"PS-1."

CHEME 7770

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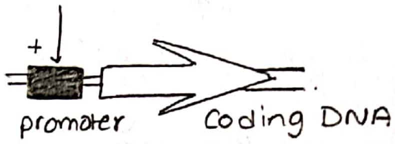
(1)



Reaction scheme:

$$G_p + R_x \underset{k_-}{\overset{k_+}{\rightleftharpoons}} (G_p:R_x)_C \qquad (1)$$

$$(G_p:R_x)_C \xrightarrow[k_I]{} (G_p:R_x)_O \qquad (2)$$

$$(G_p:R_x)_O \xrightarrow[k_A]{} G_p + R_x \qquad (3)$$

$$(G_p:R_x)_O \xrightarrow[k_E]{} G_p + R_x + m_p \qquad (4)$$

where, G_p = Gene concentration = 2500 copies/cell

R_x = RNAP concentration (Table 1)

k_+/k_- = on/off rate constant for RNAP at the promoter for gene p = Table 1

k_E = elongation rate constant for gene p (Table 1)

k_A = rate constant for abortive initiation (negligible)

Specific rate of transcription, $\hat{r}_{x,p} = r_{x,p}\, u(I)$

where, $r_{x,p}$ = Kinetic rate of transcription $= k_E (G_J:R_x)_O$

$u(I)$ = control term.

(a) Total abundance of RNAP, $R_{x,T} = R_x + (G_p:R_x)_C + (G_p:R_x)_O$

$$\frac{d}{dt}(G_p:R_x)_C = k_+(G_p)(R_x) - k_-(G_p:R_x)_C - k_I(G_p:R_x)_C \qquad (5)$$

$$\frac{d}{dt}(G_p:R_x)_O = k_I(G_p:R_x)_C - k_A(G_p:R_x)_O - k_E(G_p:R_x)_O \qquad (6)$$

At steady state,

$$(G_p:R_x)_C = \frac{K_+}{K_- + K_I}(G_p)(R_x) = \frac{1}{K_{x,p}}(G_p)(R_x)$$

$$(G_p:R_x)_O = \frac{K_I}{K_A + K_E}(G_p:R_x)_C = \frac{1}{\tau_{x,p}}\frac{1}{K_{x,p}}(G_p)(R_x)$$

where, $K_{x,p}$ = saturation constant for gene P

$\tau_{x,p}$ = time constant for gene P.

Therefore

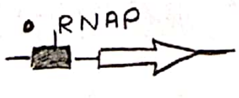
$$R_x = \frac{R_{x,T}(\tau_{x,p} K_{x,p})}{\tau_{x,p} K_{x,p} + (\tau_{x,p}+1)G_p}$$

$$(G_p:R_x)_O = \frac{R_{x,T}\, G_p}{\tau_{x,p} K_{x,p} + (\tau_{x,p}+1)G_p}$$

$$r_{x,p} = K_E R_{x,T}\frac{G_p}{[\tau_{x,p} K_{x,p} + (\tau_{x,p}+1)G_p]}$$

Determination of $u(I)$:

In this case, expressed configurations RNAP binding w/ promoter (weight factor W_1) and RNAP binding w/ promoter along w/ another molecule (weight factor W_2).

Cases	Weight	Expression
	1	—
	W_1	+
	W_2	+

$$u(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}$$ where Binding function. $f_I = \frac{I^n}{K^n + I^n}$

$$\hat{r}_{x,p} = \underbrace{K_E\, R_{x,T}}_{\mu M/s} \underbrace{\frac{G_p}{[K_{x,p}\tau_{x,p} + (\tau_{x,p}+1)G_p]}}_{\text{Dimensionless}} \cdot \underbrace{\frac{W_1 + W_2 \frac{I^n}{(K^n+I^n)}}{1+W_1+W_2\frac{I^n}{(K^n+I^n)}}}_{\text{Dimensionless}}$$

Unknown parameter values: (Reference included in table 1).

