Analysis for dimension-restricted reaction kinetics with a bacterial endonuclease movement

NORIKO HIROI*, AKIRA FUNAHASHI* and HIROAKI KITANO
ERATO-SORST Kitano Symbiotic Systems Project
Japan Science and Technology Agency
6-31-15 Jingumae, M-31 Suite 6A, Shibuya-ku, Tokyo
JAPAN
http://www.symbio.jst.go.jp/
* *These authors contributed equally to this work.*

Abstract: - To study how to express the biochemical reactions in a condensed environment like inner cellular spaces, we analyzed the EcoRV diffusion process on DNA, which is believed to be a highly correlated process. We reconstructed the targeting process of the nuclease using a kinetic model, following the theory for dimension restricted reaction kinetics. This model successfully reconstructed the reaction rate ratio of different length substrates, which cannot be reconstructed using the classic mass action equations. At the same time, this model suggested the kinetic parameters and kinetic orders of the reaction, which can be confirmed by biochemical experimentation. We also demonstrate a way to calculate the kinetic parameters and orders based on the results of such biochemical experiments. Our results suggest that the theory which we adopted to the kinetic model may be applicable in representing other dimension-restricted reactions.

Key-Words: - Dimension-restricted reaction, Kinetic model, Kinetic order, Condensed environment, Inner cellular process, 1D Diffusion, Restriction enzyme

1 Introduction

The inner cellular environment is highly condensed with skeletal proteins, ribosomes, organelle membranes, and so on [1]. This highly crowded environment restricts the diffusion dimensions of the molecules in a cell. Biochemical reactions under such conditions should be expressed taking such dimensional constraints into account, instead of assuming an idealized free reaction spatial condition. Defining fundamental reaction formulas is essential for faithful modeling and simulation of intra-cellular biochemical processes.

Our object in this study is to determine an accurate expression for dimension-restricted reactions for in vivo-oriented modeling.

In this study, we chose the EcoRV diffusion process to investigate dimension-restricted reaction kinetics with a numerical model.

EcoRV is one of the restriction enzymes of E. coli, which is a mechanism for protecting bacteria from infection [2]. There are three types of hypothesis concerning the EcoRV targeting mechanism: sliding, hopping, and jumping [3, 4]. In the process called 'sliding', the protein undergoes linear diffusion along the DNA between contiguous non-specific binding sites. Alternatively, translocation can occur in 3D space by successive cycles of dissociation / re-association of the protein with the DNA. If the dissociation is followed by re-association before the protein diffuses a threshold distance away from its initial site, the protein will re-bind at or near the original site [4]; this is called 'hopping'. 'Sliding' and 'hopping' are correlated processes; that is to say the protein at time $t + \Delta t$ is located near its position at time t [3]. Conversely, if the protein diffuses a longer distance from its initial site yet remains within the domain of the DNA, the re-association may be with an uncorrelated site elsewhere in the chain: we call this 'jumping'. So 'jumping' is an uncorrelated process. An arbitrary distinction is that hopping transfers are $< 20bp$ while jumping is $> 20bp$.

First, the EcoRV diffusion process was assumed to be sliding based on knowledge of the lac repressor-operator interaction, because the required time is too short to find their target if the process is entirely by 3D diffusion in an ideal condition, such as in a gas or well diluted solution [5]. Some experiments support this hypothesis [6, 7, 8], which analyzed the catalytic rate ratio of different length substrates to indicate that the actual catalytic rate is faster than the rate if the reaction occurred under 3D conditions. Moreover, observation for a molecular movement of a restriction enzyme [9] suggested that this molecule has the ability to slide along the DNA.

Based on these facts, we chose to analyze the targeting manner of EcoRV with our model to know how we may simulate the dimension-

restricted reaction.

We evaluated a theory for the dimension-restricted reaction in a kinetic model. The theory we adapted into a kinetic model was put forward by Kopelman [10]. It shows that a dimension-restricted bimolecular reaction can be represented with high kinetic orders of the reactants. In the case of bimolecular reactions, the overall kinetic order for each reactant is 5. They applied their theory for chemical reactions consisting of small molecules under some artificial environments and confirmed their theory is realistic [10, 11]. However, their theory has not been applied for biochemical reactions that consist of hundred-times larger molecules. The molecular size may affect their actual behavior and reactions. We applied this theory for a biomolecular reaction, in this case the EcoRV targeting process, and estimated whether the theory can be applied to biomolecular reactions.

