Computation of Diffusion Limited Controlled Actions for Gene Regulating Repressor Particles

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Abstract. Temporal changes in association and dissociation kinetics of the repressor – operator reaction were simulated by a computational approach using MATOLABO on the basis of strict mathematical description. The diffusion equation of a repressor particle has been combined with arrival probabilities of the repressor particle. Temporal behaviors of the repressor operator complex have been computed by inverting the Laplace transformed equations. The temporal kinetic data of association and dissociation obtained at different ionic strength and at different DNA length were successfully simulated. Those results could be achieved by regulating diffusion constants in medium on the DNA, a reaction radius of the repressor and a reaction rate per one repressor on the reaction sphere surface, k value. The reported values of association rate constant k_a obtained at different ionic strength were also successfully simulated. Among the regulated parameters to get these successful simulations, the reaction rate per one particle on the reaction sphere surface, k value has effective influences on the association and dissociation kinetics particularly those at varying ionic strength that were induced by high KCl condition. By an electro chemical consideration for the change of k value in combination with the screening effects of counter ions around the repressor particle and the DNA molecule, the most effective factor seems to be the Coulomb repulsive forces and or London dispersion forces. The sliding mechanism for facilitated translocation of a repressor protein on a DNA chain can be described by the present mathematical approach which describes the temporal changes in amounts of the species. To achieve such mechanism in varying ionic strength, the secondary changes in the diffusion constants, the reaction radius and the reaction rate per one particle on the reaction sphere surface k value (an electro chemical factor) seemed to have important roles.

Key words: Coulomb forces, diffusion, electro chemistry, London forces, operator, repressor

1. Introduction

There are four possible mechanisms for translocation of a repressor protein on a DNA molecule [1–4]. They are 1) macroscopic reaction within the domain of the DNA molecule, 2) microscopic reaction between closely space sites in the DNA, 3) inter segment transfer through ring closure between different segments of the DNA and 4) Sliding [5–6]. These mechanisms have been thought to facilitate the repressor protein particle to find a target operator region on the DNA molecule[2]. The validity of these possible mechanisms have been investigated

by measuring association and dissociation kinetics of a repressor particle to the DNA molecule [7–9]. Among these mechanisms, the sliding mechanism has been thought to account the association and dissociation of repressor operator complex [9]. The repressor has been assumed to slide on the contour length of the DNA molecule in one dimensional random walk [3, 6]. During such scanning, a repressor particle has remained to bind on the DNA chain non specifically [7, 8]. The sliding mechanism has been described formally by several mathematical approaches [5, 6]. These works have given Laplace transformed equations for transient changes in concentration and flux of a repressor in terms of the first time and recurrent arrival probabilities of the repressor. These theoretical studies did not, however give computed data of the temporal changes in flux and amounts of repressor-operator complex by inverting the Laplace transformed equations. In addition, some biophysical parameters were unrealistic and obscure [6]. As a result, these previous theoretical efforts could not be compared to experimental data. Hence, the validities of sliding mechanism and of the mathematical description could not be certified. Several experimental works [4, 7–9] have determined rate constants for association k_a and dissociation k_d of repressor operator reaction by measuring instantaneous concentrations of free operator, free repressor and repressor-operator complex. For each experiment however, these investigations did not show exact parameter values such as diffusion constant, radius of reaction, radius and length of a DNA molecule. Kinetic experimental studies [7–9] reported rate constants k_a , k_d on the basis of the first and the second order reactions by the least square method. The mathematical derivation for k_a and k_d was static and different from those of previous theoretical works [5, 6]. Thus, the instantaneous measured data of repressor operator interaction could not be compared to the theoretical prediction that those experimental works [7–9] have proposed. Any way, experimental data [7–9] have not been compared to computed temporal changes of species that would have been obtained by theoretical studies [5, 6]. In the experimental investigations [7–9], the decrease in k_a by an increase in concentration of KCl has been attributed to an electrostatic factor. The equations for k_a and k_d [7, 8], however did not include the electro static factor explicitly. As long as one will participate in the sliding theory, the ultimate out put of the electro static effect should be explained reasonably at least by the secondary changes in diffusion coefficients on the medium and on the DNA and in the nature of reaction k value [10].

In the present work, we examine the validity of sliding mechanism of a repressor on the DNA molecule by simulating reported experimental data [7–9]. We have inverted the Laplace transformed temporal changes [6] in concentration and flux of a repressor toward the DNA molecule with reported biophysical parameters [1, 3, 6, 7]. We have successfully simulated the experimental data measured at different ionic strength and at different length of the DNA molecules. We have discussed the biophysical significance of regulating the parameters to simulate the reported experimental data [7–9] particularly in terms of screening effects of ionic solution.

Figure 1. Cylindrical modeling of recurrent dissociation-association of Repressor repressor repressor particles around the DNA chain as a sink. Figure 1 describes an interaction space of repressor particles and the operator region of a DNA molecule in a cylindrical coordinates. R is the radius of an envelope cylinder volume that encloses the DNA molecule. b is the radius and L is the length of the DNA. z is longitudinal axis of the DNA chain. The sink is assumed to occupy the center of DNA cylinder $z = 0$.

2. Modeling

2.1. THE BASIC DIFFERENTIAL EQUATION [6]

Cylindrical coordinates r and z were introduced to characterize the straight and circular symmetric natures of the DNA molecule with radius b and length L . An envelope cylindrical volume with radius R was set around a DNA molecule (Figure 1) [6]. The radius R was set sufficiently large to enclose the whole interaction space of the repressor particle and the DNA. The operator region of the DNA to

which the repressor particle approaches was regarded as a sink at the origin $z = 0$ of the cylindrical coordinates.

In the large envelope, a repressor particle moves with a diffusion constant D . When the repressor particle approaches to the DNA, the particle will be absorbed into the DNA. Then it moves as if sliding along the DNA with one dimensional diffusion constant D_1 . The repressor particle will leave the DNA unless it has bound to the sink. We assume that a repressor particle which has dissociated from the DNA will be again lifted out within the distance $r = a$ ($R > a > b$) from the DNA to start anew [6]. The distance 'a' expresses the lifting out radius. It characterizes the movements of a repressor particle that has dissociated from the DNA at $t = 0$ will be lifted out from the DNA where it will start again [6]. We describe the arrival and leaving of a repressor particle to the DNA as a sink by the following three probabilities [6].

$u(z,t) dz$: The probability that a repressor particle is in the space interval of $(z, z+dz)$ on the DNA at time t . (1)

$G(z,t) dz dt$: The probability that a repressor particle arrives at the DNA for the first time in the space interval $(z, z+dz)$ during the time interval $(t, t+dt)$ (2)

$F(z,z', t-t') dz dt$: The probability that a repressor particle that has left the DNA from z' at time t' again returns to the DNA in the space interval $(z, z+dz)$ during the time interval $(t, t+dt)$ (3)

The diffusion equation of the repressor particle along the DNA chain is described by the space and time specified surviving probability $u(z,t)$ of the repressor particle on the DNA [6],

$$\frac{\partial u}{\partial t} = D_1 \frac{\partial^2 u}{\partial z^2} - \lambda u + \lambda \int_0^L dz' \int_0^t dt' F(z, z', t - t') u(z', t') + G(z, t) \quad (4)$$

where λ is a microscopic non specific dissociation rate constant. The first term of the right describes the diffusional displacement along the DNA by D_1 . The second term describes the repressor particle dissociation. The third describes the return of a repressor particle to the DNA that has had left the DNA at an earlier time. The last one describes the arrival of an uncorrelated repressor particle. Since the influences of pH and temperature are relatively weaker than the influence of ionic strength [7], we did not consider those factors.

