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Theoretical Study of Tryptophan Operon: Application in Microbial Technology

Somdatta Sinha
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

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A model for the tryptophan operon is formulated based on the genetic and biophysical data available on the structure of the operon and the nature of interactions between the repressor and its ligands. Studies have been done on wild-type, superrepressing, and loose-binding strains to identify conditions at which the stability of the system changes (i.e., evolves to a stable synthesis or periodic synthesis with increasing amplitude). Also, the factors that increase the yield of tryptophan are studied and predictions made, based on the results, for obtaining overproducing strains of tryptophan that can be used for the industrial production of this useful amino acid.

INTRODUCTION

Studying the control properties of regulation of gene expression in bacteria is being pursued with renewed interest due to its application in microbial technology. Production of important amino acids through genetic manipulations of bacterial strains is one of the aims of biotechnological research.[1-3] These include amino acids such as lysine, methionine, tryptophan, etc., that cannot be synthesized by man and are useful as stockfeed additives. They are particularly important to nutrition since most cereal grains are deficient in them. Amino acids are generally made by chemical synthesis, which has the disadvantages of being expensive and less efficient since it makes a mixture of both D- and L-isomers. Microbiological production (fermentation) yields only a biologically active L-isomer and hence is being introduced on a larger scale. The efficiency of the fermentation process can be increased if the respective operons and their metabolic pathways are known in detail.

It has been observed that both the genetic and metabolic components of amino acid synthesis are controlled through feedback loops, such as repression, induction, inhibition, and activation of genes and enzymes. Once the biochemical pathway for a particular amino acid is known, it is possible to study the process by making mathematical models, taking the known experimental facts into consideration. The parameters in the model then represent various coefficients of interactions between the molecular species in the process. It is possible to alter the control characteristics of the variables involved by changing these parameters, which essentially then represent mutated or altered systems. Study of such systems can yield information regarding the important points in the regulatory mechanism that limits production, and the results can be used to develop new and useful bacterial strains.

In this article a study has been undertaken to describe the expression of the tryptophan operon (*trp*) in *Escherichia coli*. One of the reasons for choosing the *trp* operon is the fact that despite many attempts using different organisms, the titers of L-tryptophan obtained by fermentation remain relatively low. The model is based on a gene–enzyme–end product control unit with a negative feedback as first described by Goodwin.[4] The tryptophan operon *trp EDCBA* structural genes encode for six enzymes that act in the metabolic pathway of the production of tryptophan. The protein *TrpR* is the aporepressor of *E. coli*, which is a product of the *trpR* gene. This protein complexed with tryptophan is involved in the transcriptional regulation of the *trp* operon by binding tightly and specifically to the operator region of *trp EDCBA* when the intracellular concentration of tryptophan is high, resulting in repression of the operon transcription. When the intracellular concentration of tryptophan is low, the *trp* repressor exists predominantly in the dissociated aporepressor form, free of bound corepressor (i.e., tryptophan). The *trpR* gene has been cloned, the DNA sequence determined, and the repressor protein purified and sequenced.[5,6] A large number of mutants of the *trpR* gene has been constructed that have a variety of properties regarding its interaction with the corepressor and the operator DNA of the *trp* operon.[7] In this article the whole process has been modeled mathematically using the above-mentioned experimental facts, and a large variety of situations have been simulated by altering different parameters. The results show that strains with mutations leading to superrepressing strains destabilize the system, and instead of returning to a stable synthesis of tryptophan, its concentration grows. Similar results can be obtained by altering the parameters that control the degradation rates of the mRNA of the operon, enzymes, and end product, i.e., tryptophan. The rate of utilization of tryptophan in protein synthesis in the cell is shown to be an important parameter in inducing instability

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in the system. Also, alteration of this parameter affected the level of tryptophan in otherwise normal strains. For certain parameter values, a "semistable" state appears where the behavior of the system depends on its position in the phase space (i.e., initial conditions). It evolves to the stable steady state in a damped periodic manner when perturbed close to the steady state; whereas it grows in amplitude when perturbed beyond a certain position. The basal values of the parameters are chosen on the basis of existing experimental data as obtained from the work of Bliss et al.,[8] but studies have been done for a range around the normal experimental parameter values to observe the effects of that parameter on the behavior of the system. The results of all these parameter variations on the behavior of synthesis of tryptophan can give an idea regarding the kinds of mutations that are needed to construct an overproducing tryptophan strain of *E. coli*. The theoretical results are compared in the light of existing strains available, and specific predictions are made following which mutations can be programmed and strains constructed.

