A MODEL FOR THE MECHANISM OF ENZYME INDUCTION

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ABSTRACT A sequence of reactions is postulated from which are derived equations describing the time course of enzyme induction. The model also yields the observed effect of the inducer concentration on the time constant and final rate of enzyme synthesis. Features of the model are: (*a*) The inducer acts first to release the protein forming template from its site of synthesis on the gene. (*b*) The inducer is involved again in the equilibrium dissociation of the free template-inducer complex which is utilized in the synthesis of the enzyme-forming unit. (*c*) The final enzyme-forming unit is unstable and must be synthesized continuously to maintain enzyme synthesis.

INTRODUCTION

Many attempts have been made to deduce the underlying mechanism of enzyme induction from measurements of the time course of enzyme synthesis. The data available have not been adequate for this purpose, however, because in some cases the process of interest was obscured by other complicating factors, and in other cases the time resolution was not sufficient to follow the rapid changes of synthetic rates.

The experiments of Boezi and Cowie (1961) provide data in which these difficulties appear to be lacking. Thus they furnish the material for one more attempt to uncover the type of reactions which are involved. Furthermore the data cover a number of experimental situations which provide a rigorous test of any postulated reaction mechanism.

The experimental data are the quantities of cells and of enzyme, measured as a function of time, through a period when the concentrations of inducers or repressors vary and the cells respond by changes in their rate of enzyme synthesis. Since the enzyme itself is stable, at least for the duration of the experiments, the quantity of theoretical interest is $(dE/dt)/Q$, the average rate of enzyme synthesis per cell. This quantity can be considered to be a measure of the number of active enzyme-forming units per cell (N). Thus all enzyme-forming units are assumed to be fully

active; the possibility that enzyme-forming units may have different rates of synthesis is ignored. Relative values of N can be determined readily from the experimental data; conversely, if N can be predicted by theory, the expected quantity of enzyme can be calculated for comparison with experimental data. This procedure is valid only when the experiments show that $(dE/dt)/Q$ does not depend upon E/Q, the quantity of enzyme accumulated.

REACTION SEQUENCE

The experimental data show that upon induction N approaches the steady-state level, N_s, as shown in equation (1).

$$N = N_s(1 - ge^{-\alpha t} - he^{-\beta t}) \tag{1}$$

where $g + h = 1$.

Furthermore, the data show that

$$N_s \sim \frac{I^2}{I^2 + K_2^2}$$

and

$$\beta \sim \frac{I}{I + K_4}$$

where I is the concentration of inducer.

The two separate exponential terms are required to allow a long time constant for induction at low levels of inducer and a rapid loss of N when the inducer is diluted out.

Such an equation also describes the appearance of material in the second component of a three component sequence of reactions:

$$\rightarrow \textcircled{1} \rightarrow \textcircled{2} \rightarrow \textcircled{3}$$

Thus the form of the induction curve suggests that: (*a*) The enzyme-forming unit is not a stable end product but is converted into an inactive form. (*b*) The enzyme-forming unit is derived from a pool of precursor material which must be accumulated.

One sequence of reactions which leads to the proper form of the induction curve is the following:

$$A + X \xrightarrow{k_1} AB \tag{2}$$

$$AB + 2I \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} ABI_2 \tag{3}$$

$$ABI_2 \xrightarrow{k_3} A + BI_2 \tag{4}$$

$$BI_2 \underset{k_4'}{\overset{k_4}{\rightleftharpoons}} BI + I \tag{5}$$

$$BI_2 + Y \xrightarrow{k_5} N \tag{6}$$

$$N \xrightarrow{k_6} Z \tag{7}$$

Reaction (2) indicates that a precursor material, X, accumulates on a template, A, and is polymerized to form a product, B, which remains complexed with the template, A.

Reaction (3) is a reversible reaction of the complex AB with two molecules of inducer, I.

Reaction (4) represents the dissociation of the complex, ABI_2. This releases the product, BI_2, and frees the template, A, for further synthesis.

Together Reactions (2), (3), and (4) determine the inducer dependence of the steady-state level of N but have no influence on the time constant.

Reaction (5) is a reversible dissociation of BI_2 and Reaction (6) is the association of the complex, BI_2, with some cellular component, Y, to make the enzyme-forming unit N. Reaction (5) introduces the inducer dependent term into the time constant without introducing any further inducer dependence in N_s.

Reaction (7) indicates that the enzyme-forming unit is unstable and disintegrates into inactive products, Z.

This system of reactions may be studied most conveniently by noting that the first three provide the mechanism of producing BI_2, while the next two are concerned with its fate.

