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A MATHEMATICAL MODEL FOR THE REGULATION OF TRYPTOPHAN PROMOTER

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Abstract—Tryptophan promoter is widely used in the industrial fermentation for the overproduction of recombinant proteins. It can be regulated by the concentrations of tryptophan and 3β-indoleacrylic acid (IAA). A mathematical model is proposed for the regulation of the promoter by the manipulation of tryptophan and IAA concentrations. The transcription initiation frequency increases with IAA concentration and reaches a plateau. The frequency decreases drastically even with the addition of a small amount of tryptophan. These behaviors agree with the previous experimental investigation.

INTRODUCTION

Initiation of transcription is a critical step for controlling gene expression. The transcription rate is primarily dependent on the rate at which mRNA synthesis is initiated and consequently on the characteristics of the promoters [1]. *Trp* promoter in *E. coli* is a strong, regulated promoter and can be easily manipulated to derive cloned-gene expression. The production of useful product proteins under the control of the *trp* promoter can thus be turned on or off by manipulating environmental conditions. The *trp* promoter is derepressed by the addition of a tryptophan analog such as 3β-indoleacrylic acid (IAA) or by the removal of tryptophan. A wide variety of mammalian and viral genes have been placed under the control of the *trp* promoter and expressed in *E. coli* [2-4]. The fermentation processes of *E. coli* containing *trp* promoter have been investigated [5-7].

The gene expression is primarily controlled at the transcriptional level and is affected by environmental conditions. It has been reported that transcription of the *trp* operon is initiated with a fixed periodicity, rather than continuously [8-11]. Initiation of transcription on the operon occurs as often as once every 6 seconds at 37℃ when the operon is on maximally [12, 13]. Under conditions of maximum repression, the rate of transcription initiation at the *trp* promoter is 1/80th the rate observed in the absence of tryptophan [14, 15].

The *trp* promoter has been extensively studied [16-18] and widely used for the production of recombinant proteins. Shina developed the mathematical model of the *trp* operon for the overproduction of tryptophan [17]. Scatchard equation was used in his model. Koh and Yap developed a genetically structured model of the *trp* operon [18]. It showed that at low aporepressor concentration, full repression was not possible even with high tryptophan levels, resulting in leaky expression. In this paper, the regulation of tryptophan promoter is mathematically formulated, based on biologically known information. When tryptophan analog, IAA is added to depress the promoter, it competes with tryptophan to bind to repressor protein. Frequency of the transcription initiation is derived as a function of tryptophan and IAA concentrations.

MATHEMATICAL MODEL

1. Regulation by Tryptophan

Regulation of the transcription on the operator is achieved by the interaction of a specific repressor with the *trp* operator site on the DNA. The *trp* repressor is a 58-kdal protein encoded by the *trpR* gene, which is far from the *trp* operon. The *trpR* polypeptide normally exists as a dimer, whether complexed with L-tryptophan or not [19]. This dimeric repressor without L-tryptophan, termed the *trp* aporepressor, does not bind to the operator. However, a complex of the aporepressor and L-tryptophan binds tightly to the operator and regulates transcription initiation.

The repressor protein has been purified and crystallized [20], and the three dimensional structure of the tryptophan-*trp* aporepressor complex has been de-

termined [21]. The dimeric protein is composed of identical half molecules. Two molecules of tryptophan bind to one dimeric molecule of aporepressor. Two ligand-binding sites are identical and independent [22]. The affinity of aporepressor for tryptophan is comparable to those for tryptophanyl-tRNA synthetase [23, 24] and the feedback-sensitive enzyme anthranilate synthetase [25, 26]. But the number of molecules of those enzymes is much less than that of tryptophan or IAA molecules in the range of our interest for the regulation of *trp* operon. This information can be described by the following equations.

$$R_0 + trp \underset{k_{-1}}{\overset{k_{-1}}{\rightleftharpoons}} R_1 \tag{1}$$

$$R_1 + trp \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} R_2 \tag{2}$$

where R_0 is the intracellular concentration of aporepressor, and R_1 and R_2 are those of repressor bound with one molecule and two molecules of tryptophan, respectively.