MODEL

The synthesis of the amino acid tryptophan in a cell is controlled through a negative-feedback process, where increased concentration of tryptophan induces a repressor-mediated interaction with the operator DNA of the *trp* operon to direct a shutdown of further mRNA synthesis for the enzymes responsible for the tryptophan biosynthetic pathway.[9] This phenomenon of repression is a two-step process: (a) end product binding to the free repressor molecules and (b) this repressor–end product complex (known as a holorepressor) then binding to the operator DNA.

This underlies the fact that the free repressor has a much lower affinity to bind to the operator and the holorepressor has a very high specific binding to the operator DNA. X-ray crystallographic studies[10] show that the *trp* repressor is a dimeric protein, and it accommodates two tryptophan molecules for the operator binding to become specific. These two binding sites are found to be of equal and identical type with respect to the ligand binding.[11] Hence, the first step in the repression process can be assumed to be given by the Scatchard equation,[12] i.e., the fraction of repressor molecules bound to tryptophan (P) is given by

$$\frac{R'}{R_t} = \frac{nP}{P + K_d} \qquad R_t = R + R' \tag{1}$$

where R, R', and R_t are the free, bound (holorepressor), and total repressor concentration, respectively, P is the end product (tryptophan) concentration, n is the number of binding sites, and K_d is the dissociation constant of the ligand binding reaction.

On reacting with the free operator sites (O^-), the holorepressor binds to the operator presumably in a linear fashion. According to the operon concept,[13] the transcription of the operon is proportional to the fraction of unrepressed operators (O^-/O_t). On transcription the mRNA (M) for the *trp* operon is translated to the enzymes, which then act in a coordinated fashion on the metabolic pathway for producing the amino acid tryptophan.[9] For the sake of simplicity (which, nevertheless, retains the essence of the processes concerned), the model is developed for a three-step process, i.e., it is assumed that the end product, tryptophan (P), production is mediated by a single enzyme E. The production and degradation functions of both E and P are assumed to be of first order. The equations describing the time evolution of this three-step process are

$$\frac{dM}{dt} = (K_m D) f\left(\frac{O^-}{O_t}\right) - K_1 M$$

$$\frac{dE}{dt} = K_e M - K_2 E$$

$$\frac{dP}{dt} = K_P E - K_D P - V_{\max} \tag{2}$$

where K_1, K_2, and K_D are the degradation and dilution rates of M, E, and P, respectively; K_m, K_e, and K_P are the production rates; D is the gene dosage; and $V_{\max}$ is the rate of end product utilization (say, use of tryptophan in protein synthesis in the cell). Also, O^-/O_t is the fraction of unrepressed operators in the cell. The function $f\ (O^-/O_t)$ is obtained from the reactions

$$R' + O^- \underset{h_2}{\overset{h_1}{\rightleftharpoons}} O^+ \qquad O_t = O^- + O^+$$

where O^-, O^+, and O_t are the free, bound, and total operators in the cell. At equilibrium

$$\frac{O^-}{O_t} = \frac{1}{1 + KR'} = \frac{1}{1 + K[nR_tP/(P + K_d)]}$$

$$= \frac{P + K_d}{K_d + (1 + nR_tK)P} \tag{3}$$

where K is the association constant ($=h_1/h_2$) for the operator–holorepressor interaction. Here R' is from Eq. (1).

Using the dissociation constant $K_0 = 1/K$ and substituting $r = nR_t/K_0$, the above function can be written as

$$\frac{O^-}{O_t} = \left(\frac{r}{1 + r}\right)\frac{K_d}{K_d + (1 + r)P} + \frac{1}{1 + r}$$

Hence Eq. (2) can be written as

$$\frac{dM}{dt} = \left(\frac{r}{1 + r}\right)\frac{DK_mK_d}{K_d + (1 + r)P} + \frac{DK_m}{1 + r} - K_1 M$$

$$\frac{dE}{dt} = K_e M - K_2 E$$

$$\frac{dP}{dt} = K_p E - K_D P - V_{\max} \tag{4}$$

The preceding set of equations now models the kinetics of the tryptophan synthesis system in a bacterial cell.

