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Journal of Biotechnology 58 (1997) 125–140

JOURNAL OF Biotechnology

Model analysis concerning the effects of growth rate and intracellular tryptophan level on the stability and dynamics of tryptophan biosynthesis in bacteria

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Received 26 February 1997; received in revised form 4 August 1997; accepted 7 August 1997

Abstract

An extended mathematical model for the biosynthesis of tryptophan (*trp*) in bacteria is proposed. The model considers both feedback inhibition of the biosynthetic enzymes and repression of the *trp* operon by tryptophan and explicitly takes into account the growth rate and the demand of tryptophan for protein synthesis. The intracellular concentration of tryptophan is found to drastically decline with the growth rate, particularly at low values of growth rate. This also applies to genetically modified strains with reduced strain levels of the repressor and/or alleviated feedback inhibition. At low growth rates the intracellular concentrations of tryptophan and the synthetic enzymes are also strongly affected by the strain level of repressor, feedback inhibition and the consumption of tryptophan for protein synthesis. It can be concluded that for an effective production of tryptophan the growth rate of cells should be kept as low as possible. Another important factor for the production of tryptophan is the stability and dynamics of the *trp* operon. Model analysis revealed that the intracellular concentration of tryptophan is determinant for the occurrence of oscillatory expression of the *trp* operon. Instability of tryptophan synthesis occurs at low intracellular tryptophan concentrations, irrespective of the growth rate. A reduction of the repressor level can reduce the instability region so that a stable operation with improved production is possible. In addition, a high demand of tryptophan for protein synthesis should be avoided which may lead to a periodic expression of the *trp* operon, even at low growth rate. © 1997 Elsevier Science B.V.

Keywords: Tryptophan; *trp* operon; Overproduction; Stability; Dynamics; Growth rate

1. Introduction

Tryptophan is one of the amino acids commercially produced by fermentation using genetically

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PII S0168-1656(97)00143-0

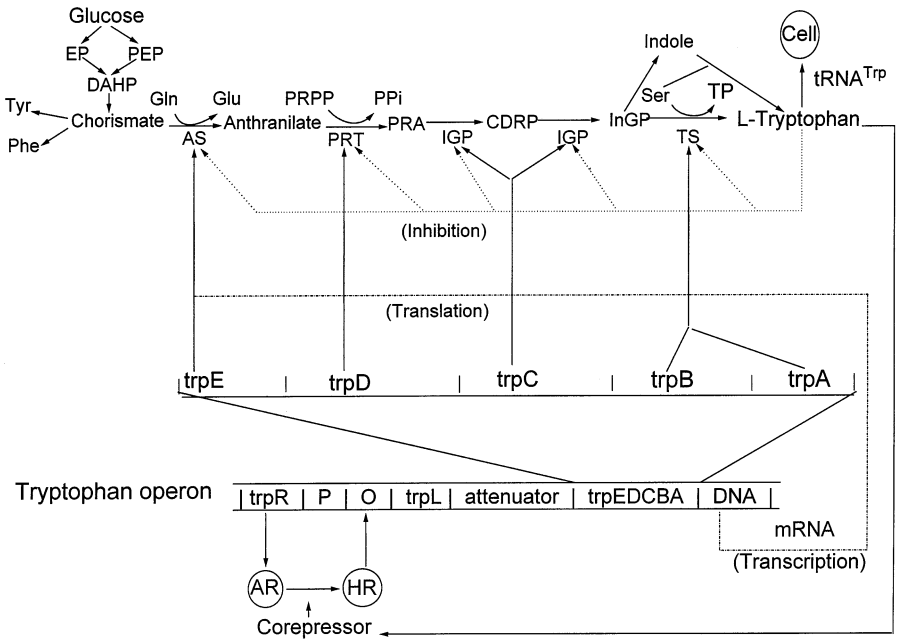

Fig. 1. The main pathway of L-tryptophan biosynthesis and regulatory and structural gene regions of the *trp* operon of *E. coli*. Abbreviations: EP, D-erythrose-4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabinoheptulosonate-7-phosphate; Tyr, tyrosine; Phe, phenylalanine; Gln, glutamine; Glu, glutamate; PRPP, 5-phosphoribosyl-1-pyrophosphate; Ppi, pyrophosphate; PRA, 5-phosphoribosyl anthranilate; CDRP, 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate; InGP, indole-3-glycerol phosphate; Ser, serine; TP, triose phosphate; AS, anthranilate synthase; PRT, phosphoribosyl anthranilate transferase; IGP, indole glycerol phosphate synthase; TS, tryptophan synthase; P, promotor; O, operator; AR, aporepressor; HR, holorepressor.

modified microorganisms. Compared to the chemical route which produces a mixture of the D- and L-forms of tryptophan the biological process yields a biologically pure active L-isomer. Different microorganisms such as *Escherichia coli*, *Corynebacterium glutamicum* and *Saccharomyces cerevisiae* have been studied for the overproduction of tryptophan. However, the productivity of tryptophan remains relatively low compared to the microbial production of other amino acids such as lysine and glutamate in spite of much efforts from both molecular biology and bioprocess engineering (Jetton and Sinskey, 1995; Krämer, 1996). This seems to be partly due to the complex multiple regulations at several steps in the biosynthetic pathway of tryptophan and the difficulty of deregulation over the whole pathway. As shown in Fig. 1, the synthesis of tryptophan from chorismate, a common precursor for aromatic amino acid, is catalyzed by four enzymes: anthranilate synthase (AS), anthranilate phosphoribosyl transferase (PRT), indole glycerol phosphate synthase (IGP) and tryptophan synthase (TS). The levels of these enzymes depend on five structural genes coded in the tryptophan operon (*trpEDCBA*). The tryptophan synthesis in bacteria such as *E. coli* and *C. glutamicum* is primarily controlled through feedback inhibitions of the end product tryptophan on the enzymes and repression of the *trp* operon at transcriptional level. Termination of the operon transcription by attenuation is also involved at sufficient supply and/or high intracellular concentration of tryptophan (Yanofsky and Crawford, 1987; Yanofsky and Horn, 1994). These regulation mechanisms give rise to complicated dynamic behaviour of the operon. It has been reported that under certain conditions such as high demand of tryptophan for protein synthesis of cells and/or loose of feedback inhibition the *trp* operon becomes unstable, lead-

ing to a reduced and/or periodic synthesis of tryptophan (Bliss et al., 1982; Sinha, 1988; Sen and Liu, 1990).

Several mathematical models have been proposed to quantitatively understand the regulation and dynamics of the *trp* operon and tryptophan biosynthesis. Goodwin (1965) was the first who postulated a gene-enzyme-endproduct control unit model with a single negative feedback control loop. This model was generalized by Griffith (1968a,b) who considered a cooperative repression. Both models did not consider the feedback inhibition of tryptophan on the enzymes. This effect was taken into account by Bliss et al. (1982). Bliss et al. (1982) also introduced time delays between the initiation of transcription and the initiation of translation, and between the initiation of translation and the appearance of the functional enzymes. Furthermore, the effect of consumption of endproduct in protein synthesis was considered. The model predictions of Bliss et al. (1982) showed qualitative agreement with experimental results showing oscillation in tryptophan synthesis. However, later X-ray crystallographic experiments showed that the first step in the endproduct repression is noncooperative (Arvidson et al., 1986). Based on this observation, an improved mathematical model for the *trp* operon was developed by Sinha (1988) in which the repression was considered to occur in two steps. First, tryptophan (as a corepressor) binds to the inactive repressor protein (aporepressor), forming a holorepressor complex. This complex then binds specifically to the *trp* operon and prevents the movement of RNA polymerase, resulting in blocking transcription of the structural genes into mRNA. As a consequence, the enzymes are not formed and the tryptophan biosynthesis stops. Sen and Liu (1990) presented a modified version and a more detailed analysis of Sinha's model by using a non-linear Michaelis–Menten function instead of a constant rate to describe the demand of tryptophan for protein synthesis. In the works of Sinha (1988) and Sen and Liu (1990) the feedback inhibition of tryptophan on the enzymes was neglected. More recently, Koh and Yap (1993) proposed a structured model for the *trp* repressor/operator interactions.