2 Reaction Kinetics in Restricted Dimensions for Biomolecules

The object of this study is to define fundamental reaction formulas for faithful modeling and simulation of biochemical processes occurring in dimension-restricted environments.

First, we introduce the basic theory of Kopelman for reaction kinetics in restricted dimensions (2.1). Second, we compare Kopelman's kinetics with classic "mass-action kinetics" (2.2). Third, we explain the difference between our application and Kopelman's experiments (2.3). Finally, we explain the details of our method to determine the kinetic order of the reaction we analyzed (2.4).

2.1 Reaction Kinetics in Restricted Space by Kopelman

Kopelman reviewed the reaction kinetics in restricted space in his 1991 paper for exciton microscopy [10]. Reactions in restricted spaces are rarely stirred vigorously by convection and are thus often controlled by diffusion. Furthermore, the compactness of the Brownian motion leads to both anomalous diffusion and anomalous reaction kinetics.

There are three major categories of simple elementary bimolecular mechanisms:

$$A + A \rightarrow P \tag{1}$$

$$A + B \rightarrow P \tag{2}$$

$$A + C \rightarrow C + P \tag{3}$$

The "trapping reaction" and the "target reaction" are both special cases of reaction (3):

$$A + C \rightarrow C \tag{4}$$

In the target reaction, A is fixed and C, which is a catalyst or catalytic site, is mobile, and only A varies in time.

Now replacing Eqs.(1), (2) and (4) by

$$A + i = P \qquad i = A, B, C \tag{5}$$

where P is any resulting product (including A and/or i), a simple way of describing many of the results is the differential rate equation:

$$-\frac{d[A]}{dt} = k(t)[A][i] \qquad i = A, B, C \tag{6}$$

where $[A]$ is the concentration of A.

The non-classical results can now be written for long times ($t \rightarrow \infty$):

$$K(t) \sim t^{-h} \qquad\qquad h < 1 \tag{7}$$

where $K(t)$ is a time-dependent parameter. In particular, for low dimensions ($d < 2$), one finds for certain $A + A$ reactions [12]:

$$h = 1 - \frac{ds}{2} \tag{8}$$

while for the same $A + B \rightarrow 0$ reactions [13],

$$h = 1 - \frac{ds}{4} \tag{9}$$

whenever the spectral (fraction) dimension (ds) is $ds < 2$ [14].

Another simple way of describing many of the results, and particularly those for steady-state reactions [15], is

$$-\frac{d[A]}{dt} = k[A]^y[i]^z \tag{10}$$

where k is a time-independent constant and the partial orders y and z may be non-integers, and the same is true for the overall order

$$x = y + z \tag{11}$$

This is for an elementary bimolecular reaction, where the molecularity of the reaction is described by Eq.(5). Eqs.(10) and (11) account for the result that $x = 5$ in random $A + B \rightarrow AB \uparrow$ batch reactions.

2.2 The difference between classic mass-action kinetics and Kopelman's method

The chemical kinetics for bimolecular reactions determined by Eq.(5) are described by the classic mass-action kinetics as follows:

$$-\frac{d[A]}{dt} = k[A][i] = \frac{d[P]}{dt} \tag{12}$$

This equates to $y = z = 1$ and $x = 2$ of Eq.(11). The beauty of classical kinetics lies in the universality of its formation. The functional form of the rate laws does not depend on the dimensions or dimensionality of the medium, initial conditions, details of source, nature of motion, relative mobility or reaction of the components, etc. Such factors only affect the values of the parameters in classical kinetics. It also implies that global and local rate laws are the same and that the particle distribution functions depend only on the concentrations, but not on whether the ensemble is in equilibrium or far from it, or in a steady state or far from it.

Naturally, we get a different value for x from classical and non-classical kinetics, respectively. Classical kinetics give us the value $x = 1 + \frac{2}{ds}$ in general, where ds is the spectral dimension.