2.2. THE MATHEMATICAL METHOD

The diffusion equation for the concentration $c(r,z,t)$ of repressor particles in the envelope cylinder is given by [6]

$$\frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial z^2} - \frac{1}{D} \frac{\partial c}{\partial t} = 0 \quad (5)$$

The initial condition is [6]

$$c(r, z, t = 0) = R_{pi}/[\pi(R^2 - b^2)L] \quad (6)$$

R_{pi} is the initial amount in Mol of the repressor particles in the envelope cylinder. The boundary conditions are [6]

$$\partial c / \partial z_{(z=L, z=0)} = 0 \quad (7-a), (7-b),$$

$$kc(r = b) = \partial c / \partial r(r = b) \quad (8)$$

$$\partial c / \partial r(r = R) = 0 \quad (9)$$

k is a parameter which is the nature of a reaction rate [10]. k elucidates the relative potency of contribution from factors other than diffusion to diffusion in a particle reaction system. A large k value means that factors other than diffusion become predominant in molecular interaction. In such situation, once a particle has reached in the reaction range, almost all the particles will react with target particles. As a result a diffusion mechanism becomes the rate limiting factor and is called diffusion controlled limited state. A large k also gives the boundary condition for an ideal sink [6]. A detailed explanation for biophysical significance of k value is given in discussion. By using the Laplace transformation [11], the solution of the equations (5) to (9) is achieved through separating the variables as

$$c(r, z, t) = v(z, t)w(r, t) \quad (10)$$

The axis z dependent part is found to [6]

$$v(z, t) = 1/L + 2/L \sum_{n=1}^{\infty} \cos(n\pi z'/L) \cos(n\pi z/L) \exp(-Dt(n\pi/L)^2) \quad (11)$$

The differential equation for radial concentration $w(r, t)$ is [6]

$$\frac{\partial^2 w}{\partial r^2} + \frac{1}{r} \frac{\partial w}{\partial r} - \frac{1}{D} \frac{\partial w}{\partial t} = 0 \quad (12)$$

The solution of equation (12) in the Laplace space is given by [6], [11]

$$\begin{aligned} w(r, s) = & [I_1(qR)K_0(qa) + K_1(qR)I_0(qa)]\{[kK_0(qb) + qK_1(qb)]I_0(qr) \\ & - [kI_0(qb) - qI_1(qb)]K_0(qr)\} \\ & / \{2\pi DI_1(qR)[kK_0(qb) + qK_1(qb)] + K_1(qR)[kI_0(qb) - qI_1(qb)]\} \end{aligned} \quad (13)$$

where $q = \sqrt{s/D}$. The z -independent flux $\psi(t)$ of repressor particles onto the DNA is [6]

$$\begin{aligned} \psi(s) = & 2\pi bD \frac{\partial w}{\partial r(r=b)} = \\ & k \frac{[I_1(qR)K_0(qa) + K_1(qR)I_0(qa)]}{\{I_1(qR)[kK_0(qb) + qK_1(qb)] + K_1(qR)[kI_0(qb) - qI_1(qb)]\}} \end{aligned} \quad (14-a)$$

$$-\partial\psi(s)/\partial s(s=0) = \tau 2 \quad (14-b)$$

is the mean time during which a repressor particle stays outside of the DNA before being reabsorbed [6]. The process for differentiating $\psi(s)$ about s is given in Appendix 1.

The back flux of the repressor particle to the DNA, $F(z, z', t)$ which enters the equation (4) is given by [6]

$$F(z, z', t) = 2\pi b D \partial c / \partial r (r = b) \quad (15)$$

This can be calculated after solving the equations (5) to (9). The form of $G(z, t)$ is given [6] by the equation (16) with the initial condition (6),

$$G(z, t) = \int_0^L dz' \int_b^R da 2\pi a F(z, z', t) / [\pi(R^2 - b^2)L] \quad (16)$$

The Laplace transform of $F(z, z', t)$ is given from the equation (15) [6, 11]

$$F(z, z', s) = \psi(s)/L + 2/L \sum_{n=1}^{\infty} \psi(s + D(n\pi/L)^2) \cos(n\pi z'/L) \cos(n\pi z/L) \quad (17)$$

The Laplace transform of $G(z, t)$ is obtained [11] by substituting $F(z, z', s)$ from the equation (17) to the equation (16) and integrating $F(z, z', s)$ over all dz' and da [6],

$$G(s) = 2bk / \{L(R^2 - b^2)q\} \text{Rpi}[I_1(qR)K_1(qb) - K_1(qR)I_1(qb)] / \{I_1(qR)[kK_0(qb) + qK_1(qb)] + K_1(qR)[kI_0(qb) - qI_1(qb)]\} \quad (18-a)$$

$$-L \partial G(s) / \partial s (s=0) = \tau 1 \quad (18-b)$$

is the mean time of the first arrival of a repressor particle on the DNA [6]. The process for differentiating $G(s)$ about s is given in Appendix 1. The Laplace transformed original differential equation (4) is

$$D_1 \partial^2 u / \partial z^2 - (s + \lambda)u + \lambda \int_0^L dz' F(z, z', s) u(z', s) + G(s) = 0 \quad (19)$$

With the Laplace transformed boundary conditions [6],

$$u(0, s) = 0, \quad \partial u / \partial z (z = L) = 0 \quad (20-a, 20-b)$$

The solution satisfying the boundary conditions (20) is given by the form of [6]

$$u(z, s) = \sum_{n=0}^{\infty} f_n(s) \sin[(2n+1)\pi z / (2L)] \quad (21)$$

A detailed process for getting $\Phi(s)$ has been already reported [12]. The Laplace transformed flux of repressor particles toward the DNA sink is given by [6]

$$\Phi(s) = D_1 \partial u / \partial z_{(z=0)} = D_1 \pi / (2L) \sum_{n=0}^{\infty} (2n+1) f_n(s) \quad (22)$$

$$= LG(s) / [1 + 2\{\lambda(1 - Rpi\psi(s)) + s\} \sum_{n=1}^{\infty} 1 / \{D_1(n\pi/L)^2 + s + \lambda(1 - Rpi\psi(s + D(n\pi/L)^2))\}] \quad (23-a)$$

$$-\partial \Phi(s) / \partial s (s=0) = \tau \quad (23-b)$$

is the mean time during which a repressor particle associates with the DNA [6]. The process for differentiating $\Phi(s)$ about s is given in Appendix 1.

3. Method for Laplace Inversion

The Laplace inversions of $w(r,s)$, $\psi(s)$, $G(z,s)$, $F(z,z',s)$ and $\Phi(s)$ can be achieved by an analytical method [11, 12] and a numerical one [12, 13].

3.1. ANALYTIC METHOD FOR LAPLACE INVERSION

Generally, the inversion of Laplace transformed function $\Phi(s)$ is given by [11]

$$f(t) = L^{-1}[F(s)] = 1/(2\pi i) \int_{\gamma-i\infty}^{\gamma+i\infty} F(s) e^{st} ds = \sum_{n=0}^{\infty} Re[s_n] \quad (24)$$

where s_n is the n^{th} pole of $F(s)$ and $Re[s_n]$ is its residual value at $s=s_n$.

By the residual theorem for $n \geq 1$, we have the residual calculation by [11]

$$\begin{aligned} \sum_{n=1}^{\infty} Re[s_n] &= \lim_{(s \rightarrow s_n)} \sum_{n=1}^{\infty} \{(s - s_n) F(s) e^{st}\} \\ &= \sum_{n=1}^{\infty} [\text{Numerator of } F(s) / \{d(\text{Denominator of } F(s)) / ds\} e^{st}] (s = s_n) \end{aligned} \quad (25)$$

An outline of applying this technique is given in Appendix 2 [12].

3.2. NUMERICAL INVERSION

The inversion of the Laplace transformed function $F(s)$ is [13]

$$f(t) = 1/t \log 2 \sum_{m=1}^N V_m F(m/t \log 2) \quad (26)$$

where coefficient V_m is

$$V_m = (-1)^{N/2+m} \sum_{n=(m+1)/2}^{\min(m, N/2)} \Gamma(2n+1) / [(N/2-n)!n!(n-1)!(m-n)!(2n-m)!] \quad (27)$$

For the reported values of b and D ,

$$qb = b\sqrt{s/D} = b10^{-7}\sqrt{s/(510^{-7})^{1/2}} = \sqrt{s}2.6810^{-3.5} \quad (28)$$

$$qR = R\sqrt{s/D} = R10^{-5}\sqrt{s/(510^{-7})^{1/2}} = \sqrt{s}2.6810^{-1.5} \quad (29)$$

We can apply the Allen's approximation formula [13] for the modified Bessel functions $I_0(qb)$, $I_1(qR)$, $K_0(qb)$, $K_1(qb)$, $K_1(qR)$ and $I_1(qb)$. For small value of x , the modified Bessel functions can be approximated by the Allen's formula at $x \leq 3.75$. For the present case, the corresponding ranges of the variables in terms of s are

$$\sqrt{s}2.6810^{-3.5} \leq 3.75 \quad \text{Hence } s \leq 10^7(3.75/2.68)^2 \quad (30-a)$$

$$\sqrt{s}2.6810^{-1.5} \leq 3.75 \quad \text{Hence } s \leq 10^3(3.75/2.68)^2 \quad (30-b)$$

The upper limits of s are sufficiently large to approximate these functions at very short t .

Precise processes for applying the Allen's approximation for the numerical Laplace inversion of $G(s)$ are given in Appendix 3. The mean number of times that a repressor particle dissociates from the DNA before the final absorption in the sink is given by [6]

$$N = \sum_{n=1}^{\infty} 2\lambda / \{D_1(n\pi/L)^2 + \lambda(1 - R\pi\psi(D(n\pi/L)^2))\} \quad (31-a)$$

$N\tau_2$ expresses the total mean time a particle spends off the DNA molecules [6]. Using this value, we can compute the total specific association rate by [6, 8]

$$ka = 1/(N\tau_2[O_T]) \quad (31-b)$$

where τ_2 can be computed from the equation (14-b) and $[O_T]$ is the total amount of the DNA in operator concentration.