None of the works mentioned above explicitly investigated the effect of growth rate on the stability and gene expression of the *trp* operon. From a process engineering point of view the growth rate of a culture is one of the important parameters affecting the productivity of a bioprocess. For a commodity chemical such as tryptophan a continuous operation with a not too low dilution rate may be desirable for an economic production. To this end, a quantitative knowledge of the stability of the expression system as affected by the growth rate is needed. This is particularly important when using genetically modified microorganisms. For recombinant plasmids it has been shown that the growth rate or dilution rate in continuous culture is one of the key environmental factors causing loss of plasmids and hence reduction of gene expression (Nancib and Boudrant, 1992; Hellmuth et al., 1994).

Another important factor for an overproduction of amino acids is the effective excretion of final products (Krämer, 1994). Among others, this may be achieved either by a high intracellular product concentration level or by an effective excretion system. An effective excretion will normally lead to a low intracellular product level. Because tryptophan is both an inhibitor for the enzymes synthesized and a corepressor of the *trp* operon it seems that an effective excretion would be a more proper strategy than a high intracellular tryptophan concentration. This is also in line with the strategy used by Ikeda and Katsumata (1994) for the overproduction of amino acids including tryptophan in *C. glutamicum*. They proposed to reduce the intracellular pool of a certain amino acid by preventing the uptake of the amino acid accumulated extracellularly. However, the results of Bliss et al. (1982) suggested that an intrinsic instability of the *trp* operon would arise from a weak feedback inhibition of tryptophan, i.e. from a reduced intracellular level of tryptophan. So far, the effect of intracellular tryptophan level on the stability and dynamics of the *trp* operon has not been systematically studied.

The objective of this work is 3-fold: first, to present a more general mathematical model for the *trp* operon in bacteria by considering both feedback inhibition of the enzyme(s) and repres-

sion of the tryptophan operon and by expressing the growth rate as an explicit variable; second, to investigate the effects of growth rate on the synthesis of tryptophan and its stability under variation of several model parameters, particularly the demand of tryptophan for protein synthesis; and finally, to examine the stability of the *trp* operon as a function of the intracellular tryptophan concentration.

2. Mathematical model

A mathematical model is set up to consider both the repression of the *trp* operon and the feedback inhibition of the enzymes by tryptophan (Fig. 1). According to Sinha (1988) the repression can be considered to be a two-step process. First, tryptophan (P) binds to a free repressor (R) to form an endproduct-repressor complex, P_nR (holorepressor). The repressor may have n binding sites. P_nR then binds to an operator (O) to form a holorepressor-operator complex P_nRO (bound operator). The reactions may be formulated as follows:

$$P + R \underset{k_2}{\overset{k_1}{\leftrightarrow}} PR \tag{1.1}$$

$$\mathrm{PR} + \mathrm{P} \underset{k'_2}{\overset{k'_1}{\leftrightarrow}} \mathrm{P_2R} \tag{1.2}$$

.....

$$P_{n-1}R + P \underset{k^n_2}{\overset{k^n_1}{\leftrightarrow}} P_nR \tag{1.n}$$

$$P_nR + O \underset{k_4}{\overset{k_3}{\leftrightarrow}} P_nRO \tag{2}$$

According to Arvidson et al. (1986) the interaction of the aporepressor with its ligand (tryptophan) can be described by the Scatchard equation, indicating that the first reaction Eq. (1.1) is the limiting step for the formation of the holorepressor P_nR. The fraction of repressor bound to tryptophan is therefore independent on the number of binding sites. In a similar way like Sinha (1988) the holorepressor and free operator concentrations at equilibrium can be derived as:

$$[P_nR] = \frac{R_t P}{P + K_d} \tag{3}$$

$$[O] = \frac{K_o O_t}{K_o + [P_nR]} = \frac{K_o O_t (P + K_d)}{K_o (P + K_d) + P R_t} = \frac{O_t (P + K_d)}{P + K_d + rP} \tag{4}$$

where R_t and O_t are the total repressor and operator concentrations, respectively. K_d and K_o are the dissociation constants of the holorepressor and the bound operator:

$$K_d = \frac{k_2}{k_1}; \quad K_o = \frac{k_4}{k_3};$$

r in Eq. (4) is defined as a dimensionless parameter characterizing the strain level of *trp* operon (repressor):

$$r = \frac{R_t}{K_o}$$

Sinha (1988) proposed a three-step process for tryptophan synthesis. First, the transcription of the operon yielded mRNA (M) which was then translated to form the enzymes. For simplicity, the production of tryptophan was assumed to be mediated by a single enzyme (E). Furthermore, the production functions for E and P were assumed to be of first order. The degradations of M, E and P were also considered. However, the dilution of M, E and P due to cell growth were not taken into account. This is considered in the present work. The influence of growth rate on the protein content is also taken into consideration. Furthermore, the inhibition of enzymes and the consumption of tryptophan for activating the aporepressor are simultaneously included in our model. The equations governing the tryptophan synthesis are formulated as follows:

$$\frac{dM}{dt} = K_m D[O] - (K_1 + \mu)M \tag{5a}$$

$$\frac{dE}{dt} = K_e M - (K_2 + \mu)E \tag{5b}$$

$$\frac{dP}{dt} = K_p E \frac{K_I^2}{K_I^2 + P^2} - (K_3 + \mu)P - nk_2[P_nR] - (P^m_{pro} + \beta\mu)\mu C \frac{P}{P + K_s} \tag{5c}$$

where M is the mRNA concentration and E represents a single enzyme in the synthetic pathway

of tryptophan for simplicity; D is the gene dosage; K_m, K_e, and K_p are rate constants for the formations of the species denoted by the appropriate subscripts, respectively; K_1, K_2 and K_3 are rate constants for the degradation of mRNA, enzyme and tryptophan; μ is the specific growth rate of cells.

Eq. (5c) describes the change of intracellular tryptophan concentration (P) as a function of time. The first term in the right-hand side of Eq. (5c) expresses the production of tryptophan catalyzed by an enzyme and the feedback inhibition of tryptophan on the enzyme. K_I is the concentration of tryptophan giving 50% inhibition of the enzyme activity. A Hill function is used to describe the cooperative inhibition and the cooperative coefficient is estimated as 2 from experimental data in the literature (Bliss et al., 1982; Tsunekawa et al., 1989; Katsumata and Ikeda, 1993). The second term is the degradation and dilution of tryptophan due to cell growth. The third one represents the demand of tryptophan bound to the aporepressor. The last term is the consumption of tryptophan for cellular protein synthesis. A hyperbolic saturation function of the Michaelis–Menten type is used (Sen and Liu, 1990). K_s is a saturation constant. The maximum demand of tryptophan for biosynthesis is expressed as a function of protein content of the cells which is in turn assumed to be a linear function of the growth rate (Calcott and MacLeod, 1974; Marr, 1991). C is the molar percentage of tryptophan in cellular protein. P^m_{pro} is the maximum protein concentration. β is a constant expressing the influence of growth rate on the protein content of cells. It may be positive or negative depending on the nature of growth limitation (Calcott and MacLeod, 1974).