Tryptophan molecule is much smaller than repressor molecule and the binding site of aporepressor (R_0) is exactly same as that of R_1 molecule. Therefore, reaction rate constants are same in Eqs. (1) and (2). Reaction rate constants for the binding of IAA are also assumed to be same each other in Eqs. (17), (19) and (20) for the same reason.

The rate equation for R_0 is obtained from Eq. (1).

$$-\frac{dR_0}{dt} = 2k_{+1}R_0trp - k_{-1}R_1 \tag{3}$$

Coefficient 2 in Eq. (2) is due to two possible ways to form R_1. Rate equation for R_2 is obtained from Eq. (3).

$$\frac{dR_2}{dt} = k_{+1}R_1trp - 2\ k_{-1}R_2 \tag{4}$$

where 2 in the right hand is due to two possible ways in which R_2 can be converted to R_1. At equilibrium, the left hand sides of Eqs. (3) and (4) become zero.

$$2\ k_{+1}R_0trp - k_{-1}R_1 = 0 \tag{5}$$

$$R_0 = \frac{R_1}{2\ trp\ K_{a1}} \tag{6}$$

$$\text{where } K_{a1} = \frac{k_{+1}}{k_{-1}} \tag{7}$$

$$k_{+1}R_1trp - 2\ k_{-1}R_2 = 0 \tag{8}$$

$$R_1 = \frac{2\ R_2}{K_{a1}trp} \tag{9}$$

The balance on R gives

$$R_t = R_0 + R_1 + R_2 \tag{10}$$

where R_t is the total concentration of repressor which is not bound to DNA.

Substituting Eq. (9) into Eq. (6),

$$R_0 = \frac{R_2}{(K_{a1}trp)^2} \tag{11}$$

Substituting Eqs. (9) and (11) into Eq. (10) and rearranging the result yield

$$R_t = \frac{R_2}{(K_{a1}trp)^2} + \frac{2\ R_2}{K_{a1}trp} + R_2 = \left(\frac{K_{d1} + trp}{trp}\right)^2 R_2 \tag{12}$$

It is believed that both ligand-binding sites must be occupied before normal repressor binding can occur at the operator [22]. The ratio between active form and total repressor is obtained from Eq. (12),

$$\frac{R_2}{R_t} = \left(\frac{trp}{K_{d1} + trp}\right)^2 \tag{13}$$

However, whether *trp* repressor containing a single bound ligand can bind operator at reduced affinity relative to the active form is still unclear. If the ratio between the specific activities of R_1 and R_2 is α ($\alpha < 1$), the ratio between active forms and total repressor is obtained from Eqs. (9) and (12). Following equation represents the ratio based on R_2-equivalent specific activity.

$$\frac{\alpha\ R_1 + R_2}{R_t} = \frac{\alpha\frac{2\ R_2}{K_{a1}trp} + R_2}{\left(\frac{K_{d1} + trp}{trp}\right)^2 R_2} = \frac{2\ \alpha\ K_{d1} + trp}{trp}\left(\frac{trp}{K_{d1} + trp}\right)^2 \tag{14}$$

2. Derepression by IAA

When IAA is added to derepress the *trp* promoter, it competes with tryptophan in binding to the *trp* aporepressor. Possible cases and notations are shown in Fig. 1. The balance equations are obtained using the notations.

$$-\frac{dR_0}{dt} = 2\ k_{+1}R_0trp - k_{-1}R_1 + 2\ k_{+2}R_0IAA - k_{-2}R_1' \tag{15}$$

$$-\frac{dR_1}{dt} = k_{+1}R_1trp - 2\ k_{-1}R_2 - 2\ k_{+1}R_0trp + k_{-1}R_1 + k_{+2}R_1IAA - k_{-2}R_2' \tag{16}$$

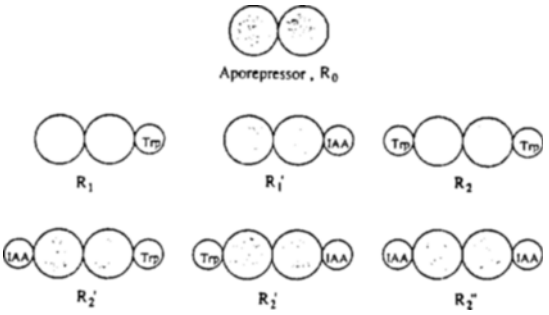

Fig. 1. Possible bindings of tryptophan aporepressor with tryptophan and IAA.