Table I. Changes in parameters for simulating association kinetics

KCl	0.1 M	0.125 M	0.15 M
D ₁	3 10 ⁻⁹ cm ² /s	2.5 10 ⁻⁹ cm ² /s	2.2 10 ⁻⁹ cm ² /s
D	5 10 ⁻⁷ cm ² /s	3.8 10 ⁻⁷ cm ² /s	3.04 10 ⁻⁷ cm ² /s
R	50 Å	40 Å	40 Å
k	10 ⁷ /cm	10 ⁸ /cm	10 ⁸ /cm

4. Method for Simulating Temporal Changes in Amounts of Species

To examine the validity of the sliding model, we have simulated reported experimental data [7–9] that were used to determine the rate constants k_a , k_d of repressor-operator association and dissociation reactions.

4.1. PARAMETER VALUES

In the first, we have selected reliable parameter values from theoretical [3, 5, 6] and reported experiments [4, 7, 8] as the standard values. They are

$$\begin{aligned}
 L &= 8.5 \cdot 10^3 \text{ Å} [1, 6], & b &= 15 \text{ Å} [6, 8, 9] \\
 a &= 20 \text{ Å} [6, 7, 8], & R &= 50 \text{ Å} [8, 9]. & \text{Å : Angstrom.}(10^{-8}) \\
 D &= 5 \cdot 10^{-7} \text{ cm}^2/\text{s} [6, 7]. & D_1 &= 3 \cdot 10^{-9} \text{ cm}^2/\text{s} [6, 7] \\
 k &= 10^7 [6, 10]
 \end{aligned} \tag{32}$$

4.2. SIMULATION FOR REPRESSOR OPERATOR ASSOCIATION KINETICS

In the reported experiments [4, 7, 8] following rate equation has been used to determine the rate constant of repressor-operator association, k_a

$$10^{12}/([R] - [O]) \text{Log}\{([O]([R] - [RO])/([R]([O] - [RO])))\} = k_a t \tag{33}$$

where $[R]$ and $[O]$ are the concentrations (in Mol) of unbound repressor particles and of unbound λ plac 5 DNA molecule (operator concentration) respectively. $[RO]$ is the concentration (in Mol) of repressor-operator complex at an instance t . Experimental values of the left side of the equation (33) have been reported [7, 8] as functions of time in sec with parametric changes in concentration of KCL of 0.1 M, 0.125 M and 0.15 M. Theoretical values of $[RO]$ at an instance t were computed on the basis of the repressor particle flux $F(t)$ to the center of the DNA axis ($z = z' = 0$). We have

$$[RO] = (G(t) + F(z, z', t))\Phi(t)(2\pi b^2 + 2\pi bL) \tag{34}$$

Table II. Changes in parameters for simulating dissociation kinetics

KCl	0.075 M	0.125 M	0.15 M
D_1	$3 \cdot 10^{-9} \text{ cm}^2/\text{s}$	$2.3 \cdot 10^{-9} \text{ cm}^2/\text{s}$	$2.25 \cdot 10^{-9} \text{ cm}^2/\text{s}$
D	$5 \cdot 10^{-7} \text{ cm}^2/\text{s}$	$3.8 \cdot 10^{-7} \text{ cm}^2/\text{s}$	$3.6 \cdot 10^{-7} \text{ cm}^2/\text{s}$
R	$50 \text{ \AA}$	$50 \text{ \AA}$	$47 \text{ \AA}$
k	$10^{6.94} / \text{cm}$	$10^7 / \text{cm}$	$10^{7.065} / \text{cm}$

bottom area of the DNA cylinder. $2 \pi b L$ is the lateral area of the DNA cylinder. According to the reported experimental conditions [7, 8], we set the initial concentration of the DNA molecule (operator concentration) as

$$[O] = 8.0 \cdot 10^{-12} \text{ Mol.}$$

To simulate the reported values of transient changes in the left side of the equation (33) at low KCl (= 0.1 M), all the parameters were set at the standard values. With an increase in concentration of KCl, the quantities of the left side of equation (33) deviated from those obtained at low KCl concentration (= 0.1 M). In those simulations, D , D_1 and R have to be decreased while k value has to be increased. The changes in these parameters were the largest at high KCl (= 0.15 M) concentration. The changes in parameters for these simulations are given in Table I.

4.3. SIMULATION FOR DISSOCIATION KINETICS

In the reported experiments [4, 7, 8] the equation (35) has been used for determining the dissociation rate constant k_d of repressor operator complex,

$$\text{Log } \{[RO]/[RO]_o\} = -k_d t \quad (35)$$

$[RO]_o$ is a normalizing constant. It is the concentration of the RO complex measured immediately after the onset of the dissociation reaction. For the present simulation, we selected the value of $[RO]$ at $t = 1.53 \text{ min}$ as the $[RO]_o$. Experimental values of $[RO] / [RO]_o$ were reported [7, 8] as functions of time in minute with parametric changes in concentration of KCL for 0.075 M, 0.125 M and 0.15 M. The initial amount of unbound λ plac 5 DNA molecule (operator concentration) was set as

$$[O] = 3.39 \cdot 10^{-11} \text{ Mol.}$$

To simulate the data obtained at moderate KCl (= 0.125 M) and high KCl (= 0.15 M), diffusion constants D , D_1 and envelope radius R have to be decreased while the k value has to be increased. The regulated parameter values are given in Table II.

4.4. SIMULATION FOR KINETIC DATA FOR LONGER DNA

We have also simulated the kinetic data for lac repressor dissociation from λ plac 5 DNA molecule (operator concentration) as a function of trapping DNA size (unlabeled Hae III-digested λ plac 5 DNA which has a longer length) [9]. The temporal changes in $[RO]/[RO]_0$ of dissociation kinetics of longer DNA molecule (Hae – 80) [7, 8] were simulated by setting $L = 8.5 \cdot 10^{3.051} \text{ \AA}$. All other parameters on the while were set at the standard values. The initial condition was set as $[O] = 3.39 \cdot 10^{-11} \text{ Mol}$ as in the standard DNA length. All the computed values were normalized by $[RO]$ at $t = 7.33 \text{ min}$.

5. Simulated Results

Figure 2 shows simulated results for the repressor-operator association kinetic data. Figure 2-a shows simulation for experimental data obtained at low KCl ($= 0.1 \text{ M}$), Figure 2-b those obtained at moderate KCl ($= 0.125 \text{ M}$) and Figure 2-c those obtained at high KCl ($= 0.15 \text{ M}$). The rate constant for association k_a has been reported [7, 8] to decrease as KCl concentration increases. The present simulation could realize the influence of an increased KCL. At the instances after $t > 100 \text{ sec}$ in low KCl condition, the computed results could not simulate the data well.

Figure 3 shows the simulated results for the dissociation kinetic data. Figure 3-a shows simulation for experimental data $[RO]/[RO]_0$ obtained at low KCl (0.075 M), Figure 3-b those obtained at moderate KCl ($= 0.125 \text{ M}$) and Figure 3-c those obtained at high KCl ($= 0.15 \text{ M}$). The rate constant for dissociation k_d has been reported [7, 8] to increase as KCl concentration increases. The present simulation could describe the effect of high KCL condition.

Figure 4 shows the simulated data for the dissociation kinetics at the standard DNA length (Figure 4-a) and at longer DNA $L = 8.5 \cdot 10^{3.051} \text{ \AA}$ (Figure 4-b). The rate constant k_d for dissociation has been reported [9] to increase with decreasing length of the operator containing DNA fragment. The present simulation could achieve the influence of shorter sized DNA on the dissociation kinetic.

These successful simulations indicate that the mathematical expression [6] can describe the temporal changes in a repressor DNA interaction under the reported conditions [7–9]. It is, however still impossible to measure the arrival probabilities of a repressor by experimental approach. Therefore, by applying the parameter values used to simulate those reported experimental data [7–9], we show only computed temporal changes in the arrival probabilities and the total flux for association. Figure 5 shows the temporal changes (in sec) of the normalized probabilities of the first time arrival $G(t)$ (Figure 5-a), of the recurrent arrival $F(z,t)$ (Figure 5-b) and of the total flux $F(t)$ to the sink (Figure 5-c) as functions of concentration of KCl. All the data in Figure 5-a, Figure 5-b and Figure 5-c are normalized by the $G(t)$, $F(z,t)$ and $F(t)$ values respectively that were computed at $t = 15 \text{ sec}$ with KCl 0.1 M condition. As the time has passed, these quantities has decreased rapidly until $t = 44 \text{ sec}$.

Figure 2. Simulated results of association kinetics data at varying KCl concentration. Figure 2-a shows the simulation for temporal changes in quantity of the left side of equation (33) as a function of time in sec. The computed values are denoted by 'simulated'. The experimental data (denoted by measured) were those obtained at low KCL (= 0.1 M). Figure 2-b shows those obtained at moderate KCl (= 0.125 M). Figure 2-c shows those obtained at high KCl (= 0.15 M). The parameters for this simulation were given in Table I.