For convenience the above equations can be rewritten in dimensionless forms by defining:

$$x = M\frac{K_eK_pT^2}{K_d};\quad y = E\frac{K_pT}{K_d};\quad z = \frac{P}{K_d};\quad \tau = \frac{t}{T};$$

$$u = T\mu;\quad T = \left[\frac{K_d}{K_mDO_tK_eK_p}\right]^{1/3}$$

where x, y, z, τ and u are the dimensionless mRNA, enzyme, tryptophan concentration, time, and growth rate, respectively. By using these notations and substituting Eqs. (3) and (4) into Eqs. (5a) and (5c), the following dimensionless equations are obtained:

$$\frac{dx}{d\tau} = \frac{z+1}{1+(1+r)z} - (\alpha_1+u)x \tag{6a}$$

$$\frac{dy}{d\tau} = x - (\alpha_2+u)y \tag{6b}$$

$$\frac{dz}{d\tau} = y\frac{k_i^2}{k_i^2+z^2} - (\alpha_3+u)z - \alpha_4\frac{z}{1+z} - \alpha_5(1+\alpha_6u)u\frac{z}{z+k} \tag{6c}$$

The dimensionless parameters appearing in Eqs. (6a), (6b) and (6c) are all positive except for α_6 and have the definitions:

$$\alpha_1 = TK_1;\quad \alpha_2 = TK_2;\quad \alpha_3 = TK_3;$$

$$\alpha_4 = \frac{nk_2R_tT}{K_d} = nk_1R_tT;\quad \alpha_5 = \frac{CP^m_{pro}}{K_d};$$

$$\alpha_6 = \frac{\beta}{P^m_{pro}T};\quad k = \frac{K_s}{K_d};\quad k_i = \frac{K_I}{K_d}$$

3. Numerical method and choice of parameters

By letting the left hands of Eqs. (6a), (6b) and (6c) be zero a steady state solution (x_0, y_0, z_0) can be obtained:

$$x_0 = \frac{1}{\alpha_1+u}\frac{1+z_0}{1+(1+r)z_0} \tag{7a}$$

$$y_0 = \frac{1}{(\alpha_1+u)(\alpha_2+u)}\frac{1+z_0}{1+(1+r)z_0} \tag{7b}$$

$$\frac{1}{(\alpha_1+u)(\alpha_2+u)}\frac{1+z_0}{1+(1+r)z_0}\frac{k_i^2}{k_i^2+z_0^2} = (\alpha_3+u)z_0 + \alpha_4\frac{z_0}{1+z_0} + \frac{\alpha_5(1+\alpha_6u)u\,z_0}{k+z_0} \tag{7c}$$

At a given growth rate and a set of fixed parameters the left hand of Eq. (7c) decreases monotonically for $z_0 > 0$; the right hand increases monotonically for positive values of z_0. Therefore, there is an unique positive steady state solution of

z_0 satisfying Eq. (7c). A Gauss–Newton least squares method supplied in the software MATLAB was used to obtain the steady state solution.

The method of stability analysis is similar to that described by Xiu et al. (1997). Local stability of a steady state can be analyzed by linearizing the differential equations of the system (Eqs. 6a, 6b and 6c) around the steady state and calculating the eigenvalues of the Jacobian matrix. The Jacobian matrix at the steady state (x_0, y_0, z_0) is as follows:

$$J = \begin{vmatrix} -(\alpha_1+u) & 0 & -v \\ 1 & -(\alpha_2+u) & 0 \\ 0 & s & -(\alpha_3+u+w) \end{vmatrix} \quad (8)$$

where

$$v = \frac{r}{(1+(1+r)z_0)^2};$$

$$w = \frac{\alpha_4}{(1+z_0)^2} + \frac{k\alpha_5(1+\alpha_6 u)u}{(k+z_0)^2} + \frac{2k_i^2 y_0 z_0}{(k_i^2+z_0^2)^2};$$

$$s = \frac{k_i^2}{k_i^2+z_0^2}$$

The eigenvalues of matrix J are the roots of the characteristic equation:

$$\lambda^3 + c_1\lambda^2 + c_2\lambda + c_3 = 0 \quad (9)$$

where

$$c_1 = \alpha_1+\alpha_2+\alpha_3+3u+w;$$

$$c_2 = (\alpha_1+u)(\alpha_2+u)+(\alpha_2+u)(\alpha_3+u+w) + (\alpha_1+u)(\alpha_3+u+w);$$

$$c_3 = (\alpha_1+u)(\alpha_2+u)(\alpha_3+u+w)+s\,v$$

According to the Routh–Hurwitz criterion, the necessary and sufficient condition of Hopf bifurcation is:

$$c_1 > 0; \quad c_3 > 0; \quad \text{and} \quad B = c_1c_2 - c_3 = 0 \quad (10)$$

The eigenvalues generally the form of conjugate pairs:

$$\lambda = p \pm q\mathrm{i} \quad (11)$$

where p and q are real numbers and i is the imaginary notation. If the real parts of all the eigenvalues are negative ($q = 0$), the steady state is said to be locally asymptotically stable. In case some or one of the real parts of the eigenvalues are positive, the steady state is unstable. If $q \neq 0$ the system will display oscillation after perturbation. Depending on the sign of p the system may have damped oscillations or excited oscillations. In the particular case where the Jacobian matrix has a pair of purely imaginary eigenvalues ($p = 0$), a Hopf bifurcation can occur which results in a sustained periodic oscillation around the steady state.

Dynamic behavior around a steady state can be also used to analyze the stability of the steady state. A steady state is said to be locally stable if the system returns to the pertinent steady state after a sufficiently small but otherwise arbitrary perturbation from the steady state. For dynamic simulation of the differential Eqs. (6a), (6b) and (6c) Runge–Kutta formulas with 4th and 5th orders in MATLAB is applied.

The kinetic constants for Eqs. (3)–(5a), (5b), (5c), (6a), (6b) and (6c) are chosen from literature which are given in Table 1. The degradation of tryptophan is omitted. The remaining parameters introduced in this work are estimated as follows.

From the definition of α_1 we can obtain the value of T as:

$$T = \alpha_1/K_1 = 0.0156 \text{ h}$$

The basal dimensionless model parameters r and α_4 can then be calculated as 10 and 0.024, respectively.

