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Determining the bistability parameter ranges of artificially induced lac operon using the root locus method ☆

N. Avcu [a,*], H. Alyürük [b], G.K. Demir [a], F. Pekergin [c], L. Cavas [d], C. Güzeliş [e]

[a] Department of Electrical and Electronics Engineering, Faculty of Engineering, Dokuz Eylül University, D1-041 Buca Izmir, 35390 Izmir, Turkey
[b] Institute of Marine Sciences and Technology, Dokuz Eylül University, 35340 Izmir, Turkey
[c] Université Paris 13, Sorbonne Paris Cité, LIPN, CNRS, Paris, France
[d] Department of Chemistry, Faculty of Science, Dokuz Eylül University, 35390 Izmir, Turkey
[e] Department of Electrical and Electronics Engineering, Faculty of Engineering and Computer Sciences, Izmir University of Economics, 35330 Izmir, Turkey

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ABSTRACT

This paper employs the root locus method to conduct a detailed investigation of the parameter regions that ensure bistability in a well-studied gene regulatory network namely, lac operon of *Escherichia coli* (*E. coli*). In contrast to previous works, the parametric bistability conditions observed in this study constitute a complete set of necessary and sufficient conditions. These conditions were derived by applying the root locus method to the polynomial equilibrium equation of the lac operon model to determine the parameter values yielding the multiple real roots necessary for bistability. The lac operon model used was defined as an ordinary differential equation system in a state equation form with a rational right hand side, and it was compatible with the Hill and Michaelis–Menten approaches of enzyme kinetics used to describe biochemical reactions that govern lactose metabolism. The developed root locus method can be used to study the steady-state behavior of any type of convergent biological system model based on mass action kinetics. This method provides a solution to the problem of analyzing gene regulatory networks under parameter uncertainties because the root locus method considers the model parameters as variable, rather than fixed. The obtained bistability ranges for the lac operon model parameters have the potential to elucidate the appearance of bistability for *E. coli* cells in *in vivo* experiments, and they could also be used to design robust hysteretic switches in synthetic biology.

1. Introduction

The lactose operon (abbreviated as lac operon) of *E. coli* bacterium is a gene regulatory network that is responsible for controlling lactose metabolism [1]. The lac operon is composed of three structural genes, *lacZ*, *lacY* and *lacA*, which encode β-galactosidase, galactoside permease and thiogalactoside transacetylase enzymes, respectively. The permease enzyme transports lactose or its non-metabolizable analogs, methyl-1-thio-β-D-galactoside (TMG) or isopropyl-β-D-thio-galactoside (IPTG), into the cell. β-Galactosidase hydrolyzes the transported lactose (a disaccharide) into galactose and glucose to be metabolized and catalyzes the conversion of internal lactose to allolactose to induce transcription of the lac operon via binding to the lac repressor. β-Galactosidase cannot metabolize TMG and IPTG, so these chemicals are ideal molecules for artificially inducing the lac operon. Thiogalactoside transacetylase, plays roles in sugar metabolism and detoxification, catalyzes the transfer of an acetyl group from acetyl-CoA to thiogalactosides [2].

The lac operon of *E. coli* is controlled by two distinct mechanisms: (i) in the absence of lactose and its analogs, the lactose repressor protein encoded by the *lacI* gene binds to the promoter region of the lac operon and prohibits the expression of *lacZ*, *lacY* and *lacA*, preventing the induction of the lac operon; (ii) when glucose is available in the extracellular medium, *E. coli* prefers to use glucose as a carbon-energy source even in the presence of inducers, so the lac operon is not induced due to two glucose-induced suppression processes, catabolite repression and inducer exclusion [3]. Herein, catabolite repression is responsible for the

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* Corresponding author. Tel.: +90 232 301 7681; fax: +90 505 643 4434.
E-mail addresses: neslihan.avcu@deu.edu.tr (N. Avcu), hakan.alyuruk@deu.edu.tr (H. Alyürük), guleser.demir@deu.edu.tr (G.K. Demir), ferhan.pekergin@lipn.univ-paris13.fr (F. Pekergin), lcavas@deu.edu.tr (L. Cavas), cuneyt.guzelis@ieu.edu.tr (C. Güzeliş).

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inhibition of the mRNA transcription in the presence of glucose. The presence of glucose results in low 3′-5′-cyclic adenosine monophosphate (cAMP) concentrations, which prevents the binding of cAMP to cAMP receptor protein (CRP), which is required to form the catabolite gene activator protein (CAP) complex required for mRNA transcription. The second suppression process, inducer exclusion, involves the inhibition of external lactose transport into the cell via the interaction of glucose with the permease, which excludes the inducer [3].

Under glucose starvation, both the glucose-stimulated suppression mechanisms become ineffective and allow lactose (or an artificial inducer such as TMG or IPTG) to induce the lac operon. Herein, this system is induced via the following molecular interactions: first, the availability of a small amount of permease due to the basal transcription of mRNA mediates the transport of a small amount of external lactose (or an artificial inducer) into the cell. Then, allolactose, which is produced from the transported lactose by β-galactosidase, or the transported artificial inducer binds to the LacI repressor protein and changes the conformation of the repressor to dissociate the repressor from the promoter region. The resulting inhibition of the LacI repressor induces the lac operon, allowing the RNA polymerase enzyme to come into contact with the free promoter site of the lac operon and initiate the synthesis of the *lacZ*, *lacY* and *lacA* genes. Then, the transcription of mRNA and the expression of permease, β-galactosidase and thiogalactoside transacetylase increase rapidly. The increase in the amount of permease due to the production of mRNA provides a positive feedback, resulting in the transportation of lactose (or an artificial inducer) and the production of mRNA in much greater amounts compared to the basal activity. This mutual amplification is limited by the saturated rate of molecular interactions. The amounts of mRNA, internal inducers and permease eventually reach steady-state values, as determined by the reductions, due to degradation and other processes.