Figure 3. Simulated results of dissociation kinetics data at varying KCL concentration. Figure 3-a shows the simulation for temporal changes in $[RO]/[RO]_0$ as a function of time in minute. The computed data (denoted by simulated) were normalized by the value $[RO]$ at $t = 1.53$ min. The experimental data (denoted by measured) were those obtained at low KCL ($= 0.075$ M). Figure 3-b shows those obtained at moderate KCl ($= 0.125$ M). Figure 3-c shows those obtained at high KCl ($= 0.15$ M). The parameters for this simulation were given in Table II.

Figure 4. Simulated results of dissociation kinetics data at longer DNA. Figure 4-a shows the simulation for temporal changes in $[RO]/[RO]_0$ as a function of time in minute. The computed data (denoted by simulated) were normalized by the value $[RO]$ at $t = 7.33$ min. The experimental data (denoted by measured) were those obtained at the standard length of the DNA molecule. Figure 4-b shows those obtained at longer DNA molecule $L = 8.5 \times 10^3$ Å.

Figure 5. Computed temporal changes (in sec) in the normalized arrival probabilities $G(t)$, $F(z,t)$ and total flux $F(t)$. Figure 5-a shows the computed temporal changes in the normalized first time arrival probability $G(t)$ of a repressor as functions of time in sec and KCl concentration. Figure 5-b shows those of the normalized recurrent arrival probability $F(z,t)$. Figure 5-c shows those of the normalized total flux to the sink $F(t)$. All the data were normalized by the $G(t)$, $F(z,t)$ and $\Phi(t)$ values respectively that were computed at $t = 15$ sec with KCl = 0.1 M.

Figure 6. Influence of k value on the temporal changes (in sec) in the normalized arrival probabilities and association rate constant k_a . Figure 6-a and Figure 6-b show the influence of changes in k value on the temporal changes of the normalized first time arrival probability $G(t)$ (Figure 6-a) and the normalized recurrent arrival probability $F(z,t)$ (Figure 6-b). All the data have been normalized by the $G(t)$ and $F(z,t)$ values respectively that were computed at $t = 10$ sec, $k = 10^7$. Figure 6-c shows the influence of k value in combination with changes in D_1 value on the association rate constant k_a .

Table III. Simulation for k_a

KCl concentration	0.1 M	0.125 M	0.15 M
Reported k_a values	$1.4 \cdot 10^{10}$ /M/sec	$1.1 \cdot 10^{10}$ /M/sec	$3.5 \cdot 10^9$ /M/sec
Computed k_a values	$1.3959 \cdot 10^{10}$ /M/sec	$1.1611 \cdot 10^{10}$ /M/sec	$3.426 \cdot 10^9$ /M/sec

In these simulations and computations, k value seems to play an important role particularly in relation to the electro chemical mechanism for the repressor DNA interaction. Thus, we have examined the influence of k value on the temporal changes in $G(t)$ and $F(z,t)$ while keeping all the parameters at the standard values. Figure 6-a and Figure 6-b show the change of k value from $10^{6.5}$ to 10^8 and the temporal changes of the normalized first arrival probability $G(t)$ (Figure 6-a) and of the normalized recurrent arrival probability $F(z,t)$ (Figure 6-b). All the data were normalized by the $G(t)$ and $F(z,t)$ values respectively that were computed at $t = 10$ sec, $k = 10^7$. As the k value has increased, the peak probability of $G(t)$ and $F(z,t)$ have been decreased, the rapid decreases in $G(t)$ and $F(z,t)$ have become gradual.

Further, to examine the influence of changes in k value and D_1 on an association rate k_a , we have taken two approaches. For the first, we computed k_a values from the equation (31-b) in which τ_2 is obtained by differentiating $\psi(s)$ by 's' and is a function of k value. N is a function of D_1 (Figure 6-c). The range of k value was restricted within the range used for simulating (Figure 2) the reported data [7, 8] in combination with ten fold changes in D_1 value. The range of computed k_a has fallen within the reported range [7, 8] from $4.5 \cdot 10^{10}$ /M/sec to $3.5 \cdot 10^9$ M/sec. Computed k_a decreased almost parallel as k value increased. There was a little decrease in k_a as D_1 has been changed within the reported upper and lower limits [3, 7, 8].

For the second, we have simulated the reported k_a values [7] obtained at KCl = 0.1 M, 0.125 M and 0.15 M respectively by computing k_a values from the equation (31-b) under the same parameter values that were used for simulating the association kinetics (Figure 2). The comparison to reported experimental values are given in Table III. The present simulation for k_a on the basis of temporal values of the radial flux $\psi(s)$ was successful.

6. Discussion

The present work has been intended to examine the validity of sliding mechanism [1–3, 6, 7] of a repressor particle which approaches to the target operator region on the DNA molecule. The present work confines only to the temporal changes of the repressor operator interactions but not to steady one. The present mathematical approach is an extension of theoretical works [5, 6] which did not, however invert the Laplace transformed flux and arrival probabilities of a repressor particle to

the DNA. The present model for sliding mechanism did not involve the electrostatic factor explicitly. In the following discussion, however we explain the way of changes in the D , D_1 , R and k in an augmented electrostatic environment from the electro chemical stand point.

6.1. PARAMETER SETTING

The length of a DNA molecule is an order of a few thousand Å but less than 10^4 Å [1, 6]. Hence we used the length $8.5 \cdot 10^3$ Å [1]. The radius of DNA chain, b has been verified to 10 Å to 11 Å [1]. The ion exclusion radius adds about 2 Å to 5 Å [1, 2]. Hence we set $b = 15$ Å [8, 9]. Value of 50 Å [8] should be regarded as an interaction radius of the repressor operator reaction. This value is compatible to the envelope cylindrical volume of the present DNA model and we set $R = 50$ Å.

Selection of the lift out distance 'a' value basis on the long range electrostatic interaction between charges on the DNA backbone and on the repressor protein [8]. An electro chemical investigation [14] has suggested that the screen out over distance does not exceed over 25 Å. Since 'a' has an inherent restriction $R > a > b$, we set 20 Å for safety.

The diffusion coefficient D_1 on the DNA molecule has been described as a hydromechanical parameter in the sliding model [3, 6, 7]. We set it as $3 \cdot 10^{-9}$ cm²/sec for simulating the instantaneous data of association and dissociation kinetics at low concentration of KCl. D_1 describes only an apparent velocity of the repressor particle on the DNA. Rigorously speaking however, D_1 must be influenced by an electro static interaction of a repressor particle and the DNA molecule. Such speculation derives from the reported experiment [15] that a repressor carries positive charges. In addition, there are negative charges on the DNA backbone which is composed of phosphate molecules [1]. The phosphate charges occupy discrete discontinuous positions on the DNA molecule [1, 3]. As a result, some parts of the mobility of a repressor on the DNA chain must be contributed from the complicated electro static interaction between the charges on the repressor and on the DNA molecule. Thus, D_1 must be influenced by the electro static circumstance around the reaction space which envelopes the repressor operator interaction.

Mathematical description of D_1 in terms of the electro chemical quantities should be linked to an Electrophoretic problem [16]. The effect to be examined is to the motion of a charged particle suspended in an electrolytic solution under the action of an electric field. The mobility of a charged particle is a complicated function of gradients of the particle concentration and of the electrostatic potential [16–19]. Such problem, however is out of scope of the present investigation. Any way, D_1 reflects the electro static effect indirectly. Therefore it is not strange that D_1 has to be changed at different KCl concentration in the present simulation.

D_1 is reported to range ten folds [7, 8]. The values from $D_1 = 8 \cdot 10^{-10}$ cm²/sec [7] to $9 \cdot 10^{-10}$ cm²/sec [7], however were too slow to simulate the reported data. Setting D_1 value faster than $5 \cdot 10^{-9}$ cm²/sec on the while could not get successful

simulation. This is consistent to the suggestion that D_1 should not exceed over $4.5 \cdot 10^{-9} \text{ cm}^2/\text{sec}$ [20].

The diffusion constant D in the medium describes free diffusion of a repressor protein in a solution of the envelope cylinder. The range of D from $2 \cdot 10^{-7} \text{ cm}^2/\text{sec}$ to $5 \cdot 10^{-7} \text{ cm}^2/\text{sec}$ derives from a hydrodynamical estimate [3, 6]. As the concentration of KCl has been increased, the electrostatic circumstance in the envelope volume will be disturbed. Hence, the standard value of $5 \cdot 10^{-7} \text{ cm}^2/\text{sec}$ has to be changed to simulate the data at high KCl condition.

k value has been determined from the reported experimental data [4], [21] to obtain the equilibrium constant K_c ($= 1.4 \mu\text{g}/\text{cm}^3$) [6] for dissociation of a repressor particle from the DNA chain. In the derivation of the minimum k value, we set $D = 5 \cdot 10^{-7} \text{ cm}^2/\text{sec}$, $D_1 = 3 \cdot 10^{-9} \text{ cm}^2/\text{s}$, $b = 15 \text{ \AA}$ and $a = 17 \text{ \AA}$ which are almost within the range of the standard parameter values.