For mathematical convenience Eqs. (4) are cast into dimensionless form by choosing

$$x = M\frac{K_e K_p t_0^2}{K_d} \qquad y = E\frac{K_p t_0}{K_d}$$

$$z = \frac{P}{K_d} \qquad T = t\frac{DK_m K_e K_p}{K_d}$$

The equations are

$$\frac{dx}{dT} = \left(\frac{r}{1+r}\right)\frac{1}{1+(1+r)z} + \frac{1}{1+r} - \alpha_1 x$$

$$\frac{dy}{dT} = x - \alpha_2 y$$

$$\frac{dz}{dT} = y - \alpha_3 z - g \tag{5}$$

where $\alpha_1 = K_1 t_0$, $\alpha_2 = K_2 t_0$, $\alpha_3 = K_D t_0$, and $g = V_{max} t_0 / K_d$. Equation (5) can now be solved for different parameter values. Since the parameter r contains the dissociation constant of the holorepressor–operator binding, our model can describe strains that may have different affinity of repressor–operator binding due to mutations at the repressor gene or operator sites. This model hence empowers one to study the behavior of such mutated or altered strains and predict their synthesis pattern.

METHOD

To study the system described by Eq. (4), first the steady states were obtained and then the stability of the real and positive steady state was examined using linear theory.[14] The behavior of the system was studied by simulating equations[4] numerically on a CDC-Cyber computer using a method to integrate a stiff system of first-order differential equations following the variable-order variable-step Gear method.[15]

The parameter values were chosen from existing experimental literature.[8,16] The basal values used for the study are

$$\alpha_1 = 1.0 \qquad \alpha_2 = 0.01 \qquad \alpha_3 = 0.01 \qquad g = 4$$

To find the value of r in wild-type *E. coli* cells, we chose[9,11]

$$R_t \simeq 10^{-9} \text{ mol/L} \qquad n = 2 \qquad K_0 \simeq 10^{-10} \text{ mol/L}$$

Therefore, r for a wild-type strain is 10, and $10 > r$ and $r > 10$ will represent loose-binding and superrepressing strains, respectively.

To study the system under conditions where the holorepressor–operator binding has changed due to mutations (which may not affect the tryptophan–aporepressor binding), K is changed to values that are both higher and lower than the wild-type. The assumption made here is that the total repressor concentration does not change due to such mutations.

RESULTS

Although a detailed study has been done for a fairly large range of K_0 ($10^{-3} < r < 10^{10}$; i.e., $10^{+6} < K_0 < 10^{-20}$) for four values of g and three sets of values of α_1, α_2, and α_3, to examine the changes in behavior from the normal case, here we present only those results that are representative of altered behavior.

Table I[(A)] shows the behavior of the system in normal parameter values but at different values of r (both lower and higher than normal values). The table shows that the normal operon (i.e., $r = 10$) evolves to stable values; i.e., when perturbed, the system comes back to the steady state in an exponential fashion. The same is true for the loose-binding strains (i.e., $r < 10$) and for some high values of r (up to 25). For $r = 50$ (i.e., $K_0 \simeq 4 \times 10^{11}$ mol/L) onward stability of the steady state changes, and increasing amplitudes of oscillations are observed in the synthesis pattern of the end product z. Figures 1(A) and (B) show the behavior of z on the simulation of the equations for $r = 25$ and $r = 50$. Table I [(B)–(F)] gives the results of the above-mentioned study for $\alpha_1 = 0.5, 1.5$, $\alpha_2 = 0.001, 1.0$, and $\alpha_3 = 0.001, 1.0$ keeping the other parameters constant to the basal level in each case. Only those values of r are presented that correspond to the normal operon and where the stability of the steady state changed. The normal operon showed stability for all the cases. Changing the value of α_1 did not affect the stability of the steady state very much compared to the normal operon, but decreasing α_2 and increasing α_3 had a stabilizing effect on the system even when repression was high.

A careful and exhaustive search was made to find out conditions at which the system may show instability for $g = 0$. But for all values of r ($0.01 < r < 10^{10}$) and the above-mentioned combinations of α_1, α_2, and α_3, the system evolved to the respective steady states.