However, when deviations from classical behavior are studied, it is important to distinguish the various factors behind such deviations and the interactions among them. This is the reason why we apply non-classical kinetics for our object, that is to determine an accurate means of expression for dimension-restricted reactions for in vivo-oriented modeling. The EcoRV catalytic rate is just such a case which cannot be explained using the classic mass kinetic model. Therefore, we chose this phenomenon for our test case.

At the same time, in order to calculate the parameters and kinetic order from experimental data, assuming a steady state condition as determined by Briggs and Haldane is reasonable. We explain the details in Section 2.4.

2.3 The difference between Kopelman's analysis and ours

The main difference between Kopelman's analysis and ours is the scale of the reactants. They applied their theory to the reactions of small molecules such as metallic ions, naphthalene exciton, and so on [10, 11]. Enzymes are actually hundreds of times larger (Table1). Many biomolecules have mesoscopic volume. This kind of molecules sometimes behave differently from microscopic scale things.

Table 1: Comparison of the diameters and volumes of each molecule and a cell

molecule	diameter(nm)	volume(nm^3)
Mg^{2+}	0.346	0.0217
naphthalene	$0.2 \times 0.7 \times 1.2$	0.168
EcoRV	$4.96 \sim 6.39$	$136.56^{\star 1}$
DNA	$2.0 \times 0.34 \times (bp)$	$4654.7^{\star 2}$
E.coli	$10^3 \times 3 \times 10^3$	2.355×10^9

$^{\star 1}$The volume of EcoRV dimer (active form)
$^{\star 2}$The case of 4361 bp linear DNA (the length of plasmid pBR322)

We are interested in whether the same theory can be applied to reactions on such a larger scale, and tested one of the behaviors of an enzyme.

2.4 Method to determine kinetic order

Especially the "target reaction" is described by the classic pseudo-monomolecular equation.

When i is a catalyst, the reaction does not depend on $[i]$. As a result, the rate equation can be described as:

$$-\frac{d[A]}{dt} = k[A] = \frac{d[P]}{dt} \tag{13}$$

At the same time, when assuming the system is given as follows;

$$A + i \quad \overset{slow}{\rightarrow} \quad Ai \quad \overset{fast}{\rightarrow} \quad P \tag{14}$$

the concentration of Ai is:

$$\frac{d[Ai]}{dt} = \frac{d[P]}{dt} = -\frac{d[A]}{dt} \tag{15}$$

This is the steady-state condition which was defined by Briggs and Haldane in 1925 [16]. From this assumption, we can analyze the kinetic order of the targeting process using experimental results. Under appropriate conditions, we can obtain the time course fluctuation of $[A]$. Based on this data, we can calculate the integral value of the reaction rate, $-\frac{d[A]}{dt}$. When the reaction is a single-order reaction, the reaction rate is (13). The integral value is:

$$ln\frac{[A]}{[A]_0} = kt \tag{16}$$

where $[A]_0$ is the concentration of A at time 0. When the reaction actually occurs in a single-order manner, the plot of time against $ln\frac{[A]}{[A]_0}$ becomes linear with a 0 intercept. In the other case

when the reaction order is more than one, the n^{th} order reaction can be described as:

$$-\frac{d[A]}{dt} = k[A]^n \tag{17}$$

and the integral value is:

$$\frac{1}{(n-1)}\left\{\frac{1}{[A]^{n-1}} - \frac{1}{[A]_0^{n-1}}\right\} = kt \qquad (n \neq 1) \tag{18}$$

When the reaction kinetic order can be estimated $n(>1)$, the plot of time against $\frac{1}{[A]^{n-1}}$ becomes linear with a $\frac{1}{[A]_0^{n-1}}$ intercept.

As a result, in both cases, whereby $n = 1$ or $n > 1$, bring about a linear plot of time against the reaction rate integral value, and an accurate intercept, when one's assumption of kinetic order (n) is appropriate.

3 The Kinetic Model for Nuclease

Here we explicate our analysis results. We evaluated whether the theoretical method for dimension-restricted diffusion could replicate the biochemical experimental results [6, 7]. (Figs.1∼3 and Tables2∼4).