6.2. INITIAL AMOUNTS OF THE DNA MOLECULES (OPERATOR CONCENTRATION)

For the association kinetics, DNA molecules (operator concentration) from $0.5 \cdot 10^{-12} \text{ M}$ to $1.0 \cdot 10^{-12} \text{ M}$ for unbound Eco RI lac and $\lambda\text{plac } 5$ DNA molecule have been used as the initial concentration [7, 8]. The amount of $\lambda\text{plac } 5$ DNA molecule required to quench the reaction at an arbitrary instance was reported [7], [8] to range for 5 folds to 50 folds excess without affecting the measured associated rate constant. Hence, we set the initial DNA amount of $8.0 \cdot 10^{-12} \text{ Mol}$ to simulate the association kinetics that is approximately within the range of reported initial concentration of $\lambda\text{plac } 5$ DNA molecules.

For the dissociation kinetics, DNA concentrations from $1 \cdot 10^{-11} \text{ M}$ to $2 \cdot 10^{-11} \text{ M}$ have been used [7, 8] and added to 50 % to 75% operators. So we set $3.39 \cdot 10^{-11} \text{ M}$ as the initial amount of the DNA molecule (operator concentration) for simulating the dissociation kinetic data. The active repressor was set $1.0 \cdot 10^{-11} \text{ M}$ according to reported data [7, 8].

6.3. COMPUTED RESULTS

6.3.1. Influence of ionic strength

The association and dissociation kinetics of a repressor and an operator region of a DNA molecule have been reported to be influenced by high concentration of KCl [7–9]. This sensitivity suggested that all the processes of the repressor operator reaction are modified by changes in the electrostatic interaction between the negatively charged DNA molecule and positive charges on the repressor. The understanding that the repressor carries positive charges has been founded on the experimental report [15] that cations are released from the DNA molecule when the repressor has bound to the DNA molecule. Thus the changes in parameters of the

present simulation for high KCl data have to be related to the electrostatic change of the reaction field around the repressor and the DNA molecule.

In the following, we discuss the way we have regulated the parameters to simulate the repressor operator kinetics at potentiated electro static circumstance induced by increasing KCl concentration. Firstly we explain the changes in D , D_1 and radius of the envelope cylindrical volume R in terms of redistribution of charges around the repressor and the DNA molecule. In the second we explain the biophysical significance of k value and its change for simulating the kinetic data of the repressor operator interaction obtained under the potentiated electrical field by high KCl circumstance.

6.3.2. *Changes in D , D_1 and R*

D , D_1 and R have to be reduced to simulate the experimental data [7, 8] for association kinetics at high KCl condition. As concentration of KCl increases, the positive charges [15] on the repressor were screened by Cl^- ions that have dissociated from the solution of KCl. On the while, the negative charges on the DNA molecule are also screened by K^+ ions. Hence, the electrostatic interaction between the repressor protein and the DNA molecule will be shielded by Cl^- ions and K^+ ions respectively and will be diminished. In the following, we discuss the mobilities of unscreened screened and screened repressor particles in relation to decreasing the diffusion constants.

In the first, we discuss the behavior of an unscreened repressor. By an increase in K^+ ions in the medium of the envelope cylinder, the motion of an unscreened repressor that has positive charges on it must be restricted due to the repulsive effect among these positive charges. Hence, the mobility of an unscreened repressor in the medium will be reduced. In addition, the access of an unscreened repressor (having positive charges) to the DNA molecule and its sliding walk on the DNA contour are also diminished by the screening effect of K^+ ions that have surrounded the surface of DNA molecule. This is because the DNA molecule exposes negative charges on its surface and K^+ ions act as counter ions. These changes in the mobility of an unscreened repressor in the medium and on the DNA result in decreases of D and D_1 .

In the second, we discuss the behavior of a screened repressor. The screened repressor particle also decreases its mobility. As an increase in Cl^- ions, an entire surface of a repressor protein is masked by the shell of aggregated Cl^- ions. In addition an apparent molecular volume of the repressor must be expanded. This shielding will reduce the effective reaction radius of the repressor in the medium [16]. More over the Cl^- ions shell around the repressor protein will reduce an electro motive excursion of the repressor particle. Finally, the mobility of an expanded screened repressor molecule must be reduced both in the medium and on the DNA.

In the present simulation, the decreases in the mobilities of screened and unscreened repressors in the medium and on the DNA contour could be realized by reducing the diffusion coefficients in the medium D and D_1 on the DNA. These

computational results are consistent to the fact that an increased repressor operator complex by reducing salt concentration evokes wider diffusional excursions of the repressor along the DNA [7, 8].

By these reductions of the diffusion coefficients D , D_1 and reaction radius R , factors other than diffusion become relatively predominant for determining behaviors of the repressor. This is consistent to the conclusion obtained by experimental approaches [7–9]. Such state on the other hand can be represented by an increase in k value [10]. In the present simulation, this situation has been realized by increasing the k value from 10^7 to 10^8 to simulate the data obtained at high KCl concentration [7, 8]. A detailed discussion for k value is given in the next section. Similar explanation will be possible for the present simulation results in the accelerated dissociation kinetics data at high KCl condition.

6.4. THE BIOPHYSICAL SIGNIFICANCE OF k VALUE AND ITS CHANGE IN TERMS OF DIFFUSION CONTROLLED LIMITED STATE

According to the diffusion theory [11], the Fick's law relates the concentration $c(r)$ of particles to the rate of reaction FM by

$$\Phi_M = 4\pi R^{*2} D (\partial c / \partial r) (r = R_c) \quad (D-1)$$

where D is a diffusion coefficient, R^* is the closest distance between two interacting particles. This means that a reaction occurs within the sphere of radius $r = R^*$, a reaction sphere. R^* is larger or equal to the sum of radius of two reacting particles A and B. In this treatment, the boundary conditions at $r = R^*$ have not been formulated. The difficulty lies in the fact that all the B particles that reach to the reaction radius do not always react with A particles. This leads that the usual boundary condition ' $c = 0$ at $r = R^*$ ' is not always adequate.

This problem can be conquered [10] by assuming that the probability by which one (B) particle reacts with another particle (A) is proportional to the probability by which B particle lies in the concentric spherical shell between $r = R^*$ and $r = R^* + dR^*$. This assumption can be formulated by introducing the nature of a specific reaction rate k such that [4]

$$\Phi_M = k 4\pi R^{*2} c_{(r=R^*)}. \quad (D-2)$$

Hence, $k = \Phi_M / (4\pi R^{*2} c_{(r=R^*)})$. Since $4\pi R^{*2}$ is the total surface area of the reaction sphere of radius $r = R^*$, $4\pi R^{*2} c_{(r=R^*)}$ indicates the total amount of particles on an entire surface area of the reaction sphere. Hence, k indicates the reaction rate per one particle on the reaction sphere surface with radius $r = R^*$. A large k value indicates high probability that the particles which reached the reaction field almost always react with the target particles. Large k value also describes that the system is reduced to be a diffusion controlled limited state and the relative potency of diffusion has been weakened. This means that factors other than diffusion has become potent to dominate the reaction system.

Among the factors other than diffusion mechanism, an electro chemical mechanism must be the most reasonable contributor for such reaction state. Hence, a k value in the present simulation must measure the relative potency of the electro chemical factor for sliding mechanism of facilitated transfer of the repressor. In the present simulations for association and dissociation of repressor operator reaction under high KCl, k value has to be increased. This means that with an increase in electro chemical influence by KCl, the electro chemical effect must play a more potent effect on the repressor operator kinetics.

In the next, we discuss possible electro chemical factors for the repressor operator interaction. The interaction of two molecular species can be treated by classical electrostatics including the Van der Waals forces. Since the radial distance between the repressor protein and the DNA falls within the range of $a - b = 20 \text{ \AA} - 15 \text{ \AA} = 5 \text{ \AA}$ at the nearest condition, the electro chemical force can be regarded as a long range force [16]. This interpretation is valid because the 'long range' is the distance which is great enough to neglect the overlap of electron distributions on the molecules [16]. Coulomb force between the molecules is one of the determinant physical quantities but it is shielded by ions of opposite sign in a medium of electrical neutrality. London forces (dispersion forces) [16] must be another candidate for the long range interaction because all atoms and molecules exert attractive forces on one another at above range of the long distance. A weaker attraction will be still effective even at very long distance extending over a hundred $\text{\AA}$ [16]. In addition the London forces act on the molecules regardless of the permanent multi poles of those interacting molecules.