In contrast, instability was observed when $g > 0$ for high values of r. Table II [(A)–(C)] shows the results for $g = 1$ for both normal parameter values and when $\alpha_1 = 0.5, 1.5$. Here too the normal operon is stable, and the steady state becomes unstable for superrepressing strains. For $g = 1$, when $\alpha_2 = 0.001$ and $\alpha_3 = 1.0$, the steady states were stable (all eigenvalues were real and negative). When $\alpha_2 = 1.0$ and $\alpha_3 = 0.001$ there were values of r where the real part of the complex eigenvalues changed sign from negative to positive, indicating that instability may arise. Integrating the equations posed a serious problem.

Table II [(D)–(I)] shows the results of the studies when g is increased, i.e., $g = 3$ for normal and altered parameter values. The normal operons (i.e., $r = 10$) for all the cases were stable. When $r = 70$ ($K_0 \simeq 3 \times 10^{-11}$ mol/L), the steady state is stable, but on simulation with a large number of initial conditions, the system showed a small attracting region in the phase space around the steady state, beyond which it shows oscillations with increasing amplitudes. Within this attracting region the system oscillates back to the steady state. In other words, the behavior of the system depended on the initial conditions. The stability property of this steady state can be termed as semistable where an unstable limit cycle may be surrounding the steady state. This behavior is seen for other combinations of parameter values also. Figure 2(a,b) represents this behavior of the system for one set of values. Here the system either returns to the

Table I. Behavior of the system in normal and altered parameter values.[a]

Serial number	g	r	Positive steady state (x, y, z)	Stability of steady state
(A)	—	1	0.5, 50.00, 4600.54	stable
	4	10	0.09, 9.11, 510.71	stable
	—	25	0.04, 4.13, 12.99	stable
	—	50	0.04, 4.00, 0.31	unstable
	—	1000	0.04, 4.00, 0.02	unstable
(B) $\alpha_1 = 0.5$—Other parameters same as basal values				
	—	10	0.18, 18.20, 1419.34	stable
	—	75	0.04, 4.02, 1.86	stable
	—	100	0.04, 4.01, 0.96	unstable
(C) $\alpha_1 = 1.5$—Other parameters same as basal values				
	—	10	0.06, 6.09, 208.70	stable
	—	25	0.04, 4.02, 1.66	stable
	—	50	0.04, 4.00, 0.45	unstable
(D) $\alpha_2 = 0.001$—Other parameters same as basal values				
	—	10	0.09, 90.92, 8691.56	stable
	—	1000	0.004, 4.00, 0.33	stable
	—	2000	0.004, 4.00, 0.14	unstable
(E) $\alpha_2 = 1.0$—Other parameters same as basal values				
			No real positive steady state	
(F) $\alpha_3 = 0.001$—Other parameters same as basal values				
	—	10	0.09, 9.1, 5092.5	stable
	—	50	0.04, 4.00, 0.92	unstable
(G) $\alpha_3 = 1.0$—Other parameters same as basal values				
	—	10	0.10, 10.37, 6.37	stable
	—	1000	0.04, 4.02, 0.02	stable
	—	2000	0.04, 4.01, 0.01	unstable

[a] Basal values of parameters are $\alpha_1 = 1$, $\alpha_2 = \alpha_3 = 0.01$.