- $K_E = \langle K_E \rangle = e_x/L = \frac{42^*}{3075} = 0.0137 \cdot s^{-1}$.
- $K_I = \frac{1}{40s^*} = 0.025\ s^{-1}$
- $\frac{K_- + K_I}{K_+ K_I} = \frac{K_{x,p}}{K_I} = 1.785\ \mu M \Rightarrow K_{x,p} = 0.0445\ \mu M$
- $R_{x,T} = 4600\ \text{copies/cell}^* = \frac{4600 \times 3075}{6.02\times10^{23}} \times \frac{10^9}{0.75^*}\ \mu M = 3.13\times10^{-8}\ \mu M$
- $\tau_{x,p} = \frac{K_E}{K_I} = 0.544$ (K_A negligible)
- Given, $G_p = 2500 \frac{\text{copies}}{\text{cell}} = \frac{(2500\times3075)\ \text{cell}}{6.02\times10^{23}\ \frac{\text{molecule}}{\text{mol}}} \times \frac{\text{cell}}{0.75\ \mu m^3 {}^*} \times \frac{10^9\ \mu M}{\frac{1\ \text{mol}}{\mu m^3}}$

$= 1.7\times10^{-8}\ \mu M$

* Table 1.

(b). Since K_E and K_I are of the same order of magnitude ($\tau_{x,p} \sim 1$) both initiation and elongation impact overall rate of reaction.

(c) Rate of change of mRNA concn.

$$\frac{dm_p}{dt} = r_{x,p}\, u(I) - (K^d_{x,p} + \mu)\, m_p$$

At steady state, $m_p = \frac{r_{x,p}\, u(I)}{K^d_{x,p} + \mu}\ (\mu M) = \frac{r_{x,p} \times u(I)}{(K^d_{x,p}+\mu)} \times \overbrace{1.17\times10^{9}\ \frac{\mu mol}{gDW}}^{\text{next page}}$

- $K^d_{x,p} = \frac{\ln 2}{t_{1/2}} = \frac{\ln 2}{4}\ min^{-1} = 0.173\ min^{-1} = 2.9\times10^{-3}\ s^{-1}$
- μ = Specific growth rate $= \frac{\ln 2}{t_d} = \frac{\ln 2}{30 min} = 3.85\times10^{-4}\ s^{-1}$.