Now, we discuss the dominancy of the probable Coulomb force and London forces in the case of the present high KCl condition. In such ionic circumstance, the surface of a repressor particle will be shielded perfectly by Cl^- ions. An entire surface of the DNA molecule, however must still expose negative charges because the DNA is a large molecule and has significantly larger surface area than that of a repressor particle. Hence the Coulomb repulsive force between the screening Cl^- ions around repressor particle and the negative charges on the DNA will be still effective than the London attractive forces. As a result, the association of the repressor to the DNA will be reduced as KCl concentration increases. For more precise discussion of the these molecular interactions, one should take the geometric factor and orientation of the molecules into consideration. Because they must contribute to the electrical dipole moment of the interacting molecules [16]. This issue is however, out of the present simulation study.

6.5. INFLUENCE OF DNA LENGTH

The temporal change in $[\text{RO}]/[\text{RO}]_0$ for the shortened DNA was successfully simulated only by decreasing the length of DNA molecule. The present simulation is consistent to the reported data [7] that the dissociation rate constant k_d increases as the size of the lac operator DNA becomes smaller. Hence, the present mathematical

modeling and sliding mechanism seem to be applicable for the kinetic analysis of the operator containing DNA molecule which has varying length.

6.6. TEMPORAL CHANGES IN $G(t)$ AND $F(z, t)$

Computed rapid decreases of the first time arrival probability $G(t)$ and the recurrent arrival probability $F(z, t)$ have continued until $t = 44$ sec and small oscillations have lasted until $t = 90$ sec (Figure 5). Hence, the repressor operator complex [RO] must be produced mainly until $t = 44$ sec and lasted in small quantity until $t = 90$ sec. On the other hand as in the Figure 2, the quantity for the association which is expressed by the equation (33) has increased until $t = 120$ sec. Let us note that the quantity of the left side of equation (33) is not an amount of the complex itself. This discrepancy must originate in the structure of the equation (33) in which there are decreasing terms [RO] (equation 34) both in the numerator and in the denominator. Other quantities that would affect on the temporal changes in the equation (33) are the free [O] and [R], because the rates of consumption of free [O] and free [R] for producing the repressor operator complex in combination with the initial conditions are different [4] for these two quantities. Hence the temporal changes in ratio between concentrations of the free [O] and the free [R] used for the association will influence on the temporal change in amount of the left side of equation (33).

As a concentration of KCl has increased, these rapid decreases of $G(t)$, $F(z, t)$ and $F(t)$ have become gradual and the peak probabilities have decreased. From these, it may be conceivable that an ionic strength may influence on the arrival probabilities and flux of the repressor particle. These changes must express that a high KCl condition may prevent rapid access of a repressor to the DNA through reducing D , D_1 , R and or increasing k value.

6.7. k_a AND k VALUE

Among the parameters to simulate kinetic data and k_a particularly in high KCl, k value has the most potent influence. This again emphasizes that the factor other than diffusion must play an important role. The computed k_a from the equation (31-b) is a function of τ_2 and N . Hence the present successful simulation for k_a indicates that the values of τ_2 and N may be comparable to probable experimental data. The reported experimental k_a values [7, 8] could be simulated by the mathematical expression for temporal changes in association kinetics of the repressor through τ_2 . The present mathematical expression was different from the static analysis [3, 7, 8] and will be available for predicting instantaneous experimental data for the repressor operator interaction.

6.8. MODELING

Steady state experiments [7–9] predicted rate constants k_a and k_d for association and dissociation on the basis of a time evolution equation for the probability $u_j(t)$ that a repressor is bound at a specific site j on the DNA molecule. They utilized the infinite time integrated variable $\int u_j(t) dt$ instead of $u_j(t)$ to obtain the rate constant. Hence, such analysis treated only the time averaged mean behavior of the repressor particle.

In the mathematical treatment for coupled diffusion [22], the effects of varying the ionic strength were involved to obtain an association constant, k_a . k_a was expressed as a complicated function of a screened electro static potential $V(r)$ [16, 23]. In the theory, however equations have been derived only for the steady state by letting the Laplace operator s to 0 but did not give an exact solution for temporal change of the system. In the second, the function $V(r)$ was not defined explicitly but approximated by the second kind modified Bessel function of 0 order $K_0(r)$.

Steady state theoretical analysis [7, 8] gave a dissociation rate Λ to obtain an association constant k_a on the basis of the mathematical work for coupled diffusion [22]. But the equation of Λ describes only for a special case where $V(r)$ approaches to 0 or the Debye screening length κ^{-1} [16] approaches to 0. Summing up, these previous works attended only for a steady state, the mean time behavior of a repressor protein and the influence of the electro static effect for deriving the association rate constant was adequate only for a limited situation. The present mathematical modeling can simulate the reported temporal changes of the repressor and operator which instantaneous values can not be simulated by a steady state approach.

7. Conclusion

Validities have been examined by computational simulation analysis for sliding mechanism of a repressor particle on a DNA molecule to find the target operator region and for the mathematical descriptions of the transient changes in repressor operator association dissociation kinetics. The theoretical prediction could simulate the reported experimental temporal changes in the quantities describing association and dissociation of repressor operator complex formation that were obtained at different ionic strength and at different DNA length. The reported values of association rate k_a also could be simulated by computing the temporal changes in the radial flux. Among the regulated parameters, the reaction rate per one particle on the reaction sphere surface, k value has significant influence on k_a values. Hence, an electro chemical factor than diffusion seems to play an important role in repressor operator reaction. Effective factors for the sliding mechanism seem to the Coulomb repulsive forces and or the London dispersion forces.

The present mathematical approach can describe the temporal behaviors of association and dissociation kinetics of the repressor and operator interaction that are obtained at different ionic strength and different DNA length.

References

1. Von Hippel, P.H.: On the Molecular Bases of the Specificity of Interaction of Transcriptional, Proteins with Genome DNA, In: R.F. Goldberger (eds.), *it Biological Regulation and Development*, Plenum Press, NY, 1979, **8**, pp. 279–347.
2. Von Hippel, P.H. and Berg, O.G.: Facilitated Target Location in Biological Systems, *J. Biol. Chem.* **264**(2) (1989), 675–678.
3. Berg, O.G., Winter, R.B. and von Hippel, P.H.: Diffusion-Driven Mechanisms of Protein Translation on Nucleic Acid. 1. Model and Theory, *Biochemistry* **20** (1981), 6929–6948.
4. Riggs, A.D., Bourgeois, S. and Cohn, M.: The *lac* Repressor-Operator Interaction. III. Kinetic Studies, *J. Mol. Biol.* **53** (1970), 401–417.
5. Richter, P.H. and Eigen, M.: Diffusion Controlled Reaction Rates in Spheroidal Geometry. Application to Repressor-Operator Association and Membrane Bound Enzymes, *Biophys. Chem.* **2** (1974), 255–263.
6. Berg, O. and Blomberg, C.: Association Kinetics with Coupled Diffusional Flows, *Biophys. Chem.* **4** (1976), 367–381.
7. Winter, R.B.: *Equilibrium and Kinetic Studies of the lac Repressor-Operator Interaction. A Dissertation*, Dept. of Chemistry and the Graduate School of the University of Oregon, December 1980.
8. Winter, R.B. and von Hippel, P.H.: Diffusion-Driven Mechanisms of Protein Translation on Nucleic Acid. 2. The *Escherichia coli* Repressor-Operator Interaction; Equilibrium Measurements, *Biochemistry* **20** (1981), 6948–6960.
9. Winter, R.B., Berg, O.G. and von Hippel, P.H.: Diffusion-Driven Mechanisms of Protein Translation on Nucleic Acid. 3. The *Escherichia coli* Repressor-Operator Interaction; Kinetic Measurements and Conclusions, *Biochemistry* **20** (1981), 6961–6977.
10. Collins, F.C. and Kimball, G.E.: Diffusion Controlled Reaction Rates, *J. Colloid. Sci.* **4** (1949), 425–437.
11. Carslaw, H.S. and Jaeger, J.C.: *Conduction of Heat in Solids*, Oxford Univ. Press, London, 1967.
12. Hirayama, H.: Transient Analysis of Stochastic Process in Gene Regulation', *Proc. 31st Int. Symp. on Stochastic Systems Theory and its Application*, pp. 283–288, 1999.
13. Stechfest, H.: Numerical Inversion of Laplace transforms, *Comm. ACM.* **13** (1970), 47–49.
14. Stigter, D.: Interactions of Highly Charged Colloidal Cylinders with Application to Double-Stranded DNA, *Biopolymers* **16** (1977), 1435–1448.
15. Record, M.T. Jr., Anderson, C.F. and Lohman, T.M.: Thermodynamical Analysis of Ion Effects on the Binding and Conformational Equilibria of Proteins and Nucleic Acids; The Roles of Ion Association or Release Screening and Ion Effects on Water Activity, *Q. Rev. Biophys.* **72** (1978), 103–178.
16. Berry, R.S., Rice, S.A. and Ross, J.: *Physical Chemistry*, Intermolecular forces. Oxford University Press, 2000, **10**, pp. 298–312.
17. Booth, F.: The Cataphoresis of Spherical, Solid Non Conducting Particles in a Symmetrical Electrolyte, *Proc. Roy. Soc A* **203** (1950), 514–.
18. Mangelsdorf, C.S. and White, L.R.: Effects of Stern-Layer Conductance on Electrokinetic Transport Properties of Colloidal Particles, *J. Chem. Soc. Faraday. Trans.* **86**(16) (1990), 2859–2870.
19. Hirayama, H.: *Computational Dynamical Method for Evaluating the Mobility of Charged Bio molecules Passing Through the Electrical Potential Field within the Ion Selective Channel on Excitable Membrane*, Technical report of Institute of Electronics, Information and Communication Engineers, OME 2002-66-78. 2002, pp. 55–60.
20. Schurr, J.M.: The One-Dimensional Diffusion Coefficient of Protein Absorbed on DNA, *Biophys. Chem.* **9** (1979), 413–414.