$$-\frac{dR_1'}{dt}=k_{+1}R_1'trp-k_{-1}R_2'+k_{+2}R_1'IAA-2\ k_{-2}R_2''-2\ k_{+2}R_0IAA+k_{-2}R_1' \quad (17)$$

$$-\frac{dR_2}{dt}=-k_{+1}R_1trp+2\ k_{-1}R_2 \quad (18)$$

$$-\frac{dR_2'}{dt}=-k_{+2}R_1IAA+k_{-2}R_2'-k_{+1}R_1'trp+k_{-1}R_2' \quad (19)$$

$$-\frac{dR_2''}{dt}=-k_{+2}R_1'IAA+2\ k_{-2}R_2'' \quad (20)$$

$$R_t=R_0+R_1+R_1'+R_2+R_2'+R_2'' \quad (21)$$

Only 6 equations are independent. At equilibrium, the left sides of the rate equations are zero. Solving the equilibrium version of Eqs. (15), (16), (18), (19) and (20) for R_0, R_1, R_1', R_2', and R_2'' as a function of R_2 and substituting the results into Eq. (21) yield

$$R_t=\left(\frac{K_{d1}^2}{trp^2}+\frac{2\ K_{d1}}{trp}+\frac{2\ K_{d1}^2IAA}{K_{d2}trp^2}+1+\frac{2\ K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}^2IAA^2}{K_{d2}^2trp^2}\right)R_2 \quad (22)$$

$$=\left(\frac{K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}}{trp}+1\right)^2R_2 \quad (23)$$

where $K_{d1}=\frac{k_{-1}}{k_{+1}}$ and $K_{d2}=\frac{k_{-2}}{k_{+2}}$

Therefore, the ratio between active form and total repressor is obtained from Eq. (23).

$$\frac{R_2}{R_t}=\left(\frac{K_{d2}trp}{K_{d1}IAA+K_{d1}K_{d2}+K_{d2}trp}\right)^2 \quad (24)$$

If the activities of R_1 and R_2' are both proportional to that of R_2, the ratio based on R_2-equivalent specific activity is expressed by the following equation.

$$\frac{\alpha(R_1+R_2')+R_2}{R_t}=\frac{2\alpha K_{d1}K_{d2}+2\alpha K_{d1}IAA+K_{d2}trp}{K_{d2}trp}\left(\frac{K_{d2}trp}{K_{d1}IAA+K_{d1}K_{d2}+K_{d2}trp}\right)^2 \quad (25)$$

The active form of the *trp* repressor binds not only at the operator of *trp* operon but also at the operators of the *aroH* operon and the *trpR* operon [25, 26]. The total concentration of *trp* repressor (R_T) is expressed by following equation.

$$R_T=(apoR)+(apoR:\text{tryptophanyl-tRNA synthetase})+(apoR:\text{anthranilate synthetase})+(R\text{ with }trp)+(R:aroH\text{ operator})+(R:trpR\text{ operator})+(R:trp\text{ operator}) \quad (26)$$

However, in the case of cell containing multicopies of *trp* operon, the amount of repressors which bind to other promoters should be negligible. The number of tryptophanyl-tRNA synthetase and anthranilate synthetase molecules is much less than that of tryptophan and IAA molecules in the range of *trp* gene regulation. So the second, third, fifth, and sixth terms are negligible in the right hand side of Eq. (26).

$$R_T=(apoR)+(R\text{ with }trp)+(R:trp\text{ operator}) \quad (27)$$

$$=R_0+R_1+R_1'+R_2+R_2'+R_2''+O_R \quad (28)$$

$$=R_t+O_R \quad (29)$$

where O_R is the concentration of the repressor bound to *trp* operator. Substituting of Eq. (23) into Eq. (29) yields.

$$R_T=\left(\frac{K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}}{trp}+1\right)^2R_2+O_R \quad (30)$$

Several assay methods for *trp* repressor-*trp* operator interaction have been developed [19, 27-30]. Since the *trp* operator site overlaps the *trp* promoter site, *trp* repressor and RNA polymerase compete each other to bind to this region. Transcription initiation is determined by this competition. *In vitro* transcription studies have established the fact that repressor prebound to the operator prevents polymerase binding and, conversely, that prebound polymerase prevents repressor action [31]. The binding of polymerase is slow even in the *trpR*$^-$ cell. Therfore we presume that the rate of the polymerase binding is proportional to O^- which is the concentration of free operator at equilibrium of the repressor binding reaction. If R_1 and R_2' are partially active, the activities of those molecules should be considered as in Eq. (25). However, we assume that the molecules are not active for the simplicity.