3.1 Catalytic rate ratio of different length substrates

Many biochemical experiments have been conducted which analyzed the catalytic rate ratio of different length substrates to demonstrate how odd the behavior of restriction enzymes looks if diffusion is completely in a 3D manner. We chose one of the most logical and elegant biochemical analyses as performed by Pingoud et al. [6, 7], to evaluate Kopelman's theory. They used $26bp$ and $958bp$ substrates and calculated the catalytic rate ratio. They analyzed the EcoRV reaction rate when the substrate was long ($958bp$) or short ($26bp$), and calculated the ratio for the reaction rate by dividing the rate for the long substrate by that of the short substrate. In this case, the ratio $(958bp/26bp) = 4.6$, indicating that the reaction rate for long substrates is 4.6 times faster than for short substrates.

3.2 Detailed construction of a Kinetic Model

Fig.1 represents a schematic of the kinetic model, while the numerical equations following the theory of restricted reaction kinetics [10] are shown in Tables2∼4. In this model, y and z were fixed at 1.0 when the expressed reaction occurs in a 3D manner (Fig.2). When the diffusion dimension of the molecules in the reaction is restricted, $y + z$ equals 5.0 in this model, following the theory for dimension-restricted kinetics explained in Section 2.1 (Fig.3).

Table 3: Parameter sets for the model

k_a	association reaction rate
k_l	$1/length$ of substrate DNA
$length$	substrate DNA length (26 or 958)
k_{d1}	$1.0E-5$
k_{d2}	1.0
k_c	4.16

Table 4: Initial conditions of the model

Species	Initial concentration (nM)
$[intactDNA]_0$	10
$[EcoRV]_0$	8
$[EcoRV\_intactDNA]_0$	0
$[EcoRV\_cleavedDNA]_0$	0
$[cleavedDNA]_0$	0

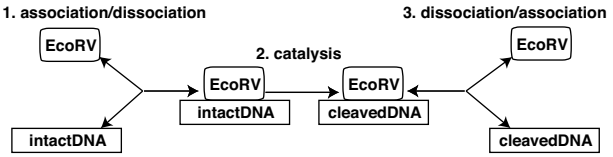

Figure 1: Kinetic model for the EcoRV enzymatic reaction consists of the targeting process, catalytic process, and dissociation process. The targeting process includes both where the enzymes associate with intact DNA and find the target sequence. This model was described by CellDesigner [17], and analyzed with MATLAB.

3.3 Results of the Kinetic Model

Our analysis using the above kinetic model demonstrated that the model which assumes the targeting process is completely undergone in an idealized condition (by fixing y and z as 1) could not explain the experimental results of Jeltsch et al. [7] (Fig.2). Fig.2 shows that both simulation results in which the substrate lengths were $26bp$ or $958bp$ were the same, so the cleavage reaction rate ratio $958bp/26bp$ remained constant at 1.0 (Fig.2). On the other hand, the model which assumes the targeting process includes a dimension-restricted reaction (meaning a 1D re-

Table 2: Differential equations for the kinetic model

$$
\begin{aligned}
\frac{d[intactDNA]}{dt} &= -k_a \times k_l \times ([intactDNA] \times length)^y \times [EcoRV]^z + k_{d1} \times [EcoRV\_intactDNA] \\
\frac{d[EcoRV]}{dt} &= -k_a \times k_l \times ([intactDNA] \times length)^y \times [EcoRV]^z + k_{d1} \times [EcoRV\_intactDNA] \\
&\quad +k_{d2} \times [cleavedDNA] - k_a \times [EcoRV] \times [EcoRV\_cleavedDNA] \\
\frac{d[EcoRV\_intactDNA]}{dt} &= k_a \times k_l \times ([intactDNA] \times length)^y \times [EcoRV]^z + k_{d1} \times [EcoRV\_intactDNA] \\
&\quad -k_c \times [EcoRV\_intactDNA] \\
\frac{d[EcoRV\_cleavedDNA]}{dt} &= k_{d2} \times [cleavedDNA] - k_a \times [EcoRV] \times [EcoRV\_cleavedDNA] \\
\frac{d[cleavedDNA]}{dt} &= k_c \times [EcoRV\_intactDNA] - k_{d2} \times [cleavedDNA] + k_a \times [EcoRV] \times [EcoRV\_cleavedDNA]
\end{aligned}
$$

action) could explain the experimental results (Fig.3). As shown in Fig.3, the cleavage reaction rates differed when the substrates' lengths were different. In this case, we could find the appropriate parameter k_a, which is related to the association reaction rate, for the reconstruction of experimental results through parameter searching (Fig.3). These results indicate that the kinetics for the dimension-restricted reactions can represent a biochemical phenomenon which could not be reconstructed by classical mass-action kinetics.