Marr (1991) derived an equation describing the relationship between the molar total amino acids of protein (based on the intracellular volume of cells) and the specific growth rate of *E. coli*:

$$P_{\text{pro}} = (2.64 - 0.32\mu) \times 10^6 \ (\text{mol l}^{-1}) \quad (12)$$

Based on Eq. (12), the following parameter values are obtained:

$$P_{\text{pro}}^{\text{m}} = 2.64 \times 10^6 \text{ M}; \ \beta = -0.32 \times 10^6 \text{ M h}^{-1};$$

$$\alpha_6 = \beta / P_{\text{pro}}^{\text{m}} T = -7.5$$

Table 1
Kinetic parameters chosen for Eqs. (3)–(5a), (5b) and (5c) associated with the *trp* operon and tryptophan biosynthesis in bacteria

Parameter	Reported value	Value used in this study	Reference
C	1.1%	1.1%	Neidhardt et al. (1990)
K_1	57.6 h^{-1}	57.6 h^{-1}	Bliss et al. (1982)
k_2	7200 h^{-1}	7200 h^{-1}	Bliss et al. (1982)
K_d	4×10^{-5} mol l^{-1}	10^{-5} mol l^{-1}	Arvidson et al. (1986)
K_o	10^{-10}, 2×10^{-10} mol l^{-1}	10^{-10} mol l^{-1}	Sinha (1988), Hurlburt and Yanofsky (1992)
n	2	2	Arvidson et al. (1986)
R_t	10^{-9} mol l^{-1}	10^{-9} mol l^{-1}	Sinha (1988)
α_1	1	0.9[a]	Sinha (1988)
α_2	0.01	0.02[a]	Sinha (1988)
α_3	0.01	0[a]	Sinha (1988)
k	0.002–0.008	0.005	Sen and Liu (1990)

[a] The parameters α_1, α_2, and α_3 used in this work have different meanings from those of Sinha (1988).

The maximum tryptophan demand (i.e. $\alpha_5(1+\alpha_6 u)u$) in Eq. (6c) was taken as 4 by Sen and Liu (1990) for a wild-type strain of *E. coli* at a growth rate 0.72 h^{-1}. According to these authors we can obtain the value for α_5:

$$\alpha_5 = 4/(0.01\times(1+0.01\alpha_6)) = 430$$

In fact, the cellular protein content of *E. coli* varies not only with growth rate but also with nutritional status (Calcott and MacLeod, 1974). For example, in an ammonium-limited chemostat culture the cellular protein content increased as growth rate shifted up. In this case β and α_6 are positive.

K_I appearing in Eq. (5c) is difficult to determine because four enzymes in the biosynthetic pathway of tryptophan are subject to the feedback inhibition of tryptophan. Each enzyme has a respective K_I value. For instance, K_I for TS in *E. coli* is 22.83 mM; K_I for AS and PRT in *C. glutamicum* mutant are 4.0 and 4.8 mM, respectively (Katsumata and Ikeda, 1993). In this study K_I is chosen as 22.83 mM and the corresponding dimensionless parameter $k_i = 2283$.

The maximum specific growth rate (μ_m) of *E. coli* is normally lower than 2 h^{-1}. Therefore, the corresponding maximum dimensionless growth rate $u_m = T\mu_m = 0.0312$. This value is quite small. For convenience, a relative growth rate (u_R) is defined here to replace the dimensionless growth rate (u):

$$u_R = \frac{\mu}{\mu_m} = \frac{u}{u_m} \tag{13}$$

4. Results and discussion

In the model Eqs. (6a), (6b) and (6c) there are all together nine dimensionless parameters which have different physical meanings. In this work, emphasis is laid on investigating the effect of growth rate on the tryptophan production and the stability of tryptophan operon under variation of each of the following parameters r, α_4, k_i, α_5 and α_6 with a fixed set of parameter values $\alpha_1 = 0.9$, $\alpha_2 = 0.02$, $\alpha_3 = 0$, $k = 0.005$.

The steady state solutions of Eqs. (6a), (6b) and (6c) at different growth rates and strain levels are depicted in Fig. 2. The intracellular tryptophan concentration decreases as the growth rate increases, particularly profound at low growth rate (Fig. 2A). The enzyme concentration decreases at first and then increases as the growth rate increases (Fig. 2B). The concentration of mRNA remains constant at low growth rate but begins to increase above a certain growth rate which corresponds the growth rate at which enzyme concentration increases (Fig. 2C). At a strain level of $r = 10$ which corresponds to that of a typical wild-type strain of *E. coli* the concentration of mRNA increases with dilution rate when $u_R > 0.3$. This is in accordance with the experimental find-