$$R_2+O^- \rightleftarrows O_R \quad (31)$$

$$pol+O^- \rightleftarrows O_p \quad (32)$$

Dissociation constant for the first reaction is

$$K_{d3}=\frac{R_2O^-}{O_R} \quad (33)$$

The total concentration of *trp* operator, O_T, is the sum of the concentrations of free operator (O^-), repressor bound operator (O_R), and polymerase bound operator (O_p). Since binding frequency of the polymerase is low and bound polymerase moves quickly along DNA, the concentration of polymerase bound operator can be neglected,

$$O_T=O^-+O_R+O_P \quad (34)$$

$$=O^-+O_R \quad (35)$$

If the frequency of transcription initiation is assumed to be proportional to the concentration of free operator, it can be expressed by the following equation.

$$\text{Frequency of Transcription Initiation}=k\frac{O^-}{O_T}D \quad (36)$$

where D is the number of trp operons.
From Eq. (33)

$$O_R=\frac{R_2O^-}{K_{d3}} \quad (37)$$

Substituting Eq. (37) into Eq. (35) yields

$$O_T=O^-+\frac{R_2O^-}{K_{d3}} \quad (38)$$

Substitution of Eq. (37) into Eq. (30) yields

$$R_T=K^2R_2+\frac{R_2O^-}{K_{d3}} \quad (39)$$

$$\text{where } K=\frac{K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}}{trp}+1$$

Subtracting Eq. (39) from Eq. (38) and rearranging the result yield

$$R_2=\frac{O^--O_T+R_T}{K^2} \quad (40)$$

Substituting Eq. (40) into Eq. (38) and solving for O^-, we obtain

$$O^-=\frac{K_{d3}K^2}{2}\left\{-\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)+\sqrt{\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)^2+\frac{4\ O_T}{K_{d3}K^2}}\right\} \quad (41)$$

$$\frac{O^-}{O_T}=\frac{K_{d3}K^2}{2\ O_T}\left\{-\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)+\sqrt{\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)^2+\frac{4\ O_T}{K_{d3}K^2}}\right\} \quad (42)$$

$$\text{where } K=\frac{K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}}{trp}+1 \quad (43)$$

If the concentrations of total repressor and total operator are known, Eq. (42) is a function of intracellular concentrations of tryptophan and IAA. By substituting Eq. (42) into Eq. (36) the frequency of transcription initiation is expressed by Eq. (44) as a function of tryptophan and IAA concentration.

Frequency of Transcription Initiation

$$=kD\frac{K_{d3}K^2}{2\ O_T}\left\{-\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)+\sqrt{\left(1+\frac{R_T-O_T}{K_{d3}K^2}\right)^2+\frac{4\ O_T}{K_{d3}K^2}}\right\} \quad (44)$$

$$\text{where } K=\frac{K_{d1}IAA}{K_{d2}trp}+\frac{K_{d1}}{trp}+1 \quad (45)$$

RESULTS AND DISCUSSION

Tryptophan in the medium represses the *trp* promoter. The production of recombinant protein can be automatically induced when the tryptophan is consumed by the host organism. This means that the concentration of active repressor decreases as the tryptophan concentration decreases. The ratio of active and total repressors can be calculated by Eq. (14). Dissociation constant $K_{d1}(=1/K_{a1})$ is 1.6×10^{-4} M at 37℃ [22]. The effect of temperature on L-tryptophan binding is significant. K_{d1} is 28 μM at 6.5℃, while it is 217 μM at 40℃. In contrast to temperature, neither salt nor pH exhibited a significant effect on L-tryptophan binding by *trp* aporepressor. The relatively little change in the dissociation constant as a function of ionic strength suggests that the binding of L-tryptophan is not primarily ionic. As discussed earlier, it is believed that both ligand-binding sites of aporepressor must be occupied for the repressor-operator binding ($\alpha=0$). However, whether the repressor containing a single bound ligand has a partial activity is unclear ($0<\alpha<1$). Fig. 2 shows the ratio of active and total repressors as a function of tryptophan concentration at various value of α.

