow Gilbert and Müller-Hill. They attempted to relate K_D to the constants in the other three reactions by incorrectly assuming that the two D reactions can be separately in equilibrium. In any case K_D is not physically determined by the rates in the other reactions.)



Therefore the time rate of change of the total repressor-operator complex, $D\hat{R} \equiv DR + DRI$, is given by

$$d(D\hat{R})/dt = \rho \cdot D \cdot \hat{R} - k_s \cdot DR - k_f \cdot DRI$$

where $\hat{R} \equiv R + RI$, and $k_f >> k_s$ are the fast and slow disassociation rates. (We will see that the source term $\rho \cdot D \cdot \hat{R}$, neglected by Gilbert and Müller-Hill (1967) makes the dominant contribution to the time constant for induction at low inducer concentrations.) By expressing DR and DRI in terms of $D\hat{R}$: $DR = D\hat{R}/(1 + \iota_D)$, $DRI = D\hat{R} \cdot \iota_D/(1 + \iota_D)$, and expressing D and $\hat{R}$ in terms of total repressor and total operator: $R_t = R + D\hat{R}$, $D_t = D + D\hat{R}$, we obtain a quadratic differential equation in the single unknown $D\hat{R}(t)$: $d(D\hat{R})/dt = \rho \cdot (D_t - D\hat{R}) \cdot (R_t - D\hat{R}) - D\hat{R} \cdot f(I)$, where $f(I) = (k_s + \iota_D k_f)/(1 + \iota_D)$, $\iota_D = I \cdot K_D$. The solution to this equation corresponding to a sudden change in inducer level from I_0 to I at time t = 0 is

$$D\hat{R}(t) = \tfrac{1}{2} \cdot \frac{\sqrt{q(I)}\left(\left[B(I_0) - B(I) - \sqrt{q(I_0)}\right]\left[e^{\lambda(I)t} + e^{-\lambda(I)t}\right] - \sqrt{q(I)}\left[e^{\lambda(I)t} - e^{-\lambda(I)}\right.\right.}{\sqrt{q(I)}\left[e^{\lambda(I)t} + e^{-\lambda(I)t}\right] - \left[B(I_0) - B(I) - q(I_0)\right]\left[e^{\lambda(I)t} - e^{-\lambda(I)t}\right]}$$

$$+ B(I)/2$$

where $B(I) = \rho(R_t + D_t) + f(I)$, $q(I) = B(I)^2 - 4\rho^2 R_t \cdot D_t$, and $\lambda(I) = \sqrt{q(I)}/2$.

In equilibrium $(d[\hat{DR}(t)]/dt = 0)$ the total number of operators with repressors bound to them is $\hat{DR}(I) = \frac{1}{2}\rho\left(B(I) - \left(B(I)^2 - 4\rho^2 D_t \cdot R_t\right)^{\frac{1}{2}}\right)$ which varies monotonically between the two limiting values

$\hat{DR}(I=0) \cong D_t(1 - k_s/\rho(R_t - D_t))$ and $\hat{DR}(I=\infty) \cong \rho D_t \cdot R_t/(\rho(R_t + D_t) + k_f)$.

The equilibrium rate of enzyme synthesis should be proportional to the fraction of operators free of repressor: $S(I) = [D_t - \hat{DR}(I)]/D_t$ which in the two limiting cases becomes

$S(I=0) \cong k_s/(\rho(R_t - D_t))$ and $S(I=\infty) \cong (\rho D_t + k_f)/[\rho(R_t + D_t) + k_f]$.

From Gilbert and Müller-Hill (1967) we can take the numbers, $\rho = 5 \times 10^7$ $\underline{M}^{-1}$ sec^{-1}, $k_s/\rho = 10^{-11}$ $\underline{M}$, $R_t = 2 \times 10^{-8}$ $\underline{M}$, $D_t = 2 \times 10^{-9}$ $\underline{M}$, and calculate that $S(I=0) = 5.5 \times 10^{-4}$. If we now require that the maximum rate be 900 times the basal rate we get that $k_f/\rho = 1.8 \times 10^{-8}$ $\underline{M}$. The time constant for induction from I = 0 to I is given to a good approximation by $t_{\frac{1}{2}}(I) \cong (\ln 2)/2\lambda(I) = (\ln 2)/\sqrt{q(I)}$, which varies between the limits:

$$t_{\frac{1}{2}}(I=0) \cong (\ln 2)\left[\frac{1}{\rho(R_t - D_t)} - \frac{\rho(R_t + D_t)}{\rho^3(R_t - D_t)^3} k_s\right] \cong .76 \text{ sec.}$$

and

$$t_{\frac{1}{2}}(I=\infty) \cong (\ln 2)\left(\frac{[\rho(R_t + D_t) + k_f]^2 + 2\rho^2 R_t \cdot D_t}{[\rho(R_t + D_t) + k_f]^3}\right) \cong .36 \text{ sec.}$$

Thus this model predicts an upper limit on the time constant for induction of less than one second. It is more convincing to observe that the time constant for de-induction is also given by the expression $t_{\frac{1}{2}}(I=0)$ and that this quantity must be less than twenty seconds or so, from the pulse induction experiments of Kepes (1963, 1967) and Kepes and Beguin (1966). In any model the de-induction time constant must equal the $I \to 0$ limit of the

induction constant. So if the induction time constant increases monotonically with decreasing I, it cannot exceed the time constant for de-induction.

Of course, the response of the lac operon to induction cannot be adequately described by a model having a single inducer site (or n equivalent sites) on the repressor (Gilbert and Müller-Hill, 1967). In our model, the inducer concentration must increase by more than four decades before the equilibrium rate of synthesis increases by approximately nine hundred times.

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