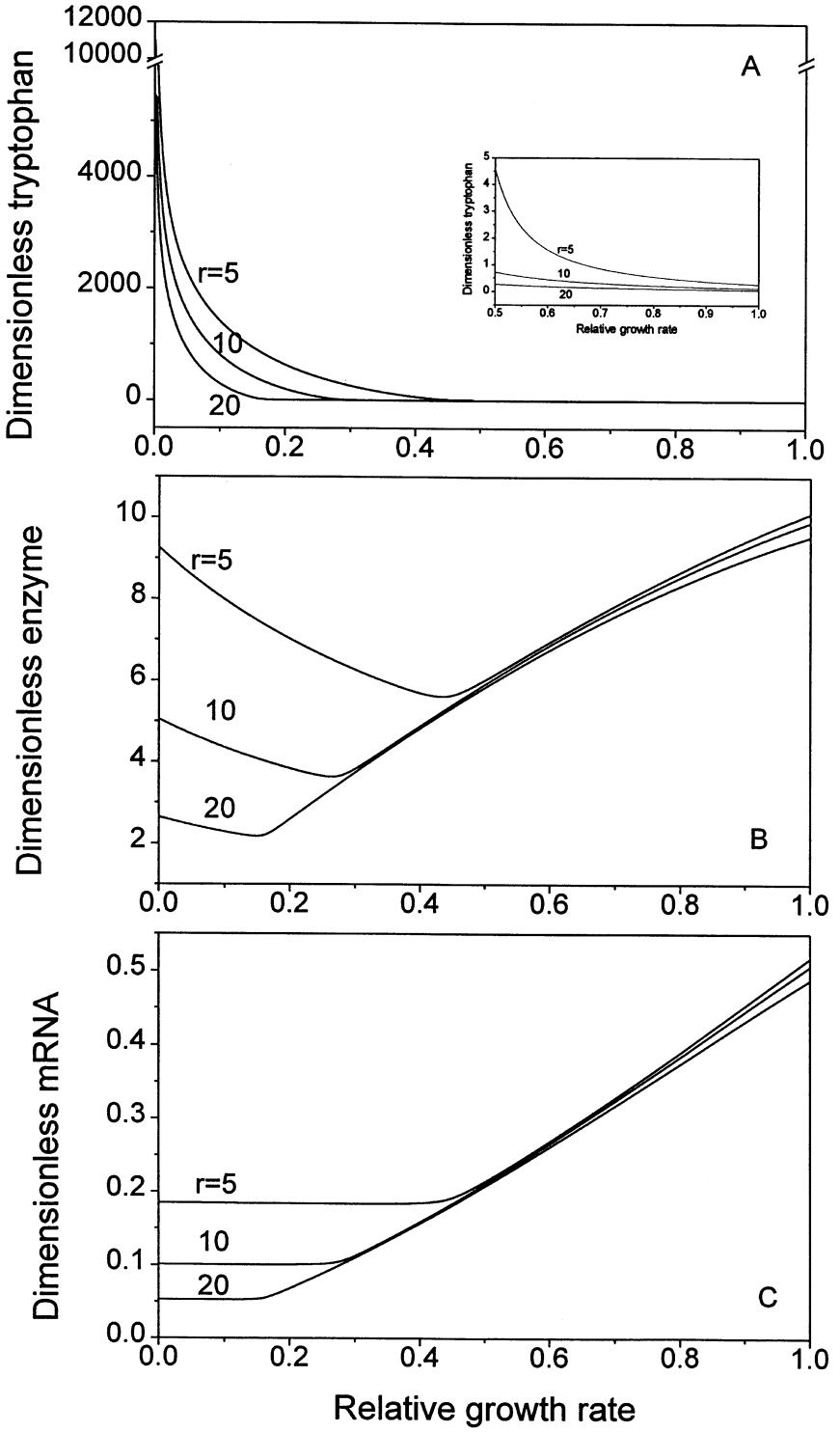

Fig. 2. Effect of growth rate on the concentrations of intracellular tryptophan (A), enzyme (B), and mRNA (C) at three different strain levels of the *trp* operon repressor *r*. Results are calculated with Eqs. (7a), (7b) and (7c) and the following dimensionless parameters: $\alpha_1 = 0.9$, $\alpha_2 = 0.02$, $\alpha_3 = 0$, $\alpha_4 = 0.024$, $\alpha_5 = 430$, $\alpha_6 = -7.5$, $k = 0.005$, $k_i = 2283$.

ings for wild-type strains of *E. coli* that the ribosome content increased after the growth rate was shifted up (Bremer and Dennis, 1987; Yun et al., 1996). Because the strain level r was considered to be one of the effective genetic approaches in strain improvement for tryptophan production (Aiba et al., 1982; Sinha, 1988), the dependencies of tryptophan, enzyme and mRNA concentrations on the growth rate are also compared at different values of r. As shown in Fig. 2, r merely affects the concentrations of tryptophan, enzyme and mRNA at relatively low growth rates. It should be mentioned that according to the definition r depends on both the total aporepressor concentration (R_t) and the dissociation constant of the holorepressor-operator complex (K_0). Assuming that the reduction of r is achieved by lowering R_t the value of $\alpha_4(\alpha_4 = nk_1R_tT)$ will also be reduced. A corresponding adjustment of α_4 values gave similar results as shown in Fig. 2 (data not shown).

The effect of k_i on the tryptophan concentration at different growth rate is shown in Fig. 3A. The effect of k_i is remarkable at relatively low growth rate (e.g. < 0.2). Improved production of tryptophan can be achieved by increasing the k_i value, i.e. by preventing the sensitivities of enzymes in the tryptophan pathway to the inhibition of tryptophan. The model prediction is in qualitative agreement with the experimental results obtained for mutants of *E. coli* (Aiba et al., 1982) and *C. glutamicum* (Katsumata and Ikeda, 1993) which have rendered AS and PRT sensitivities to tryptophan inhibition. The intracellular tryptophan concentration is also very sensitive to the demand of tryptophan for protein synthesis as reflected by the value of α_5 value (Fig. 3B). Increasing α_5 results in a drastic decrease in tryptophan productivity. On the other hand, α_6 which reflects the influence of growth rate on the protein content has little effect on tryptophan production (data not shown).

From the above analysis it can be stated that the growth rate is an important process parameter in the tryptophan production, both for wild-type and genetically modified strains. In general, the intracellular tryptophan concentration decreases significantly with the growth rate, particularly at low growth rates. Obviously, for an effective production the growth rate of cells should be controlled at very low level. Cell immobilization and/or cell recycle might be a proper choice. In addition, the influences of genetic modifications of strains on their sensitivity to growth rate should be considered when developing a fermentation process.

The effect of growth rate on the tryptophan production is further discussed below with respect to stability and dynamic behavior of the *trp* operon. Fig. 4 shows a division of the operational region on a growth rate and tryptophan concentration plane according to the dynamic behavior of the steady-state solutions at three different strain levels. For the construction of Fig. 4, α_5 is assumed as a variable. Considering the eigenvalues of the Jacobian matrix (Eqs. 8 and 9) and the Routh–Hurwitz criterion (Eq. 10) the z-u_R plane can be divided into three domains. In domain I, the eigenvalues of the stability matrix have only negative real parts and the steady states are locally asymptotically stable. In domain II, the eigenvalues are complex numbers with negative real parts and the steady states exhibit damped oscillatory transient behavior. Domain III represents a region of instability in which the steady state solution is unstable, leading to excited oscillatory behavior. In contrast to its effect on tryptophan synthesis the growth rate has little influence on the stability of the system. Instead, the intracellular concentration of tryptophan appears to be a determinant factor for the instability. Whereas the stable domains I and II encompass both high and very low intracellular tryptophan concentrations, the instability region is found at relatively low intermediate intracellular tryptophan concentrations, irrespective of the growth rate and strain level. It is remarkable that the instability region enlarges at first as r decreases from 10 to 5 but then declines when r is further reduced to 2.3. For values of $r < 2$ domain III disappears (data not shown). The transient lines between domains I and II move up as r is reduced.

The results of Fig. 4 appear to agree well with the experimental results of Bliss (1979) who found oscillating intracellular concentration of tryptophan with a feedback-resistant *E. coli* strain