The *trp* promoter can be also derepressed by the addition of IAA and IAA competes with tryptophan in binding to the aporepressor. The ratio of active and total repressors can be calculated by Eq. (25) when

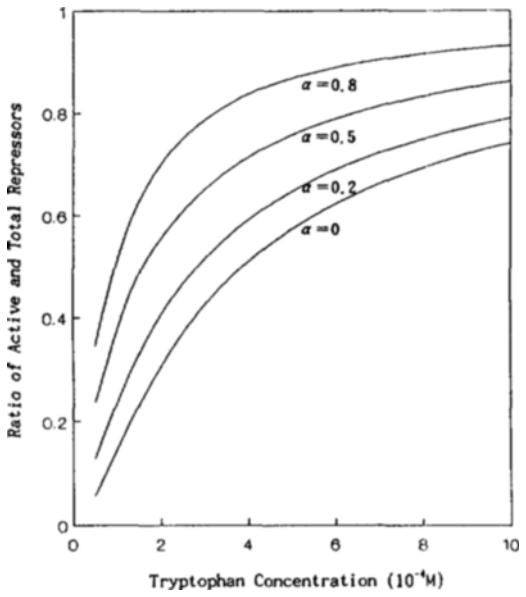

Fig. 2. Effect of tryptophan concentration on the ratio of active and total repressors.

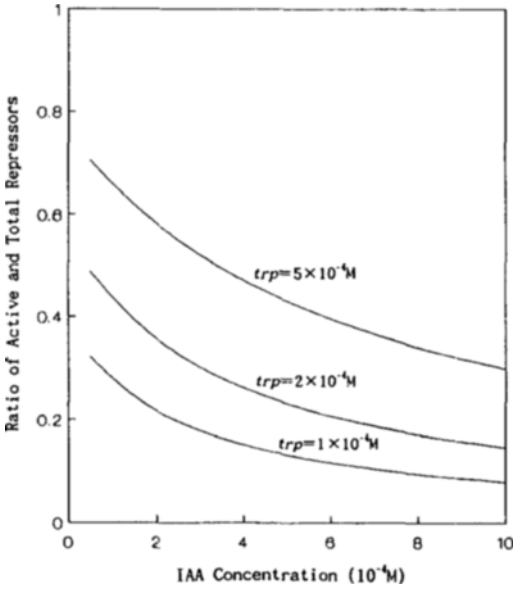

Fig. 3. Effect of IAA concentration on the ratio of active and total repressors at various values of α. Tryptophan concentration is 2×10^{-4} M.

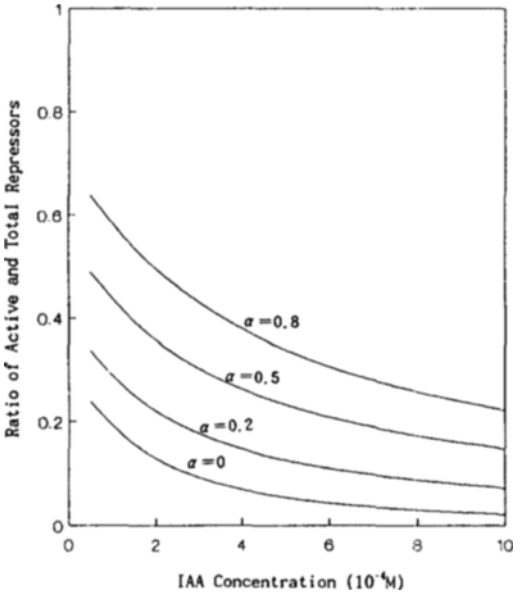

Fig. 4. Effect of IAA concentration on the ratio of active and total repressor at various tryptophan concentrations. α is 0.5.