21. Lin, S. and Riggs, A.D.: The General Affinity of lac Repressor for *E. Coli* DNA, *Cell* **4** (1975), 107-1111.
22. Berg, O.G. and Blomberg, C.: Association Kinetics with Coupled Diffusion III. Ionic Strength Dependence of the Lac Repressor-Operator Association, *Biophys. Chem.* **8** (1978), 271-280.
23. Israelachvill, J.N.: *Intermolecular and Surface Forces*, Academic press, 1992.

Appendix 1

$$\begin{aligned}
 G'(s) &= \partial G(q(s))/\partial s = \partial G/\partial q \partial q/\partial s \\
 &= \partial[G_N(q)/G_D(q)]/\partial q \partial(\sqrt{s}/\sqrt{D})/\partial s \\
 &= [G_D(q)\partial G_N(q)/\partial q - G_N(q)\partial G_D(q)/\partial q]/G_D(q)^2 \partial(\sqrt{s}/\sqrt{D})/\partial s \quad (\text{A-1})
 \end{aligned}$$

where the differentiation of denominator is

$$\begin{aligned}
 \partial G_D(q)/\partial q &= \\
 &= kR(K_0(qb)I_0(qR) - I_0(qb)K_0(qR)) - (k/q + bq)(K_0(qb)I_1(qR) \\
 &\quad + I_0(qb)K_1(qR)) + qR(K_1(qb)I_0(qR) + I_1(qb)K_0(qR)) \\
 &\quad + (bk + 1)(I_1(qb)K_1(qR) - K_1(qb)I_1(qR)) \quad (\text{A-2})
 \end{aligned}$$

and the differentiation of the numerator is

$$\begin{aligned}
 \partial G_N(q)/\partial q &= \\
 &= [-3I_1(qR)K_1(qb) + 3K_1(qR)I_1(qb) - qb(I_1(qR)K_0(qb) \\
 &\quad + K_1(qR)I_0(qb)) + qR(K_1(qb)I_0(qR) + I_1(qb)K_0(qR))]/q^2 \quad (\text{A-3})
 \end{aligned}$$

The differentiation of $\psi(s)$ about s can be obtained by differentiating numerator $\psi_N(q)$ because the denominator of $\psi(s)$ is the same with that of $G(s)$. The operation on the essential part is

$$\begin{aligned}
 \partial \psi_N(q)/\partial q &= \\
 &= a[-I_1(qR)K_1(qa) + K_1(qR)I_1(qa)] - [K_0(qa)I_1(qR) \\
 &\quad + I_0(qa)K_1(qR)]/q + [K_0(qa)I_0(qR) - I_0(qa)K_0(qR)]R \quad (\text{A-4})
 \end{aligned}$$

The differentiation of $\Phi(s)$ about s can be obtained by differentiating its denominator $\Phi_D(s)$, because the numerator of $\Phi(s)$ is the same with that of $G(s)$. The operation on the essential part is

$$\begin{aligned}
 \partial \Phi_D(s)/\partial s &= \\
 &= 2(1 - \lambda\psi'(s)) \sum_{n=0}^{\infty} 1/\{D1(n\pi/L)^2 + s + \lambda(1 - \psi(s + D(n\pi/L)^2))\} \\
 &\quad - (1 + 2\lambda(1 - \psi(s)) + 2s) \sum_{n=0}^{\infty} (1 - \lambda\partial\psi(s + D(n\pi/L)^2)/\partial s)/ \\
 &\quad \{D1(n\pi/L)^2 + s + \lambda(1 - \psi(s + D(n\pi/L)^2))\}^2 \quad (\text{A-5})
 \end{aligned}$$

Appendix 2

The n th root of the characteristic equation of $\psi(s)$ is given by the denominator of $\psi(s)$

$$I_1(qR)[kK_0(qb) + qK_1(qb)] + K_1(qR)[kI_0(qb) - qI_1(qb)] = 0 \quad (\text{B-1})$$

where $q = \sqrt{s/D}$. The pole of the denominator can be expressed by $s_n = -D\omega_n^2/a^2$ where ω_n is the n^{th} root of equation A-1. By the use of inter change formula of Bessel and modified Bessel functions and putting $q = \sqrt{s/D} = i\omega/a$ as in usual setting,

$$\begin{aligned} K_0(qa) &= K_0(i\omega) = \pi/2(-Y_0(\omega) - iJ_0(\omega)) \\ K_0(qb) &= K_0(i\omega b/a) = \pi/2(-Y_0(\omega b/a) - iJ_0(\omega b/a)) \\ K_1(qR) &= K_1(i\omega R/a) = \pi/2(-J_1(R/a\omega) + iY_1(R/a\omega)) \\ K_1(qb) &= K_1(i\omega b/a) = \pi/2(-J_1(b/a\omega) + iY_1(b/a\omega)) \end{aligned} \quad (\text{B-2})$$

substituting equation (A-2) into the denominator of $\psi(s)$, we have

$$\begin{aligned} &(\text{the denominator of } \psi(s))/(\pi/2) \\ &= iJ_1(R/a\omega)\{k(-Y_0(\omega b/a) - iJ_0(\omega b/a)) + i\omega/a(-J_1(\omega b/a) \\ &+ iY_1(\omega b/a))\} + (-J_1(R/a\omega) + iY_1(\omega R/a)) \\ &\{k(J_0(\omega b/a) - i\omega/a iJ_1(\omega b/a))\} \end{aligned} \quad (\text{B-3})$$

By putting

$$\begin{aligned} B1 &= J_1(R/a\omega)Y_0(\omega b/a), \quad B2 = Y_1(R/a\omega)J_0(\omega b/a) \\ B3 &= J_1(\omega R/a)Y_1(\omega b/a) \text{ and } B4 = J_1(\omega b/a)Y_1(\omega R/a) \end{aligned} \quad (\text{B-4})$$

(the denominator of $\psi(s))/(\pi/2)$

$$= -k(B1 - B2) - \omega/a(B3 - B4) \quad (\text{B-5})$$

in which only $\omega = \omega_n$ satisfies (A-5) = 0. Then, setting the n^{th} root of (A-5) as $\omega = \omega_n$ and to calculate the residuals at the poles $s = s_n$ of $\psi(s)$, we set

$$\begin{aligned} q &= \sqrt{s/D} = i\omega/a, \quad q_n = \sqrt{s_n/D} = i\omega_n/a \\ qa &= i\omega, \quad qb = i\omega b/a, \text{ and } qR = i\omega R/a \end{aligned} \quad (\text{B-6})$$

For inverting the Laplace transformed $\psi(s)$, firstly we differentiate the denominator of $\psi(s)$ (D = Denominator of $\psi(s)$) with respect to s through ω as

$$\begin{aligned} &\partial [(\text{the denominator of } \psi(s))/\partial \omega/(i\pi/2)] \\ &= -k\partial[B1 - B2]/\partial \omega - \omega/a\partial[B3 - B4]/\partial \omega - (B3 - B4)/a \end{aligned} \quad (\text{B-7})$$

The differentiation can be achieved by using the formula of the Bessel functions.

$$\begin{aligned} J_0(az)/\partial z &= -aJ_1(az) & \partial Y_0(az)/\partial z &= -aY_1(az) \\ \partial J_1(az)/\partial z &= aJ_0(az) - J_1(az)/z, \\ \partial Y_1(az)/\partial z &= aY_0(az) - Y_1(az)/z \end{aligned} \quad (\text{B-8a, B-8b, B-8c, B-8d})$$

we, get

$$\begin{aligned}
\partial[B1]/\partial\omega &= R/a J_0(R/a\omega) Y_0(\omega b/a) - 1/\omega J_1(R/a\omega) Y_0(\omega b/a) \\
&\quad - b/a J_1(R/a\omega) Y_1(\omega b/a) \\
\partial[B2]/\partial\omega &= R/a Y_0(R/a\omega) J_0(\omega b/a) - 1/\omega Y_1(R/a\omega) J_0(\omega b/a) \\
&\quad - b/a J_1(b/a\omega) Y_1(\omega R/a) \\
\partial[B3]/\partial\omega &= R/a J_0(R/a\omega) Y_1(\omega b/a) - 2/\omega J_1(R/a\omega) Y_1(\omega b/a) \\
&\quad + b/a Y_0(b/a\omega) J_1(\omega R/a) \\
\partial[B4]/\partial\omega &= R/a Y_0(R/a\omega) J_1(\omega b/a) - 2/\omega Y_1(R/a\omega) J_1(\omega b/a) \\
&\quad + b/a J_0(b/a\omega) Y_1(\omega R/a) \quad (\text{B-9a, B-9b, B-9c, B-9d})
\end{aligned}$$