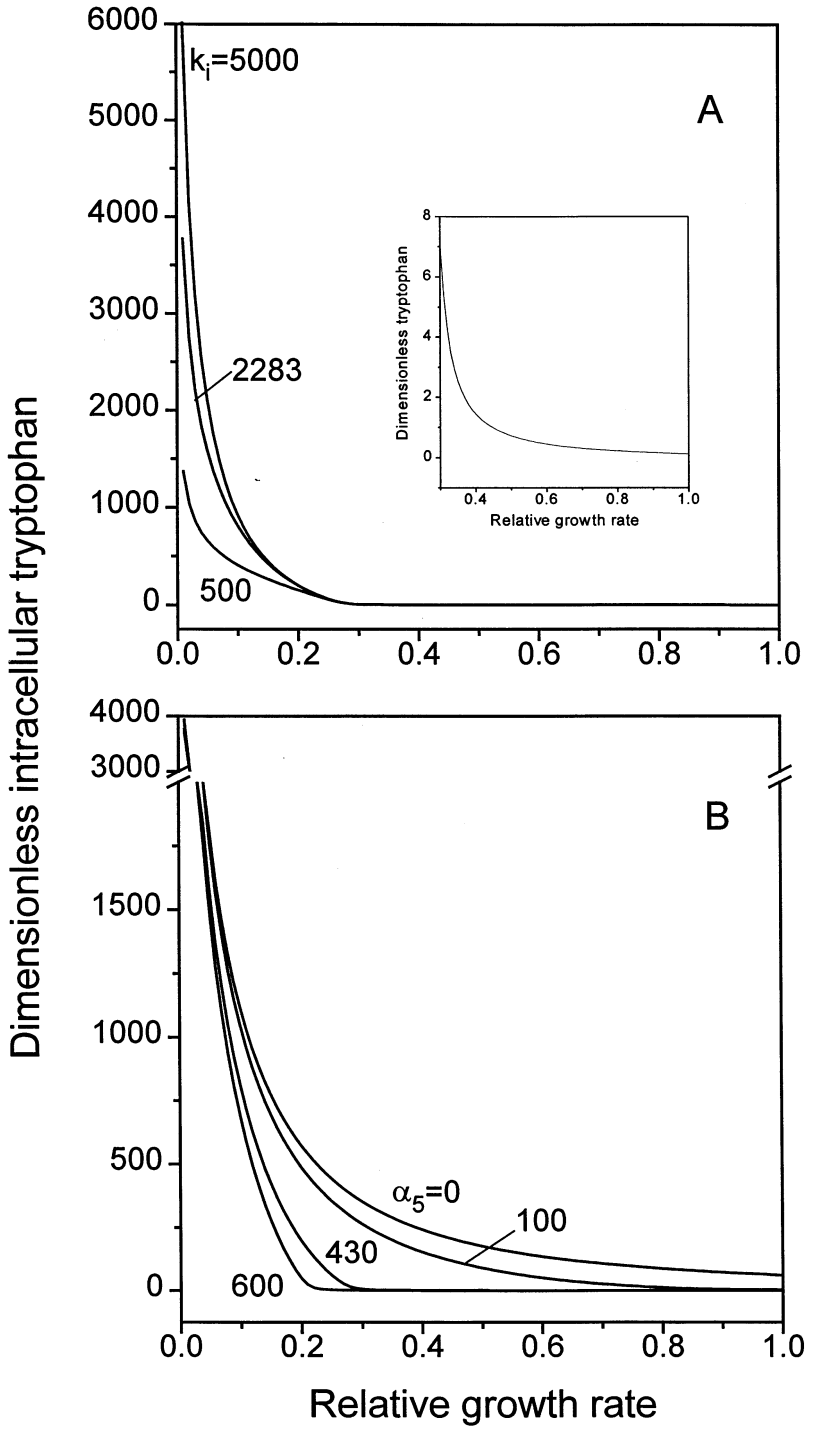

Fig. 3. Influences of model parameters on the intracellular tryptophan concentration at different growth rates. The parameters α_1, α_2, α_3, α_4, α_6, k are the same as those in Fig. 2. Other parameters except those denoted in the figures are as follows: (A) $\alpha_5 = 430$; $r = 10$; (B) $k_i = 2283$; $r = 10$.

1–42 in a concentration range of about 0.2–0.5 mmol l^{-1}. No oscillation was found at both higher (about 1.2 mmol l^{-1}) and lower (about 0.1 mmol l^{-1}) tryptophan concentrations in the strain 1–42 and another *E. coli* strain W1485 tna^{-1}. Bliss et al. (1982) interpreted the experimental results with changed values of the feedback inhibition constant K_I (Eq. 5c). They concluded that the *trp* operon becomes unstable as the intrinsic feedback inhibition is lost. Our results suggest that the loose of feedback inhibition is not the only reason for the observed instability. In fact, the model of Sen and Liu (1990) did not consider feedback inhibition but could still predict the occurrence of instability. Sen and Liu (1990) argued the occurrence of instability as a consequence of increased demand of tryptophan for biosynthesis (α_5) and/or increased strain level r. An analysis of the results presented by Sen and Liu (1990) revealed that the instability region on their α_5-r plane corresponds to a region with relatively low intracellular concentrations of tryptophan as well. Thus, our results are consistent with those of Sen and Liu (1990) in this aspect. This becomes more clear if the z-u_R plane of Fig. 4 is transferred into a corresponding α_5-u_R plane as shown in Fig. 5. Note that the critical lines for subdomains I(a), I(e), II(b) and II(d) on the α_5-u_R plane decline significantly as the growth rate raises. As in Fig. 4 instability of the *trp* operon is only found in a certain region of the α_5-u_R. Considering a typical α_5 value of about 430 for wild-type strains of *E. coli* the critical values of α_5 for the occurrence of instability as shown in Fig. 5 are relatively high.

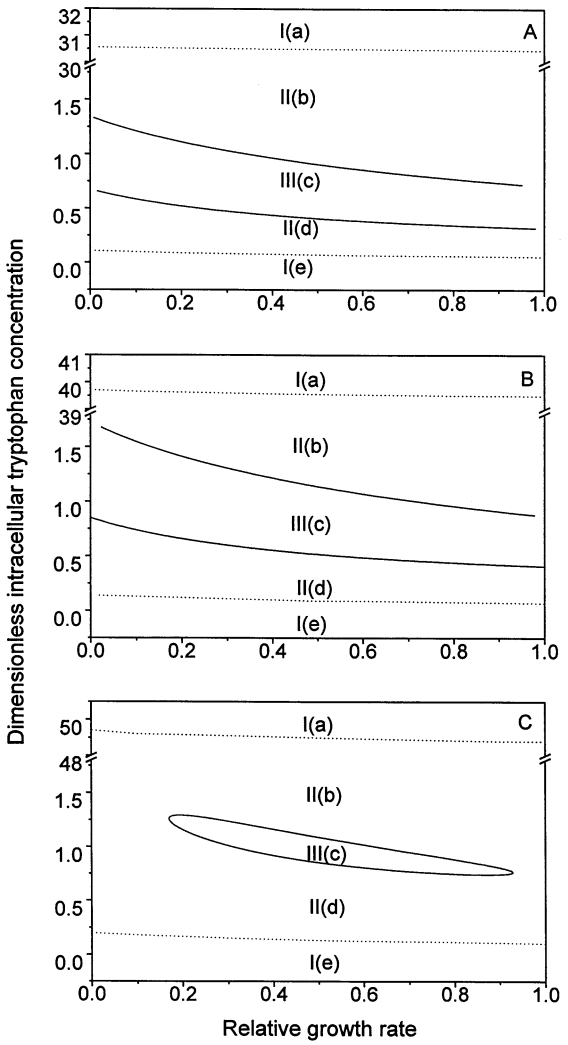

Fig. 4. Division of the operational region on a growth rate and intracellular tryptophan plane according to the eigenvalues and dynamic behavior of the steady state solutions at the different strain levels. (A) $r = 10$; (B) $r = 5$; and (C) $r = 2.3$. The dimensionless model parameters except α_5 are the same as in Fig. 2. The eigenvalues are negative real parts in domain I, complex number with negative real parts in domain II, and complex number with positive real parts in domain III, respectively.

The use of the z-u_R plane (Fig. 4) for examining the stability of the *trp* operon is more straightforward. It underlines the importance of the intracellular tryptophan concentration, the reduction of which can be caused not only by α_5 and r but also by other means such as an increased secretion rate. The latter aspect is of particular importance for the improvement of amino acid fermentation (Krämer, 1994). Although the product secretion is not explicitly included in our model the analysis presented above may still give some indications to possible effects of an increased product secretion. In fact, the last term on the right-hand side of Eq. (5c) may be considered as an accumulative term describing both the tryptophan consumption and tryptophan secretion. It is understood that an enhanced secretion rate will certainly rise the value of α_5 and hence reduce the intracellular product concentration. The results shown in Figs.