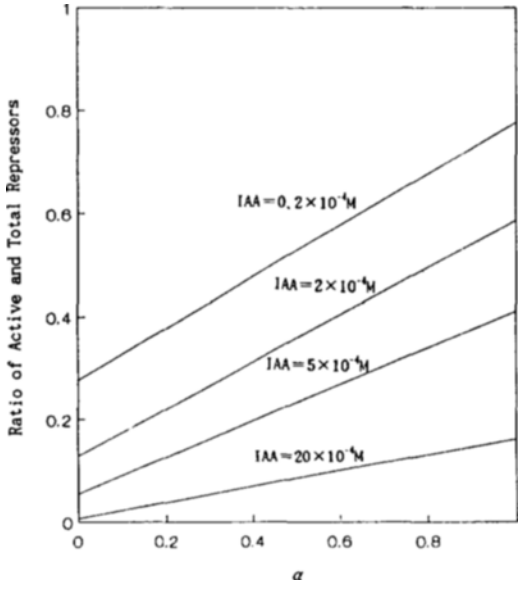

Fig. 5. Effect of α on the ratio of active and total repressors. Tryptophan concentration is 2×10^{-4} M.

IAA is added for product induction. $K_{d1}=K_{d2}=1.6\times10^{-4}$ M was used for the calculation. Fig. 3 and 4 show the ratio as a function of IAA concentration at various values of α and tryptophan concentration, respectively. The ratio decreases with IAA concentration. Decreasing rates are higher at low IAA concentration.

Fig. 5 shows the ratio between active and total repressors as a function of α at various values of IAA

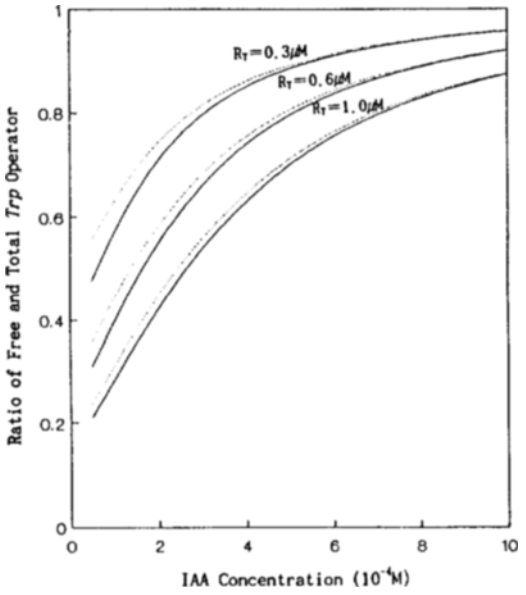

Fig. 6. Effect of IAA concentration on the ratio between the concentrations of free and total *trp* operator. Tryptophan concentration is 0.2×10^{-4} M.
Solid lines and dotted lines represent the values for low copy number (10) and high copy number (100), respectively.

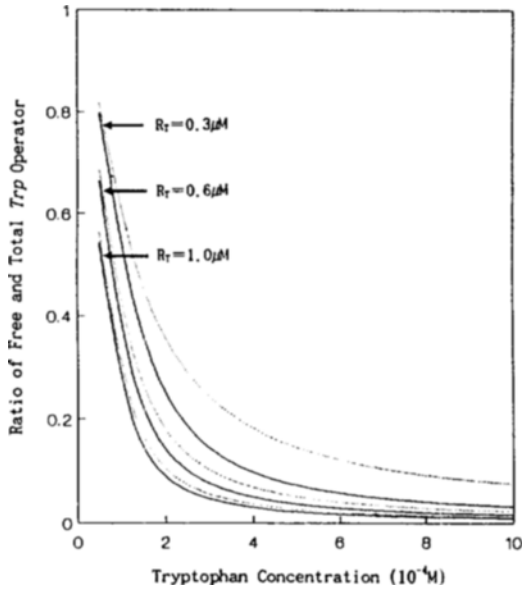

Fig. 7. Effect of tryptophan concentration on the ratio between the concentrations of free and total *trp* operator. IAA concentration is 1.0×10^{-3} M.
Solid lines and dotted lines represent the values for low copy number (10) and high copy number (100), respectively.

concentration. The ratio is linearly proportional to α and the slope of the line increases as IAA concentration decreases. The increasing rate of the slope is higher at the higher range of IAA concentration while the slopes are almost constant in low IAA concentrations.