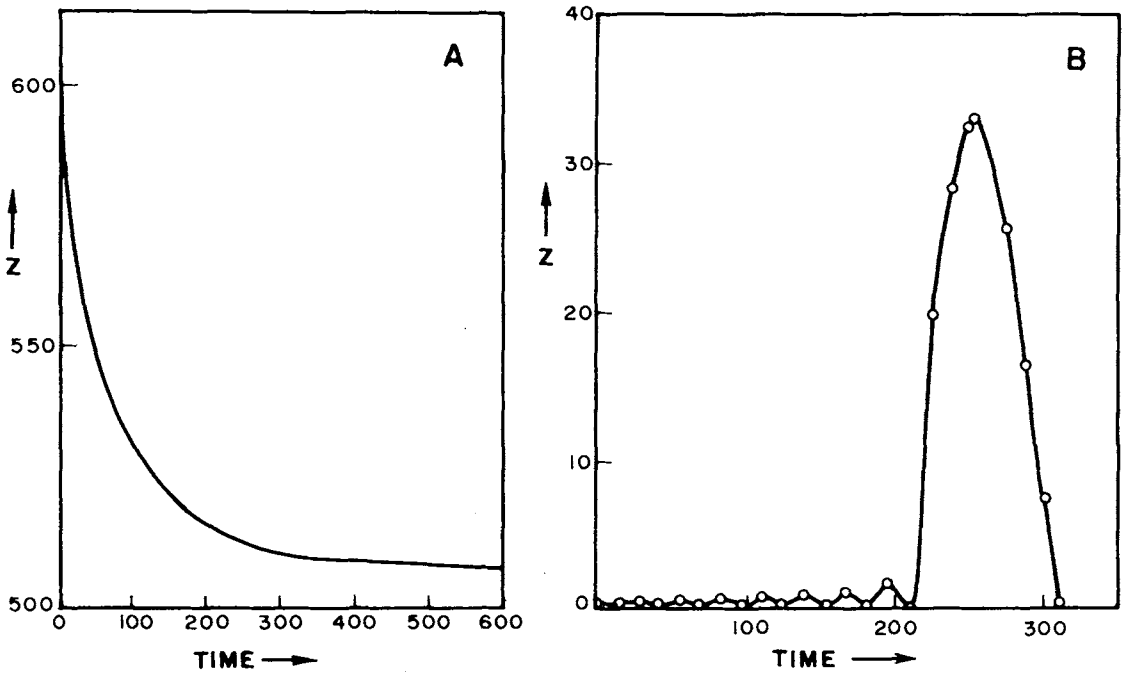

Figure 1. Tryptophan synthesis for strains showing (A) stable ($r = 25$), and (B) unstable ($r = 50$) pattern. Value of z becomes negative beyond time shown in curve in (B). Other parameter values are $g = 4$, $\alpha_1 = 1$, $\alpha_2 = \alpha_3 = 0.01$.

Table II. Behavior of the system on variation of g for normal and altered parameter values.[a]

Serial number	g	r	steady state	stability of steady state
(A)	1	10	0.09, 9.1, 810.1	stable
	—	200	0.01, 1.01, 0.96	stable
	—	500	0.01, 1.00, 0.25	unstable
(B) $\alpha_1 = 0.5$ — Other parameters same as basal values				
	—	500	0.01, 1.01, 0.65	stable
	—	1000	0.01, 1.00, 0.25	unstable
(C) $\alpha_1 = 1.5$ — Other parameters same as basal values				
	—	100	0.01, 1.02, 1.81	stable
	—	500	0.01, 1.00, 0.15	unstable
(D)	3	10	0.09, 9.1, 610.4	stable
	—	70	0.03, 3.0, 0.85	stable
	—	75	0.03, 3.0, 0.75	unstable
(E) $\alpha_1 = 0.5$ — Other parameters same as basal values				
	—	100	0.03, 3.02, 1.88	stable
	—	500	0.03, 3.00, 0.15	unstable
(F) $\alpha_1 = 1.5$ — Other parameters same as basal values				
	—	50	0.03, 3.01, 0.73	stable
	—	60	0.03, 3.00, 0.55	unstable
(G) $\alpha_2 = 0.001$ — Other parameters same as basal values				
	—	1000	0.003, 3.00, 0.49	stable
	—	5000	0.003, 3.00, 0.07	unstable
(H) $\alpha_3 = 0.001$ — Other parameters same as basal values				
	—	50	0.03, 3.0, 1.83	stable
	—	100	0.03, 3.0, 0.48	unstable
(I) $\alpha_3 = 1.0$ — Other parameters same as basal values				
	—	1000	0.03, 3.03, 0.03	stable
	—	5000	0.03, 3.01, 0.01	unstable

[a] Basal values of parameters are as in Table I.