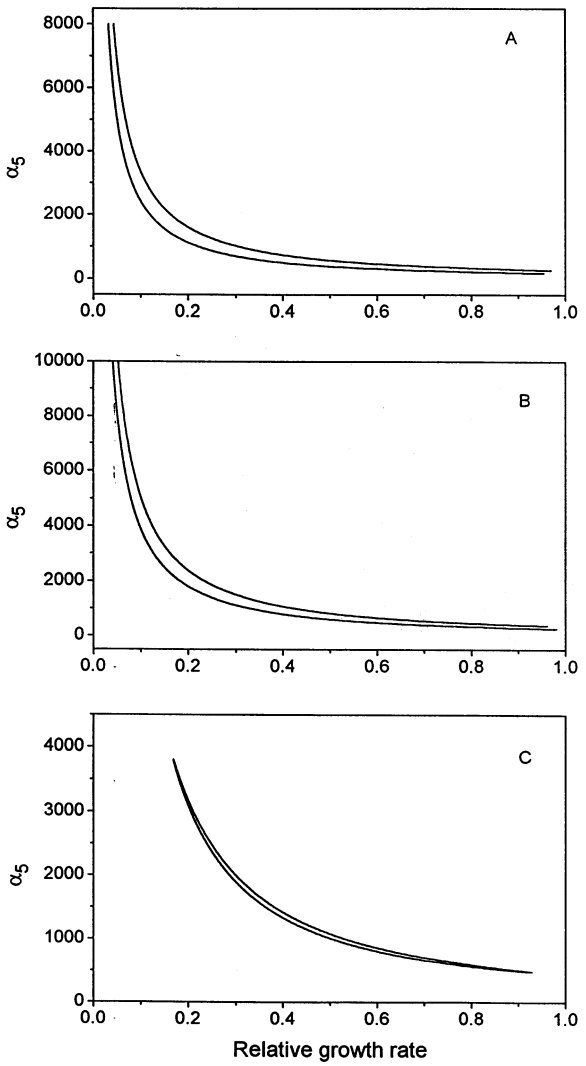

Fig. 5. The unstable region on a growth rate and α_5 plane corresponding division of Fig. 4 according to the eigenvalues and dynamic behavior of the steady state solutions at the different strain levels. (A) $r = 10$; (B) $r = 5$; and (C) $r = 2.3$.

4 and 5 imply that an excessive secretion of the product should be avoided in order to prevent instability of the *trp* operon.

The dynamic behavior of steady-state solutions in the subdomains I and II on the z-u_R plane (Fig. 4) is depicted in Fig. 6 for a growth rate shift from 0.3 to 0.31 at $r = 5$. Fig. 6A represents a stable transient response from one steady state to another in domain I(a). The dynamic behavior of steady states in Domain I(e) is similar to that in domain I(a). Notably different dynamic behavior is found for steady states of the domains II(b) and II(d) which exhibit damped oscillation. Although the eigenvalues in both domains are the same type, i.e. complex with negative real parts, the period of the damped oscillation declines as α_5 increases (Fig. 6B,C). The reason for this is that the absolute values of the imaginary parts of the

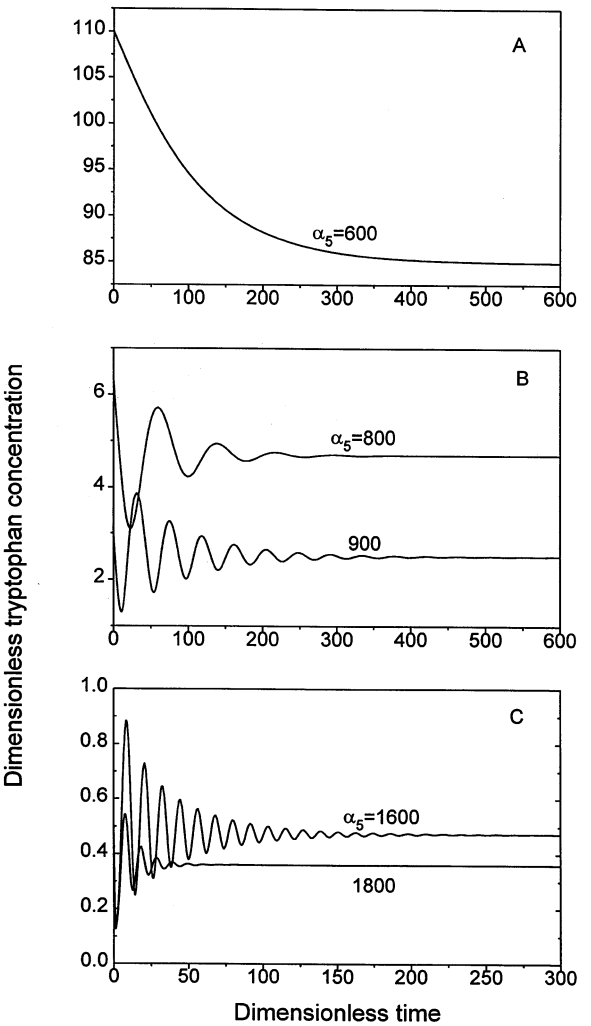

Fig. 6. Transient behavior of the intracellular tryptophan concentration after the relative growth rate is shifted from 0.3 to 0.31 at $r = 5$ in the different subdomains of domains I and II of Fig. 4. (A) Steady states in subdomain I(a); (B) steady states in subdomain II(b); and (C) steady states in subdomain II(d).

