CHEMICAL INSTABILITIES OF "ALL-OR-NONE" TYPE IN β - GALACTOSIDASE INDUCTION AND ACTIVE TRANSPORT

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1. Introduction

The investigations of Jacob and Monod [1] and Novick and Weiner [2] have shown that at the cellular level, the induction of β-galactosidase in *E. Coli* is an "all-or-none" phenomenon.

Bacterial populations grown at low concentration of inducer in a fixed medium are composed of individuals which are in either of two possible steady states: maximally induced or non-induced. The bacteria can be shifted from one stable state to the other by transitory variations in the environment. The "all-or-none" character can be understood in terms of the functioning of "galactoside permease" * [2,3]. This protein is an integral part of the transport system by which lactose is accumulated and utilised in the organism. Permease is a genetically controlled protein and is a product of Y gene of lac operon which also controls the synthesis of β-galactosidase itself. Thus lac permease is induced by its own product: the induction phenomenon is autocatalytic.

The existence of multiple steady states in open systems far from equilibrium and undergoing chemical reactions described by non-linear kinetics has been established in the framework of "dissipative structures" [4]. These are spatial or temporal or coherent organisations arising in non-linear chemical systems far from thermodynamic equilibrium. Such states can only appear beyond a critical point of instability of the solutions of the kinetic equations. The relations between multiple steady states and some general features of biological structures and functions have been investigated recently. These are metabolic processes [4], Jacob–Monod [5] type models for differentiation, membrane excitation [6] and polymer synthesis [7]. In the region of existence of multiple steady states, the system is driven discontinuously from one type of regime with low concentration of a given product to another with high concentration.

In this paper, following the existing experimental data on kinetics of induction of β-galactosidase, we construct a simplified model and show that at a critical value of inducer concentration, the quantity of enzyme in the bacteria jumps discontinuously from the uninduced state to an induced one. We also show that in the induced state the permeation is achieved via active transport.

The lactose operon is one of the most studied problems in bacterial genetics. There is a wealth of experimental data on β-galactosidase induction kinetics [8]. The process of permeation of inducer through bacterial membrane has also been widely investigated [11]. It seems to us that the following simplified model (scheme 1) can describe the most important features of induction processes.

Here R and R′ are the active and inactive (precursor) forms of repressor molecules [9]. O^+ and O^- are, respectively, the probabilities that the regulatory gene be opened or closed (with the obvious physical condition $O^+ + O^- = 1$) I_i is the concentration of "gratuitous" inducer inside the bacteria and I the concentration outside. E and M are enzyme and permease concentrations, respectively.

* Also called M protein [11,8].

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Scheme 1

$$R' \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R \qquad 1)$$

$$R + O^+ \underset{k_4}{\overset{k_3}{\rightleftharpoons}} O^- \qquad 2)$$

$$R + I_i \underset{k_6}{\overset{k_5}{\rightleftharpoons}} F \qquad 3)$$

$$\alpha + O^+ \xrightarrow{k_7} M + E + O^+ \qquad 4)$$

$$M + I \underset{k_9}{\overset{k_8}{\rightleftharpoons}} I_i + M \qquad 5)$$

$$M \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} F' \qquad 6)$$

$$E \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} F'' \qquad 7)$$

The first step describes the activation of repressor molecules [9]. The second and third steps denote, respectively, operator–repressor and repressor–inducer complex formation [10]. The next step is a short cut to describe protein synthesis on DNA templates.

The lactose permease system for transport of inducer is a much more involved and extensively studied mechanism [11]. However it does not seem to contain relevant non-linearities as far as β-galactosidase induction process is concerned. We summarise the inducer transport by the simple step 5). The asymmetry of binding of inducer by permease on the exterior as compared to the interior membrane has been thought to be the primary cause of the internal accumulation of the galactosides [11,8]. To describe this fact we take $k_8 > k_9$. The last two steps account for the protein loss.

Scheme 1 is described by the following kinetic equations if the system is assumed to be homogeneous:

$$\frac{dR}{dt} = k_1 R' - k_2 R + k_4 O^- - k_3 R O^+ - k_5 R I_i + k_6 F \qquad a)$$

$$\frac{dO^+}{dt} = -k_3 R O^+ + k_4 O^- \qquad b)$$

$$\frac{dM}{dt} = k_7 \alpha O^+ - k_{10} M + k_{11} F' \qquad c)$$

$$\frac{dE}{dt} = k_7 \alpha O^+ - k_{10} E + k_{11} F'' \qquad d)$$

$$\frac{dI_i}{dt} = k_8 I M - k_9 I_i M - k_5 R I_i + k_6 F \qquad e)$$

The steady state of these equations has been studied numerically on a C.D.C. computer for a wide range of values of different coefficients. A typical result is shown in fig. 1.