By virtue of the above described control mechanisms, the lac operon defines a biological switch controlled by two inputs (i.e., external glucose and lactose-artificial inducer) so that it is open (induced) only when lactose (or an artificial inducer), but not glucose, is available in the extracellular medium. In experiments using TMG and IPTG at the population and cell level, the induction status of *E. coli* clearly depends on its previous induction status in addition to the levels of extracellular glucose and inducer concentrations [4–8]. *E. coli* cells maintain their induced and uninduced states (i.e., if they are induced or uninduced, they have a tendency to remain in that state). A large number of theoretical studies have attempted to explain how two alternative equilibrium states (i.e., the induced and uninduced states) exist as steady-state levels for the same concentrations of extracellular glucose and inducer [6,9–19]. Research generally states that this characteristic is due to the bistable behavior of a nonlinear dynamical system possessing two stable equilibria. This induction mechanism of the lac operon has been observed as the response to changing extracellular concentration levels of glucose and inducer, and it has been described in a number of models derived from enzyme kinetics using the well-known engineering concept of hysteresis [6].

Although the above described operation of the lac operon as a bistable hysteretic switch under glucose starvation has been observed in many experimental studies using the gratuitous inducers, TMG and IPTG, no experimental evidence has been reported using lactose as a substrate [6,18–20]. The existence of bistability for both gratuitous inducers and natural inducer, such as allolactose, has also been studied using a number of theoretical models. Most lac operon models are derived from mass action kinetics employing Hill and Michaelis–Menten approaches, and they are defined by ordinary differential equations (ODEs) [3,6,11–16,18–25]. *In silico* studies revealed that these ODE models exhibit bistable behavior for both artificial and natural inducers, and while bistability is possible for natural inducer, it is less likely to occur compared to artificial inducers.

A portion of the theoretical research suggests that induction of the lac operon in the natural setting with lactose as substrate lacks the bistable phenomenon [13,15,18–20]. Metabolism of the natural inducer attenuates the positive feedback due to the permease [15,18–20], and the increased stabilization of the dilution rate of permease, which is squarely proportional to the permease activity of the metabolism of the natural inducer [13], represent two major biological reasons why bistability is not apparent when lactose is used in *in vivo* experiments. The literature also suggests [16,24] that bistability does not disappear for lactose, although much higher lactose concentrations are required for it to be observed compared to artificial inducers. Therefore, there is still a need to test theoretical predictions with suitably designed *in vivo* experiments.

ODE models, which are described in terms of molecule concentrations rather than molecule numbers, effectively represent molecular interactions only at high concentration levels, which is a valid assumption for inducers, but not for mRNA and permease variables. Stochastic models of the lac operon, which are usually expressed as the chemical master equation defining time evolution of the probability distribution of molecule numbers, have also been used to obtain more realistic predictions [16,24,26–30]. One report suggested [16] that the existence of bistability in the ODE model is implied by the bimodality observed in the steady-state solution of the corresponding master equation under the same parameter setting, but the reverse may not always be true. Other reports state [26,29] that bistability and bimodality do not necessarily imply the presence of each other. Thus, ODE and stochastic models for predicting bistable behavior of the lac operon must be analyzed by new theoretical studies and numerical simulations. Moreover, testing the present hypotheses and introducing new ones, such as studying the mechanisms governing lac operon dynamics *in vivo*, warrant further investigation [7,8,31–34].

ODE models derived from mass action kinetics and stochastic models derived from stochastic chemical kinetics are defined by a number of internal parameters (e.g., reaction rate constants) and external parameters (e.g., external inducer and glucose concentrations) [28]. The quantitative and qualitative behaviors of lac operon models depend on the values of the model parameters. However, there is a significant amount of uncertainty regarding the estimation of the biophysically realistic values of these model parameters due to the differences between *in vivo* and *in vitro* kinetics, laboratory conditions, parameter heterogeneity within a population, a lack of direct measurements, and inaccuracies in data fitting used for parameter calculations, among others [20,29,35,36]. To validate or invalidate the existence of bistable behavior for the lac operon in natural settings, one can attempt to determine the entire bistability ranges of the model parameters and check whether the values falling within these ranges are biophysically realistic.

This paper utilizes the root locus method to determine the bistability parameter ranges of the ODE model of the lac operon. The root locus method is applicable to address this problem because of the following facts: (i) ODE models derived based on mass action kinetics can be obtained as nonlinear state equations whose right hand sides are rational functions of molecule concentrations when Michaelis–Menten and Hill approximations employed; (ii) the equilibrium equation for these ODE models can be written as polynomial equations of the concentrations; and (iii) the model parameters originated from Michaelis–Menten and Hill kinetics constitute the linear coefficients of the polynomial equilibrium equation, so each of these linear coefficients can be considered as a control parameter, such that the number and positions of the equilibrium points can be determined as a function of the chosen control parameter using the root locus method. Thus, the bistability range with respect to a specific model parameter is obtained by specifying the parameter