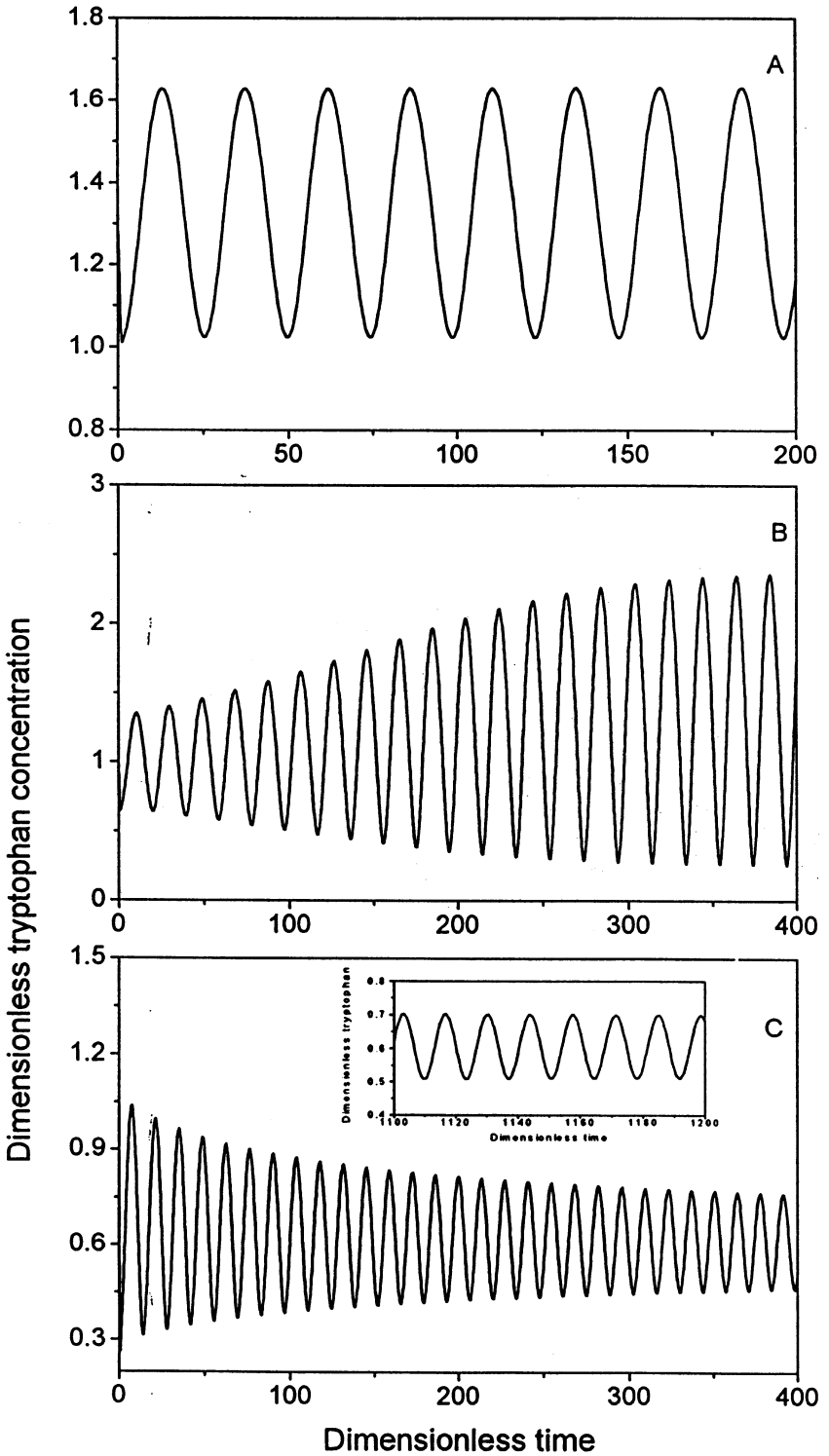

Fig. 7. Sustained oscillations of the intracellular tryptophan concentration after the relative growth rate is increased from 0.3 to 0.31 for 1 dimensionless time and then set back to 0.3 at $r = 5$ for operational states on the boundaries between domains II and III and within domain III of Fig. 4. (A) Upper boundary between domains II(b) and III ($\alpha_5 = 1125$); (B) within domain III ($\alpha_5 = 1250$); (C) lower boundary between domains II(d) and III ($\alpha_5 = 1507$).

eigenvalues become larger if the α_5 value raises. In domain II(b) the damped oscillation with a high α_5 value becomes more frequent than that with a low α_5 value (Fig. 6B). However, the opposite situation appears in domain II(d) (Fig. 6C). Fig. 7 illustrates the dynamic behavior of steady states in domain III for a growth rate pulse from 0.3 to 0.31 for 1 dimensionless time and then back to 0.3. Hopf bifurcation appears on the top critical curve of domain III in Fig. 4 (Fig. 7A). Within domain III the dynamic response is initially an excited oscillation and finally becomes a stable Hopf bifurcation (Fig. 7B). On the bottom critical curve of domain III the dynamic response is first a damped oscillation and becomes gradually a Hopf bifurcation (Fig. 7C).

Fig. 8 represents, respectively, the phase-planes of three cases in domain II(d) and domain III, which are subject to a pulse perturbation, for instance, the growth rate is raised from 0.3 to 0.31 for 1 dimensionless time and then set back. Fig. 8A shows that the system jumps off a steady state and comes finally back to the original state after several cycles of damped oscillations. This steady state is stable. However, the response of a steady state within domain III to a pulse perturbation is a limit cycle after excited oscillations (Fig. 8B). The phase-plane of a steady state on the bottom critical curve of domain III, which corresponds to Fig. 7C, is also a limit cycle from outside to inside (Fig. 8C). The two systems in domain III can not return to the original states.

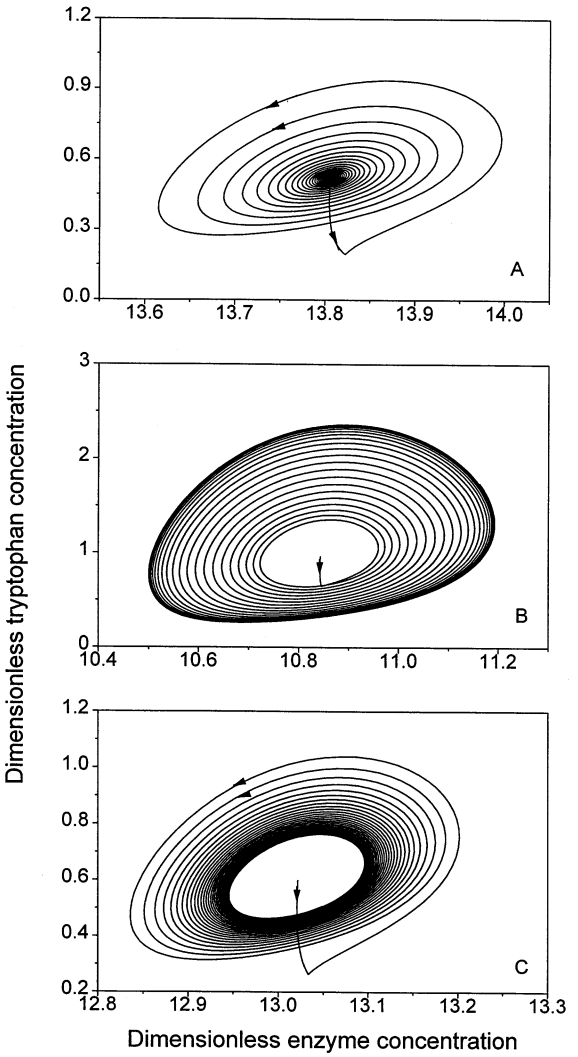

Fig. 8. Phase-planes of intracellular concentration of tryptophan and enzyme concentration at $r = 5$ for a pulse perturbation of the growth rate that is increased from 0.3 to 0.31 for 1 dimensionless time and then set back to 0.3. (A) $\alpha_5 = 1600$; (B) $\alpha_5 = 1250$; (C) $\alpha_5 = 1507$. Other parameters are the same as in Fig. 2.

Appendix A. Nomenclature

C	molar percentage of tryptophan in cellular protein
D	gene dosage
E	intracellular enzyme concentration (mol l^{-1})
J	Jacobian matrix
K_1, K_2, K_3	degradation rate constants of mRNA, enzyme and tryptophan, respectively (h^{-1})
k_1, k_2, k_3, k_4	constants for the reactions of formation and dissociation of holorepressor and bound operator, respectively
K_d, K_o	dissociation constants of holorepressor and bound operator, respectively (mol l^{-1})
K_e, K_m, K_p	rate constants of formations of enzyme, mRNA and tryptophan, respectively (h^{-1})

K_I, K_s	inhibition and saturation constants of tryptophan, respectively (mol l^{-1})
k, k_i	dimensionless saturation and inhibition constants of tryptophan
M	intracellular mRNA concentration (mol l^{-1})
n	number of binding sites of aporepressor
O, O_t	free and total operator, respectively
P	intracellular tryptophan concentration (mol l^{-1})
P^m_{pro}	maximum cellular protein concentration based on intracellular cell volume (mol l^{-1})
R, R_t	free and total aporepressor, respectively
r	dimensionless constant indicating strain level of the *trp* operon repressor
T	dimensionless parameter
t	time (h)
u, u_m, u_R	dimensionless growth rate, maximum and relative dimensionless growth rate
x, y, z	dimensionless concentrations of mRNA, enzyme, and tryptophan, respectively

Greek symbols

α_1, α_2, α_3	dimensionless degradation rate constants of mRNA, enzyme, and tryptophan, respectively
α_4, α_5, α_6	dimensionless parameters
β	a constant indicating the effects of growth rate on protein content of cells
μ, μ_m	specific growth rate and maximum specific growth rate (h^{-1}), respectively
λ	eigenvalues of the system (Eqs. 6a, 6b and 6c)

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