Then,

$$\begin{aligned}
\partial(B1 - B2)/\partial\omega &= R/a [J_0(R/a\omega) Y_0(\omega b/a) - Y_0(R/a\omega) J_0(\omega b/a)] \\
&\quad + (B2 - B1)/\omega + b/a (B4 - B3) \quad (\text{B-10a})
\end{aligned}$$

$$\begin{aligned}
\partial(B3 - B4)/\partial\omega &= R/a [J_0(R/a\omega) Y_1(\omega b/a) - Y_0(R/a\omega) J_1(\omega b/a)] \\
&\quad + 2(B4 - B3)/\omega + b/a (B1 - B2) \quad (\text{B-10b})
\end{aligned}$$

Associating these

$$\begin{aligned}
&\partial [(the denominator of \psi(s))]/\partial\omega/(i\pi/2) \\
&= -R/a [k\{J_0(R/a\omega) Y_0(b/a\omega) - Y_0(R/a\omega) J_0(\omega b/a)\} \\
&\quad + \omega/a \{J_0(R/a\omega) Y_1(\omega b/a) - Y_0(R/a\omega) J_1(\omega b/a)\}] \\
&\quad + k(B1 - B2)/\omega + k(B3 - B4)b/a - \omega/ab/a (B1 - B2) \\
&\quad + \omega/a 2/\omega (B3 - B4) - (B3 - B4)/a \quad (\text{B-11})
\end{aligned}$$

Since $\omega = \omega_n$ is the n^{th} root of the characteristic equation of $\psi(s)$ (A-5), we have the relation,

$$\begin{aligned}
&k[J_1(R/a\omega_n) Y_0(\omega_n b/a) - Y_1(R/a\omega_n) J_0(\omega_n b/a)] \\
&+ \omega_n/a [J_1(\omega_n R/a) Y_1(\omega_n b/a) - J_1(\omega_n b/a) Y_1(\omega_n R/a)] = 0 \quad (\text{B-12})
\end{aligned}$$

Thus,

$$[k(B1 - B2) + \omega/a (B3 - B4)](\omega = \omega_n) = 0 \quad (\text{B-13})$$

Using this relation, we have the differentiated denominator of $\psi(s)$ at $\omega = \omega_n$

$$\begin{aligned}
&\partial [(the denominator of \psi(s))]/\partial\omega/(i\pi/2)(\omega = \omega_n) \\
&= -R/a [k\{J_0(R/a\omega_n) Y_0(\omega_n b/a) - Y_0(\omega_n R/a) J_0(\omega_n b/a)\} \\
&\quad + \omega_n/a \{J_0(\omega_n R/a) Y_1(\omega_n b/a) - J_1(\omega_n b/a) Y_0(\omega_n R/a)\}] \\
&\quad - b/(a^2 \omega_n) (k^2 a^2 + \omega_n^2) [J_1(\omega_n R/a) Y_0(\omega_n b/a) - J_0(\omega_n b/a) Y_1(\omega_n R/a)] \quad (\text{B-14})
\end{aligned}$$

The Numerator of $\psi(s)$ at the pole is

$$\text{Num} = i\pi/2 [Y_1(\omega_n R/a) J_0(\omega_n) - J_1(\omega_n R/a) Y_0(\omega_n)] \quad (\text{B-15})$$

since $q = \sqrt{s/D} = i\omega/a$, the exponential term of the final inverted Laplace transform has

$$\exp(st) = \exp(-D\omega_n^2/a^2t) \quad (\text{B-16})$$

Then, we have the residuals for a given n^{th} root ω_n of the characteristic equation (A-5).

Summing those residuals for each ω_n completes the inversion of Laplace transform of $\psi(s)$.

8. Appendix 3

Thus, we have following approximation forms for sufficiently large s (short time period)

$$1) I_0(qb) = \sum_{k=0}^6 a'_k (qb/3.75)^{2k} = \sum_{k=0}^6 a_k q^{2k} \quad (\text{C-1})$$

$$a'_0 = 1.0, a'_1 = 3.5156229, a'_2 = 3.08994, a'_3 = 1.2067, \\ a'_4 = 0.2659, a'_5 = 0.03607, a'_6 = 0.00458 \text{ and putting} \\ a'_k (qb/3.75)^{2k} = a_k$$

$$2) I_1(qb) = qb \sum_{k=0}^6 a'_k (qb/3.75)^{2k} = \sum_{k=0}^6 b_k q^{2k+1} \quad (\text{C-2})$$

$$a'_0 = 0.5, a'_1 = 0.8789, a'_2 = 0.5149, a'_3 = 0.15084, \\ a'_4 = 0.02658, a'_5 = 0.003015, a'_6 = 0.000324 \text{ and putting} \\ a'_k (b/3.75)^{2k} b = b_k$$

$$2) I_1(qR) = qR \sum_{k=0}^6 a'_k (qR/3.75)^{2k} = \sum_{k=0}^6 B_k q^{2k+1} \quad (\text{C-3})$$

$$a'_k (R/3.75)^{2k} R = B_k$$

$$3) K_0(qb) = -\log((qb)/2) I_0(qb) + \sum_{k=0}^6 a'_k (qb/2)^{2k} \\ = -\log((qb)/2) I_0(qb) + \sum_{k=0}^6 c_k q^{2k} \quad (\text{C-4})$$

$$a'_0 = -0.5772, a'_1 = 0.42278, a'_2 = 0.23069, a'_3 = 0.034885, \\ a'_4 = 0.002626, a'_5 = 0.0001075, a'_6 = 0.0000074 \text{ and putting} \\ a'_k (b/2)^{2k} = c_k$$

$$4) K_1(qb) = \log((qb)/2) I_1(qb) + \sum_{k=0}^6 a'_k (qb/2)^{2k} / b$$

$$= \log((qb)/2) I_1(qb) + \sum_{k=0}^6 d_k q^{2k-1} \quad (\text{C-5})$$

$a'0 = 1.0, a'1 = 0.15443, a'2 = -0.6727, a'3 = -0.1815,$
 $a'4 = -0.01919, a'5 = -0.0011, a'6 = -0.000046868$ and putting
 $a'_k(b/2)^{2k}/b = d_k$

$$\begin{aligned} 5) K_1(qR) &= \log((qR)/2) I_1(qR) + \sum_{k=0}^6 a'_k(qR/2)^{2k}/R \\ &= \log((qR)/2) I_1(qR) + \sum_{k=0}^6 e_k q^{2k-1} \end{aligned} \quad (\text{C-6})$$

$$a'_k(R/2)^{2k}/R = e_k$$

5) Using these series expansions, the Laplace inversion of the Denominator of $G(s)/q$ is

$$\begin{aligned} &= I_1(qR) [k(-\log((qb)/2) I_0(qb) + \sum_{k=0}^6 c_k q^{2k}) \\ &+ q(\log((qb)/2) I_1(qb) + \sum_{k=0}^6 d_k q^{2k-1})] \\ &+ (\log((qR)/2) I_1(qR) + \sum_{k=0}^6 e_k q^{2k-1}) [k I_0(qb) - q I_1(qb)] \end{aligned} \quad (\text{C-7})$$

$$\begin{aligned} &= \log(qb/2) I_1(qR) [-k I_0(qb) + q I_1(qb)] \\ &+ \log(qR/2) I_1(qR) [k I_0(qb) - q I_1(qb)] \\ &+ k I_1(qR) \sum_{k=0}^6 c_k q^{2k} + I_1(qR) \sum_{k=0}^6 d_k q^{2k} + k I_0(qb) e_k q^{2k-1} \\ &- I_1(qb) \sum_{k=0}^6 e_k q^{2k} \end{aligned} \quad (\text{C-8})$$

the Laplace inversion of Numerator of $G(s)$ is given by

$$\begin{aligned} &= I_1(qR) [\log(qb/2) I_1(qb) + \sum_{k=0}^6 d_k q^{2k-1} \\ &- [\log(qR/2) I_1(qR) + \sum_{k=0}^6 e_k q^{2k-1}] I_1(qb) \end{aligned}$$

$$\begin{aligned}
&= \log(b/R) \sum_{k=0}^6 B_k q^{2k+1} \sum_{k=0}^6 b_k q^{2k+1} + \sum_{k=0}^6 B_k q^{2k+1} \sum_{k=0}^6 d_k q^{2k-1} \\
&\quad - \sum_{k=0}^6 b_k q^{2k+1} \sum_{k=0}^6 e_k q^{2k-1}
\end{aligned} \tag{C-9}$$