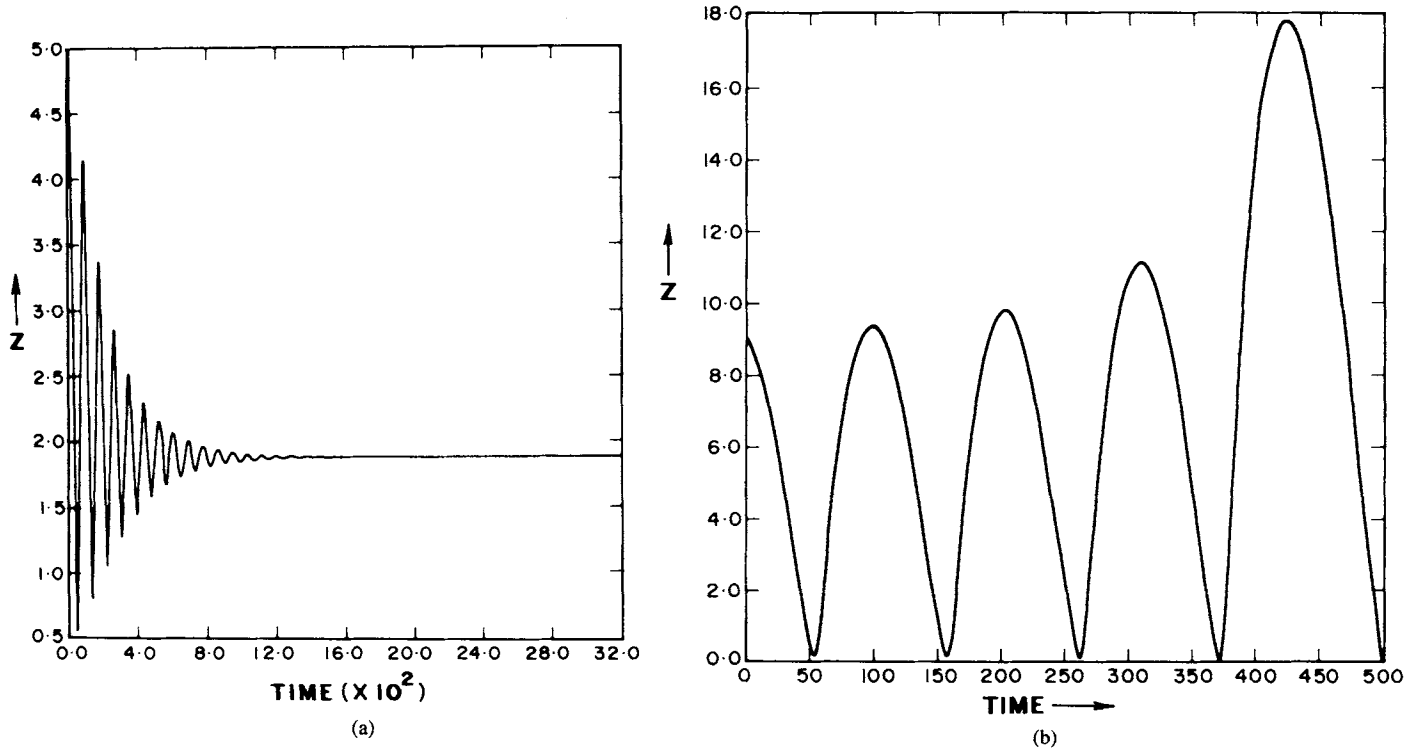

Figure 2. Pattern of tryptophan synthesis in semistable state. Stable steady state is $\bar{x} = 0.03, \bar{y} = 3.02$, and $\bar{z} = 1.88$. (a) Evolves to stable steady state periodically when perturbed close to it ($x = 0.03, y = 3.02, z = 5.0$). (b) Spirals outward when perturbed away ($x = 0.03, y = 3.02, z = 7.0$). Other parameter values are $g = 3$, $\alpha_1 = 0.5$, $\alpha_2 = \alpha_3 = 0.01$ and $r = 100$.

steady state (which is stable according to the linear stability analysis) or spirals outward for the same parameter values ($g = 3$, $\alpha_1 = 0.5$, $\alpha_2 = \alpha_3 = 0.01$) when perturbed to different extents (i.e., the initial conditions are different: $\bar{x} = 0.03$, $\bar{y} = 3.02$, for both, but $z = 5$ for Figure 2(a) and $z = 7$ for Figure 2(b). For $\alpha_2 = 1.0$, the system did not possess any positive steady state up to $r = 10^5$.

From Tables I and II it is clear that the lower the value of g, the higher the value of r needed for instability to occur. In Fig. 3 the behavior of z for the normal operon ($r = 10$) is plotted for different values of g. The figure shows that the system reaches the steady state for all values of g, but the level of z falls with increasing values of g.