First, let us find a relationship for the production of BI_2 by the first three reactions. We shall assume that X and I are present in sufficient quantity so that their concentrations are not significantly altered by the reactions. We shall also assume that the usual "steady-state" approximation will be adequate. This simply means that the reaction system adjusts to a change of conditions sufficiently rapidly so that the intermediates are always at their steady-state concentrations, or, in other words, that transients are quickly damped out. Then we have for the rate of production of BI_2,

$$R_{BI_2} = k_3(ABI_2)$$

with the conditions:

$$A_T = (A) + (AB) + (ABI_2) \qquad \text{conservation of } A$$

$$k_1(X)(A) = k_3(ABI_2) \qquad \text{steady state for } A$$

$$k_2''(AB)(I)^2 = k_1(X)(A) + k_2'(ABI_2) \qquad \text{steady state for } AB$$

where A_T is the total available A in the system. Thus

$$R_{BI_2} = K_3 A_T \frac{(I)^2}{(I)^2 + K_2^2},$$

where we define

$$K_3 = \frac{k_1(X)k_3}{k_1(X) + k_3}$$

and

$$K_2^2 = \frac{k_1(X)[k_3 + k_2']}{k_2''[k_1(X) + k_3]}.$$

Now, turning to the question of the time dependence of BI_2 we note that

$$\frac{d[(BI_2) + (BI)]}{dt} = R_{BI_s} - k_5(Y)(BI_2).$$

We now assume that Y is available in sufficient excess so that its concentration is not sensibly time-dependent and that Reaction (5) is sufficiently fast so that it is always essentially at equilibrium, *i.e.*,

$$(BI) \simeq \frac{K_4}{(I)}(BI_2) \quad \text{with} \quad K_4 = k_4''/k_4'.$$

Then we have

$$\frac{K_4 + (I)}{(I)} \frac{d(BI_2)}{dt} = R_{BI_s} - k_5(Y)(BI_2)$$

which integrates, with the initial condition that $(BI_2) = 0$ at $t = 0$, to give (BI_2) as a function of time. This is now substituted in the equation for the number of enzyme-forming sites, N,

$$\frac{dN}{dt} = k_5(Y)(BI_2) - k_6 N,$$

which is integrated, with the initial condition $N = 0$ at $t = 0$, to give

$$N = N_s\left[1 - \frac{\beta}{\beta - \alpha} e^{-\alpha t} - \frac{\alpha}{\alpha - \beta} e^{-\beta t}\right]$$

where

$$N_s = \frac{K_3 A_T}{k_6} \frac{(I)^2}{(I)^2 + K_2^2}$$

$$\alpha = k_6$$

$$\beta = k_5(Y) \frac{(I)}{(I) + K_4}$$

Thus we have obtained an equation for N of the form called for in equation (1).

To compare with the actual data we must now find the concentration of the enzyme, E. Let us consider the case where the times involved are sufficiently short that the cell density, Q, is essentially unchanged. Then

$$\frac{dE}{dt} \sim QN$$

which integrates, with the initial condition $E = 0$ at $t = 0$, to give

$$E \sim QN_s\left[t + \frac{\beta(e^{-\alpha t} - 1)}{\alpha(\beta - \alpha)} + \frac{\alpha(e^{-\beta t} - 1)}{\beta(\alpha - \beta)}\right].$$

This equation predicts that, after a "time lag" there will be a linear rise in enzyme concentration and the rate will be proportional to $(I)^2/[(I)^2 + K_2^2]$, in agreement

with Boezi and Cowie. If the linear portion of the curve is extrapolated back to $E = 0$, then it will intersect the abscissa at a time T_c given by

$$T_c = \frac{\alpha + \beta}{\alpha\beta}.$$

Thus

$$\frac{1}{T_c} = \frac{k_6 k_5(Y)(I)}{k_6 K_4 + [k_6 + k_5(Y)](I)}$$

$$\sim \frac{I}{I + K'},$$

again in agreement with Boezi and Cowie.

In the more general case there will be some cell growth during the process and this must be taken into account in integrating to find the enzyme concentration. The method is given in the Appendix.

The reaction sequence postulated was based solely upon the kinetics of induction and the loss of induction upon dilution of the inducer. It was gratifying therefore to find that the same reaction sequence provided a mechanism for the temporary repression caused by glucose. When the inducer concentration is high, glucose affects the time constant of induction but not the final rate of synthesis. It appears to compete with the inducer in Reaction (5) but not in Reaction (3). Thus the complex AB seems to show a greater specificity than does BI.

When glucose is added to cells which are already induced there is a temporary depletion of BI_2 by glucose substitution for one I, thereby shutting off the production of enzyme-forming units. Those already present decay and the rate of enzyme synthesis drops exponentially. Glucose does not influence the production of new BI_2; hence BI accumulates until the level of BI_2 is restored.

At higher ratios of glucose to inducer the rate of enzyme synthesis is also reduced, presumably by competition in Reaction (3).

The kinetics of induction using TMG in place of IPTG are correlated by making suitable changes in K_2 and K_4. K_2 must be decreased by a factor of 35 and K_4 by a factor of 6. Again Reaction (3) shows a greater specificity than does Reaction (5).