where, t_d = Doubling time = 30 min

$t_{1/2}$ = Half-life (Table 1) = 4 min

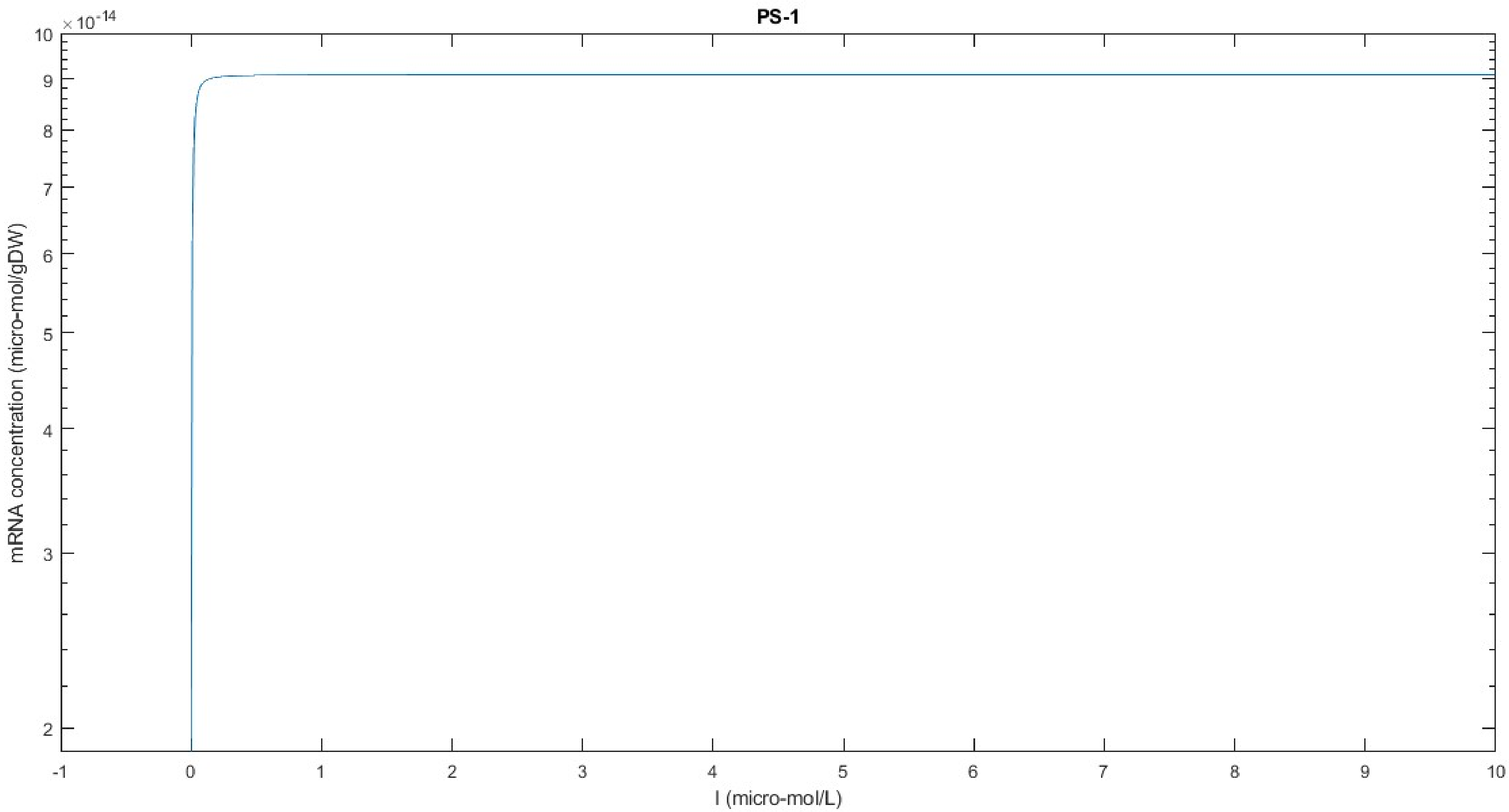
To convert μM to $\frac{\mu mol}{gDW}$:

$$\frac{1\,\mu mol}{L} = 1\,\frac{\mu mol}{L} \times \frac{10^3 L}{1 m^3} \times \frac{1 m^3}{10^6 \mu m^3} \times \frac{0.75\,\mu m^3}{cell} \times \frac{cell}{641 \times 10^{-15}\,gDW} = 1.17 \times 10^9\,\frac{\mu mol}{gDW}$$

$$\therefore\ m_p = \frac{r_{x,p} \times u(I)}{(K^d_{x,p} + \mu)} \times 1.17 \times 10^9 \left(\frac{\mu mol}{g}\right).$$

Table 1 (reference)

Property (symbol)	Value (unit)	Source	Reference
Elongation constant (e_X)	42 nt/s	BIONUMBERS	PubMed ID 20413502
Rate constant for initiation (k_I)	0.025 s^{-1}		(McClure) PubMed ID6160577
Saturation constant ($K_{X,P}$)	0.0445 μM		(McClure) PubMed ID6160577
RNAP concentration in *E. coli* ($R_{X,P}$)	4600 copies/cell	BIONUMBERS	PubMed ID 22624875
E. coli cell volume	0.75 μm^3		https://doi.org/10.1006/jmbi.1999.3056
Median half-life of *E. coli* mRNA ($t_{1/2}$)	~4 min	BIONUMBERS	PubMed ID 14981237
Dry mass of *E. coli*	641 fg/cell	BIONUMBERS (103892)	Bremer, H., Dennis, P. P. (1987) Modulation of chemical composition and other parameters of the cell by growth rate. Neidhardt, et al. eds. Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, 1st ed. chapter 96, Table 2 pp.1530-1


PS-1
×10^-14
mRNA concentration (micro-mol/gDW)
I (micro-mol/L)
-1
0
1
2
3
4
5
6
7
8
9
10