4 Conclusion

The results from our kinetic model suggest that the EcoRV targeting process including some dimension-restricted reaction processes could be represented by a theory for dimension-restricted reactions.

In conclusion, we demonstrated that biochemical reactions, which occur in dimension-restricted environments, can be expressed and simulated accurately with the dimension-restricted reaction kinetics of Kopelman and the data introduced by in vitro experiments.

References:

[1] O. Medalia, I. Weber, A. S. Frangakis, D. Nicastro, G. Gerisch, and W. Baumeister. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. *Science*, 298(5596):1209–1213, Nov. 2002.

[2] G. V. Kholmina, B. A. Rebentish, IuS. Skoblov, A. A. Mironov, and N. K. Iankovskii. Isolation and characteristics of the new site-specific endonuclease ecorv. *Dokl Akad Nauk SSSR*, 253(2):495–497.

[3] O. G. Berg, R. B. Winter, and P. H. von Hippel. Diffusion-driven mechanisms of protein translocation on nucleic acids. 1. models and theory. *Biochemistry*, 20(24):6929–6948, Nov. 1981.

[4] O. G. Berg. On diffusion-controlled dissociation. *Biochemistry*, 31(1):47–57, Jun. 1978.

[5] A. D. Riggs, S. Bourgeois, and M. Cohn. The lac repressor-operatorinteraction. iii. kinetic studies. *J. Mol. Biol*, 53(3):401–417, Nov. 1970.

[6] A. Jeltsch, C. Wenz, F. Stahl, and A. Pingoud. Linear diffusion of the restriction endonuclease ecorv on dna is essential for the in vivo function of the enzyme. *EMBO J.*, 15(18):5140–5111, Sep. 1996.

[7] A. Jeltsch and A. Pingoud. Kinetic characterization of linear diffusion of the restriction endonuclease ecorv on dna. *Biochemistry*, 37(8):2160–2169, Feb. 1998.

[8] N. P. Stanford, M. D. Szczelkun, J. F. Marko, and S. E. Halford. One- and three-dimensional patheways for proteins to reach specific dna sites. *EMBO J.*, 19(23):6546–6557, Dec. 2000.

[9] H. Kabata, W. Okada, and M. Washizu. Single-molecule dynamics of the ecori enzyme using stretched dna: its application to in situ sliding assay and optical dna mapping. *Jpn. J. Appl. Phys*, 39(12B):7164–7171, Dec. 2000.

[10] R. Kopelman. Exciton microscopy and reaction kinetics in restricted spaces. *Physical and Chemical Mechanisms in Molecular Radiation Biology.*

[11] E. Monson and R. Kopelman. Nonclassical kinetics of an elementary a + b -¿ reaction-diffusion system showing effects of a speckled initial reactant distribution and eventual self-segregation: Experiments. *Physical Review E*, 69(2):021103–1–021103–12, Feb. 2004.