The ratio between the concentrations of free and total *trp* operator can be calculated by Eq. (42) as a function of intracellular concentrations of tryptophan and IAA, if the concentrations of total repressor and total operator are known. $K_{d3}=2\times10^{-9}$ M was used for the calculation [19]. The repressor level in cells growing with excess tryptophan is approximately 50 molecules per cell [32]. The *trpR* operon is regulated autogeneously but the rate of synthesis of aporepressor varies only 4- or 5-fold in response to changes in the intracellular concentration of tryptophan. The *trpR* promoter is about one-tenth as active as the *trp* operon promoter and the *trpR* coding region is translated inefficiently. Therefore, the level of repressor per cell is relatively low (50-250 molecules/cell=25-125 dimers/cell) [33]. If cell volume is assumed to be 0.7×10^{-15} L, the repressor concentration is 0.06 to 0.3 μM. When the *trpR* gene is cloned the repressor concentration can be increased. Total operator concentration depends on plasmid copy number and the concentration is 2×10^{-9} M for one copy number plasmid.

Fig. 6 shows the ratio between the concentrations of free operator and total *trp* operator as a function of IAA concentration at various values of total repressor concentrations for low and high copy number plasmids of 10 and 100. The ratio increases with IAA concentration. Park et al. [16] showed the effect of IAA concentration on the production of recombinant protein experimentally. The production of recombinant protein increased with IAA concentration and reached a plateau. Although the IAA concentration is extracellular in the experiment, the simulation results shown in Fig. 6 show similar behaviors to the experimental ones.

The effect of tryptophan concentration on the ratio at various values of total repressor concentrations is shown in Fig. 7 for low and high copy number plasmids. Experimental results by Park et al. [16] showed that the production of recombinant protein decreased drastically when a small amount of tryptophan was added. The ratio between the concentrations of free and total *trp* operator decreases with tryptophan con-

centration in a similar manner in Fig. 7.

The frequency of the transcription initiation of the *trp* promoter is proportional to the plasmid copy number and the ratio between the concentrations of free operator and total *trp* operator. It is expressed by Eq. (44) as a function of tryptophan and IAA concentration.

This model is based on the intracellular concentrations which are difficult to be measured. More research about the relation between the intra and extracellular concentrations by considering membrane transport experimentally and theoretically, is needed to validate the model in more detail.

CONCLUSIONS

A mathematical model for the regulation of *trp* promoter is developed as a function of tryptophan and IAA concentrations. The transcription initiation frequency of the *trp* promoter can be predicted quantitatively using this model. This model is based on biologically known information for the intracellular concentrations of total repressor and total operator. The frequency increases with IAA concentration and reaches a plateau, while it decreases drastically with the addition of the small amount of tryptophan. This model is based on the intracellular concentrations and needs to be extended for the practical use of fermentation in which extracellular concentrations of IAA and tryptophan are manipulation variables.

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NOMENCLATURE

D : number of *trp* operon
IAA : concentration of IAA
K : defined in Eq. (43)
K_a : association constant
K_d : dissociation constant
k : constant defined in Eq. (36)
k_+ : forward reaction rate constant
k_- : backward reaction rate constant
O_p : concentration of operator bound with RNA polymerase
O_R : concentration of operator bound with repressor
O_T : concentration of total *trp* operator
O^- : concentration of free operator
pol : concentration of RNA polymerase
R_T : concentration of total aporepressor defined in Eq. (26)
R_t : concentration of total aporepressor which is not bound to DNA
R_0 : concentration of free aporepressor
R_1 : concentration of aporepresor bound with one molecule of tryptophan
R_1' : concentration of aporepressor bound with one molecule of IAA
R_2 : concentration of aporepressor bound with two molecules of tryptophan
R_2' : concentration of aporepressor bound with each molecule of tryptophan and IAA
R_2'' : concentration of aporepressor bound with two molecules of IAA
trp : concentration of tryptophan

Greek Letter

α : constant ($\alpha<1$)