LIMITATIONS OF THE MODEL

These equations are by no means unique. In particular there is no way to distinguish by kinetic analysis whether the inducer plays a direct active role as indicated or whether the inducer inactivates or removes a repressor. The form of the equations can remain unchanged when written in terms of interactions between inducer molecules and a hypothetical repressor.

Furthermore, these reactions are undoubtedly too simple and represent only those reactions which predominate in the range of experimental conditions tested. For example, Reaction (5) indicates the partial dissociation of a complex contain-

ing two molecules of inducer but the complete dissociation (*i.e.*, $BI \rightleftharpoons B + I$) is neglected. Similarly the possibility that one molecule of I might strip B off A is ignored. The divergence between the theoretical and experimental curves at very low concentrations of inducer may well be due to such omissions. The effective concentration of the inducer is assumed to be the external concentration. The time required for the inducer to penetrate the cell is ignored.

Neither have we found it necessary to include any additional reaction of the inducer with the enzyme-forming unit itself. Such a reaction might well be present but would have no influence on the experimental data if it saturated at extremely low concentrations of inducer. In short, we believe that these reactions represent one of the simplest formulations adequate to describe the prominent features of induction of β-galactosidase in the mutant ML 3. In other systems, other reactions might become rate-limiting.

IDENTIFICATION OF THE CELLULAR COMPONENTS

The reactions postulated become much more meaningful if the symbols *A, B, etc.* can be associated with known cellular components. However, this correlation is quite independent of the validity of the equations themselves and is much more speculative. The correlations may be quite wrong even though the equations are correct.

It seems quite certain that A represents DNA. Pardee *et al.* (1959) have shown that induction is initiated when genetically competent DNA enters a cell. It seems equally certain that N must represent the 70S ribosomes as they have been shown to be the principal sites of protein synthesis and to carry a small portion of the enzyme (McQuillen *et al.*, 1959; Cowie *et al.*, 1961).

B and Y are then the components needed to form an active protein-synthesizing unit. B, the unit formed on the DNA template, has the characteristics of the "messenger RNA" postulated by Jacob and Monod (1961). Accordingly Y must represent the bulk of the active 70S particle. Presumably an ample supply of Y exists before induction because the time course of ribosome synthesis is slow compared to the rapidity of enzyme induction. Y must therefore be non-specific or only partially specific.

Alternatively B could represent an early stage of ribosome synthesis during which these precursors of the ribosomes acted as templates for protein synthesis. The half-life of the first precursor corresponds closely to the half-life of the enzyme-forming unit (Britten, 1961).

Z represents the inactive particles after the "messengers" are destroyed or the ribosome precursors are converted to ribosomes.

Since B combines specifically with the inducer it is attractive to postulate that B also carries at least the active site of the enzyme. If so, X must represent the amino acid precursors of the enzyme in addition to the nucleotide precursors of the RNA.

CONCLUSION

We have no confidence that this model will survive long. It is quite definite and therefore highly vulnerable to experimental disproof. It does, however, unify a wide variety of experimental data. Furthermore it indicates an additional reaction which might take part in the regulation of enzyme synthesis, namely in the association of the newly formed RNA with an existing particle to create an enzyme-forming unit. This association could be blocked or facilitated by alterations in the protein of the ribosomes and thus controlled by genes remote from the genes determining the structure of the enzyme.

APPENDIX

In the case of experiments covering a time long enough to necessitate correcting for cell growth, a somewhat different analysis is required. In this case

$$Q = Q_0 e^{at}$$

and

$$\frac{dE}{dt} \sim QN,$$

using N as before, now integrates, with $E = 0$ at $t = 0$, to give

$$E \sim N_s Q_0 \left[\frac{e^{at} - 1}{a} + \frac{\beta\{e^{-(\alpha - a)t} - 1\}}{(\beta - \alpha)(\alpha - a)} + \frac{\alpha\{e^{-(\beta - a)t} - 1\}}{(\alpha - \beta)(\beta - a)} \right].$$

This may better be represented, for comparison with experimental data, by replacing e^{at} by Q/Q_0 to get

$$E \sim N_s \left[\frac{Q - Q_0}{a} + \frac{\beta(e^{-at}Q - Q_0)}{(\beta - \alpha)(\alpha - a)} + \frac{\alpha(e^{-\beta t}Q - Q_0)}{(\alpha - \beta)(\beta - a)} \right].$$

If E is now plotted against Q, there will appear to be a "lag" and then E will grow linearly with Q according to the relation

$$E \sim N_s \left[\frac{Q - Q_0}{a} - \frac{BQ_0}{(\beta - \alpha)(\alpha - a)} - \frac{\alpha Q_0}{(\alpha - \beta)(\beta - a)} \right].$$

Extrapolating the linear part back to $E = 0$, we will find an intercept on the Q axis at Q_e. Then

$$\frac{Q_e - Q_0}{aQ_0} = \frac{\alpha + \beta - a}{\alpha\beta - (\alpha + \beta)a + a^2}.$$

Incidentally, it may be noted that in the limit as $a \to 0$, all these equations reduce to the results given earlier for the case of no growth.

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