Before concluding the results, it is probably necessary to mention that wherever instability occurs, this system shows the tendency to spiral out of the steady state for all parameter values; but very soon integration of the equations become impossible, possibly due to very fast changes in the dynamics of the variables concerned, which cannot be taken care of by the method of integration used. The other phenomenon observed was that the system, while spiraling out, enters the negative z phase space. Since negative concentrations are meaningless in our study, integration is stopped in such situations.

DISCUSSION

A mathematical study of the tryptophan synthesis system in *E. coli* can give information about the control elements that play an important role in increasing the yield of tryptophan in the cell. Based on these results, one can develop a tailor-made industrial bacterium from the wild type by changing its genetic information in a way that eliminates undesirable properties, accentuates the desirable ones, or introduces entirely new ones by taking advantage of mutations (self or induced).

The model developed in this article is confined to the intracellular system and is by no means complete, but it retains the essential features of the tryptophan synthesis pathway without being too complicated. There has been a detailed study of the tryptophan operon[8] where time delays and enzyme inhibition have been incorporated. But recent experiments with purified repressor protein and its interaction with L-tryptophan[11] and the crystal structure data[10] showed that the nature of interaction of the end product–operator assumed there are not correct. The two major differences with the existing model are (a) the end product–operator interaction have been replaced with a more realistic two-step process—end product–free repressor and this complex–operator interactions—and (b) the end product–repressor binding is noncooperative with two molecules of L-tryptophan binding to the dimer repressor protein as against the cooperative interaction with the four end product molecules used earlier. Introduction of these factors make our model more generally capable of describing varied situations that can be used for genetic manipulations.

Study of such models can have two aspects: (a) predictions leading to experimental applications and (b) investigation of systems from a stability point of view.

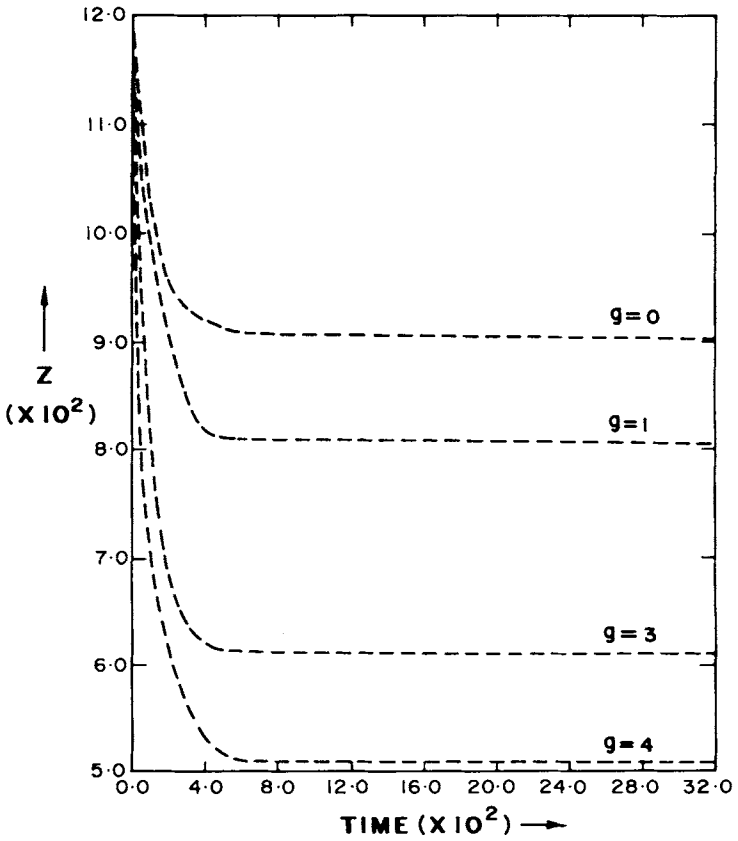

Figure 3. Stable pattern of tryptophan synthesis in normal strain ($r = 10$) for different values of g, i.e., $g = 0, 1, 3, 4$.

Since, for industrial use, overproduction of the particular amino acid (tryptophan here) is the objective, our results show various methods by which it can be achieved. Figure 3 shows one important parameter whose variation has quite strong effect on the steady-state level of tryptophan concentration in a normal operon. Here g denotes the utilization of tryptophan in protein synthesis in a cell, and among others, one way of altering g could be due to mutation in the tRNA gene resulting in the altered charged tRNA of tryptophan. Hence, a lower g results in lower charged tRNA, which incidentally also relieves the operon from "attenuation,"[17] and therefore, an increased level of tryptophan synthesis can occur. Increasing the g value also induces instability in strains with lower r, but only when g is 15, the normal operon ($r = 10$) loses its stability, though such a high value of g is very unrealistic.