Subscripts

1 : reaction with tryptophan
2 : reaction with IAA

REFERENCES

1. Rosenberg, M. and Court, D.: *Ann. Rev. Genet.*, **13**, 319 (1979).
2. Emtage, J. S., Tacon, W. C. A., Catlin, G. H., Jenkins, B., Porter, A. G. and Carey, N. H.: *Nature*, **283**, 171 (1980).
3. Leung, D. W., Capon, D. J. and Goeddel, D. V.: *Bio/Technol.*, **2**, 458 (1984).
4. Rosenberg, S. A., Grimm, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K. and Mark, D. F.: *Science*, **223**, 1412 (1984).
5. Park, T. H., Seo, J.-H. and Lim, H. C.: *Biotechnol. Bioeng.*, **34**, 1167 (1989).
6. Yoon, S. K., Kang, W. K. and Park, T. H.: *Biotechnol. Bioeng.*, **43**, 995 (1994).
7. Kawai, S., Mizutani, S., Iijima, S. and Kobayashi, T.: *J. Ferment. Technol.*, **64**, 503 (1986).
8. Baker, R. F. and Yanofsky, C.: Proc. Natl. Acad. Sci. U. S. A., **60**, 313 (1968).
9. Imamoto, F.: Proc. Natl. Acad. Sci. U. S. A., **60**, 305 (1968).
10. Morse, D. E., Mosteller, R. D. and Yanofsky, C.: Cold Spring Harbor Symp. Quant. Biol., **34**, 725 (1969).
11. Baker, R. and Yanofsky, C.: Cold Spring Harbor Symp. Quant. Biol., **35**, 467 (1970).
12. Baker, R. and Yanofsky, C.: *J. Mol. Biol.*, **69**, 89

(1972).
13. Bertrand, K., Squires, C. and Yanofsky, C.: *J. Mol. Biol.*, **103**, 319 (1976).
14. Jackson, E. N. and Yanofsky, C.: *J. Mol. Biol.*, **69**, 307 (1972).
15. Yanofsky, C., Kelley, R. L. and Horn, V.: *J. Bacteriol.*, **158**, 1018 (1984).
16. Park, T. H., Seo, J.-H. and Lim, H. C.: *Biotechnol. Lett.*, **11**, 87 (1989).
17. Shinha, S.: *Biotechnol. Bioeng.*, **31**, 117 (1988).
18. Koh, B. T. and Yap, M. G. S.: *Biotechnol. Bioeng.*, **41**, 707 (1993).
19. Joachimiak, A. R., Kelley, L., Gunsalus, R. P., Yanofsky, C. and Sigler, P.: Proc. Natl. Acad. Sci. U. S. A., **80**, 668 (1983).
20. Joachmimiak, A. R., Schevitz, R. W., Kelley, L., Yanofsky, C. and Sigler, P. J.: *Biol. Chem.*, **258**, 12641 (1983).
21. Schevitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L. and Sigler, P. B.: *Nature (London)*, **317**, 786 (1985).
22. Arvidson, D. N., Bruce, C. and Gunsalus, R. P.: *J. Biol. Chem.*, **261**, 238 (1986).
23. Joseph, D. R. and Muench, K. H.: *J. Biol. Chem.*, **246**, 7602 (1971).
24. Joseph, D. R. and Muench, K. H.: *J. Biol. Chem.*, **246**, 7610 (1971).
25. Pabst, M. J., Kuhn, J. C. and Somerville, R. L.: *J. Biol. Chem.*, **248**, 910 (1973).
26. Zalkin, H.: "Multifunctional Enzymes", eds. Besswanger, H. and Schmincke-Ott, E., 1213, John Wiley and Sons, Inc., New York (1980).
27. Das, A., Drawfold, I. P. and Yanofsky, C.: *J. Biol. Chem.*, **257**, 8795 (1982).
28. Haydock, P. V. and Somerville, R. L.: *Biochem. Biophys. Res. Commun.*, **119**, 926 (1984).
29. Rose, J. K., Squires, C. L., Yanofsky, C., Yang, H. L. and Zubay, G.: *Nature (London) New Biol.*, **245**, 133 (1973).
30. Zubay, G., Morse, D. E., Schrenk, W. J. and Miller, J. H.: Proc. Natl. Acad. Sci. U. S. A., **69**, 1100 (1972).
31. Squires, C. L., Lee, F. and Yanofsky, C.: *J. Mol. Biol.*, **92**, 93 (1975).
32. Kelley, R. L. and Yanofsky, C.: Proc. Natl. Acad. Sci. U. S. A., **79**, 3120 (1982).
33. Yanofsky, C. and Crawford, I. P.: in "E. coli and Salmonella typhimurium vol. 2", eds. Neidthard, F. C., Ingraham, J. L., Low, K. B., Magasanik, B. and Schaechter, M., 1453, American Society for Microbiology, Washington DC (1987).