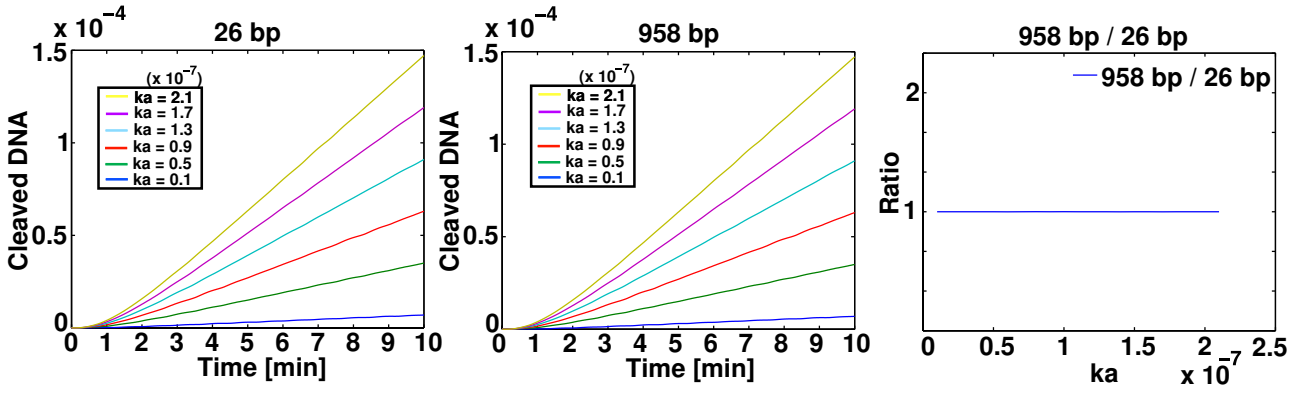

Figure 2: 3D reaction cases: The time course simulation results for the concentration of cleaved 26*bp* DNA and 958*bp* DNA (left and middle panels), and the correlation between parameter k_a (x-axis) and the cleavage reaction rate ratio, 958*bp*/26*bp* (y-axis) (right panel).

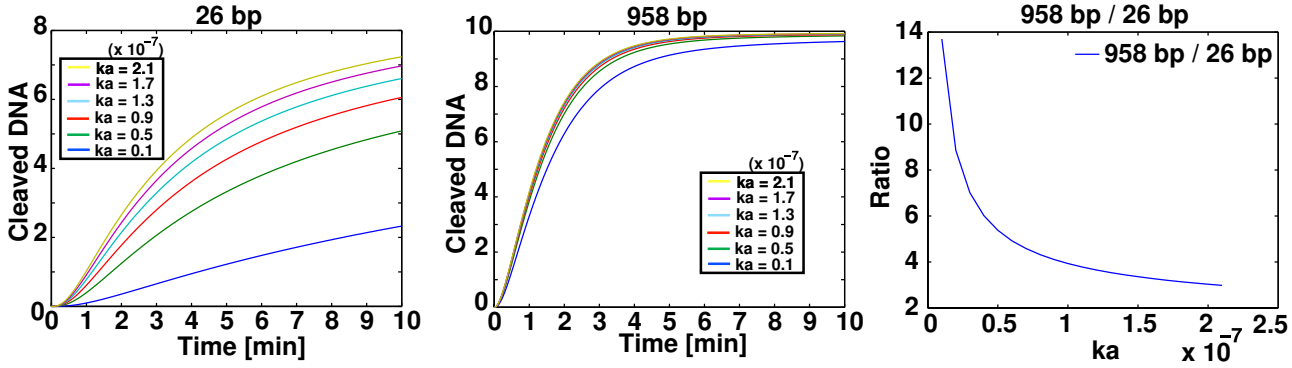

Figure 3: 1D reaction cases: The time course simulation results for the concentration of cleaved 26*bp* DNA and 958*bp* DNA (left and middle panels), and the correlation between parameter k_a (x-axis) and the cleavage reaction rate ratio, 958*bp*/26*bp* (y-axis) (right panel).

[12] R. Kopelman. Rate process on fractals: Theory, simulation and experiments. *J. Stat. Phys.*, 42(1/2):185–200.

[13] K. Kang and S. Render. Scaling approach for the kinetics of recombination processes. *Phys. Rev. Lett.*, 52(12):955–958, Mar. 1984.

[14] S. Alexander and R. Orbach. Density of states of fractals: Fractions. *J. Phys. Lett.*, 43.

[15] R. Kopelman. Fractal reactor kinetics. *Science*, 241(4873):1620–1626, Sep. 1988.

[16] G. E. Briggs and J. B. S. Haldane. A note on the kinetics of enzyme action. *Biochem. J.*, 19.

[17] A. Funahashi, N. Tanimura, M. Morohashi, and H. Kitano. CellDesigner: a process diagram editor for gene-regulatory and biochemical networks. *BioSilico*, 1(5):159–162, Nov. 2003.