One begins with a very low concentration of inducer and adds gradually substrate into the system. The computed concentration of β-galactosidase is low. This corresponds to the uninduced bacteria and is shown by branch (a) of fig. 1*. When a threshold concentration I_c is reached the concentration of enzyme jumps to branch (b). In this state the bacteria are induced.

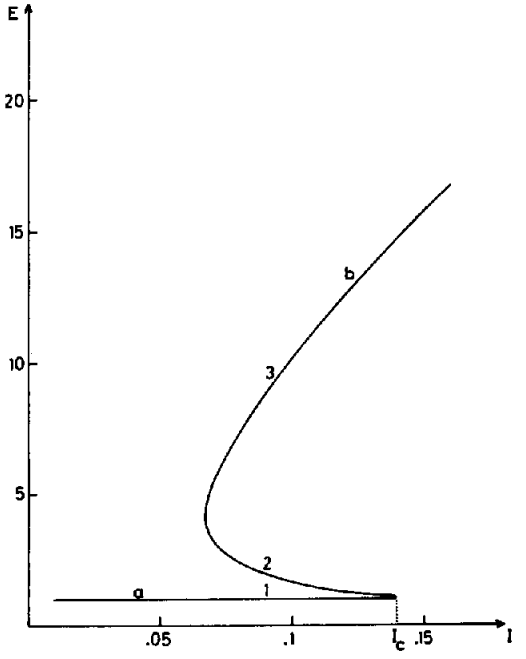

Fig. 1. Multiple steady states of E versus inducer concentration. $k_1 = 1; k_2 = .01; k_3 = 10; k_4 = .01; k_5 = 5; k_6 = 1; k_7 = 1; k_8 = 5; k_9 = .1; k_{10} = .01; k_{11} = 1; \alpha = 4; F = .1; F' = .01; F'' = .01; R' = .8$ (in proper units).

* For other choice of values of constants one can get sigmoidal curves.

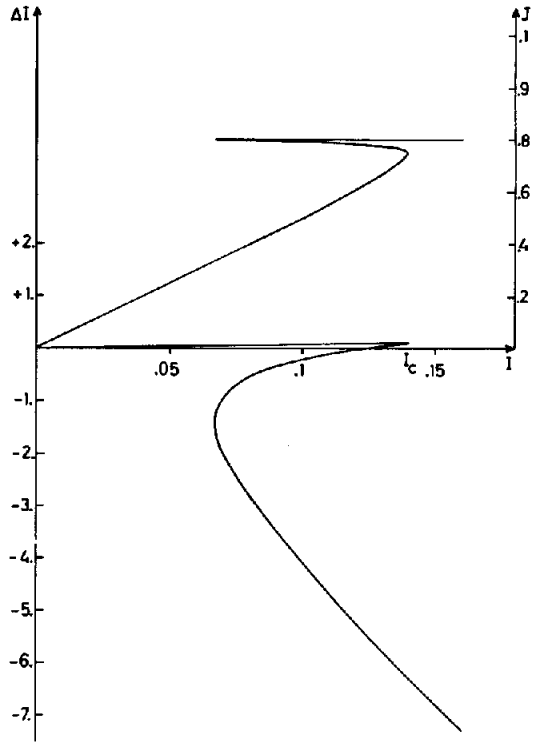

Fig. 2. $\Delta I = I - I_i$ and $J = M(k_8 I - k_9 I_i)$ as a function of I.

The stability of these steady states has been investigated by normal mode analysis. It is shown that states 1 and 3 are stable and 2 is unstable. In fig. 2 we plot the flux of inducer through bacterial membrane $J = M(k_8 I - k_9 I_i)$ and the gradient $\Delta I = I - I_i$ as a function of inducer concentration. It is seen that for uninduced bacteria before the critical point of transition the inducer entry is carrier mediated. ΔI and J have the same sign.

In the region of multiple steady states, where the bacteria are induced, there is a sudden jump of ΔI towards negative values while J remains positive. The change of sign of ΔI shows that for these bacteria there is an uphill transport of inducer after the point of instability of branch (a) is reached. We have here an example of active transport in the particular case of the lactose transport system.

Recent work [12] in the study of repressor–inducer interaction suggest that the repressor is an allosteric protein which, in the absence of inducer, combines with the operator and which in its presence assumes a form where this affinity is lost. The operator can again synthetise protein normally. Moreover, the interaction between the repressor and inducer is quadratic [13]. These additional features of operator–repressor interaction add new types of non-linearities to the system. Here they would merely amplify the distance separating multiple steady states* but their role is prominent in the occurrence of logistic shaped kinetic curves for the induction in permeaseless strains.

These aspects of the problem together with a more realistic model for transport process [11,8] are currently under investigation.

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* We verified this conjecture for the case $R + 2I_i \rightleftharpoons F$.