There are other ways where alterations at the translational control level can have favorable effects toward increased tryptophan synthesis. Table I shows that tryptophan synthesis can be increased by 2.75 times in an otherwise normal strain if the mRNA stability is increased ($\alpha_1 = 0.5$). Though it is not very clear, mRNAs of different stabilities are known that can offer a measure of translational control.[18] Table I also shows that if the tryptophan degradation rate is reduced ($\alpha_3 = 0.001$), the synthesis can be increased almost 10 times. Mutants lacking tryptophanase are available and have already been used in strain constructions.[19] A careful analysis of Tables I and II can offer combinations of g, α_1, α_2, and α_3 with r that may yield high levels of tryptophan. Both superrepressing and loose-binding strains of *E. coli* are available obtained by mutating the *trpR* gene.[7] Controls can also be loosened at different points in the branched metabolic pathway for hyperproduction of tryptophan by altering the enzymes and common intermediates,[20] a feature not included in the present model.

The unstable strains do not seem to be particularly interesting from the production point of view; but their behavior would be interesting to study both theoretically and experimentally. It was observed that in most cases the positive steady state is accompanied by another steady state where only z is negative. Whenever the positive steady state is unstable, the other one is stable and the system gets attracted toward it. Hence, one observes the behavior shown in Fig. 1(b).

The semistable state [Fig. 2(a,b)] is an interesting example of behavior. Here the pattern of tryptophan synthesis depends on its initial concentration. The steady state is stable according to linear stability analysis, and z does spiral inward to the stable point when perturbed close to it. But this attracting region is quite small in the phase space, and the system spirals outward when simulated at that region showing unstable behavior. It essentially gets attracted toward the other steady state (with negative z). Although, during the study, we never encountered a stable limit cycle behavior of z, this semistable situation can be compared to an unstable limit cycle surrounding the stable point.

Studies on mathematical models describing gene expression in bacteria, such as the one here, give an insight into the regulatory processes and allows one to decide on future experiments or production plans. The implementation of the predictions depend on available technology. With recombinant DNA techniques one can produce strains with altered genetic and metabolic circuit of tryptophan synthesis in *E. coli* following a proper mutational program. But there are many other factors that should be kept in mind: the ability of the cell to excrete the oversynthesized product, the tolerance and survival of the organism under associated accumulation of toxic products, the reduction in vitality of the cell following several rounds of mutagenesis, and of course, the necessity to screen large numbers of mutants for the right combination of traits sequentially obtained that lead to overproduction. One method to avoid some of these problems is to assemble the appropriate characteristics in genetic backgrounds that have not been subjected to repeated mutagenesis.

To have a more complete description of affairs in the cell, many more factors, such as (a) attenuation, (b) autogenous regulation of trp repressor gene, (c) leakage and transport through cytoplasmic membrane, (d) kinetics of the enzyme in the metabolic pathway, etc., should be taken into account, some of whose mode of action are still not clearly understood. Some of these, e.g., (c), can be effectively included in the terms which describe the dilution and utilization of tryptophan in the cell. In practice, inducing the cells to excrete the amino acid in large quantities is performed by allowing the membrane to develop leaks through chemical means. When mechanisms governing the excretion are well understood, it should be possible to apply recombinant DNA techniques to create leaky membranes. In such a situation the model should include a description of the excretion phenomena explicitly, since it would not allow the buildup of the intracellular amino acid pool concentration, which may have considerable effect on the dynamics of the system.

The maximum production of L-tryptophan in *E. coli* using genetic engineering methods has not been high enough as compared to other amino acids, such as lysine or glutamate; and the constructed strain loses its phenotype soon.[21] Although instability of the genetically engineered strains seems to be a problem, other methods of strain construction, such as transduction, transformation, conjugation, etc., are also being used to overcome the difficulties.

The results obtained from analytical and numerical studies on the model of the tryptophan synthesis system could possibly help in indicating new alterations that may help in designing the right kinds of organisms for overproduction.

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