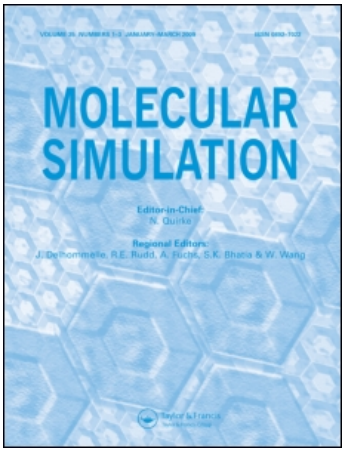


Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

Brownian Dynamics Simulation of DNA Gel Electrophoresis

Mitsuhiro Matsumoto[a]; Masao Doi[a]
[a] Department of Applied Physics, School of Engineering, Nagoya University, Nagoya, Japan

To cite this Article Matsumoto, Mitsuhiro and Doi, Masao(1994) 'Brownian Dynamics Simulation of DNA Gel Electrophoresis', Molecular Simulation, 12: 3, 219 — 226
To link to this Article: DOI: 10.1080/08927029408023032
URL: http://dx.doi.org/10.1080/08927029408023032

Molecular Simulation, 1994, Vol. 12(3-6), pp. 219-226
Reprints available directly from the publisher
Photocopying permitted by license only

BROWNIAN DYNAMICS SIMULATION OF DNA GEL ELECTROPHORESIS

MITSUHIRO MATSUMOTO and MASAO DOI

Department of Applied Physics, School of Engineering, Nagoya University, Nagoya 464-01, Japan

(Received February 1993, accepted May 1993)

Brownian dynamics computer simulation technique was applied to investigate DNA dynamics in gel electrophoresis. Under a constant electric field of moderate strength, large DNA chains take stretched and contracted conformations alternatively during the migration. The conformation change is quasi-periodic under certain conditions, and its frequency is closely related to the experimentally-found suitable frequency of pulse field gel electrophoresis.

KEY WORDS: *Brownian dynamics, DNA, gel electrophoresis, polymer dynamics*

INTRODUCTION

DNA gel electrophoresis is one of the standard techniques in biotechnology and genome engineering. DNA molecules are highly charged (or ionized) in aqueous solutions, and their mobility during electrophoresis depends on various external conditions, such as the molecular weight, the electric field strength, and the gel concentration. Many types of electrophoresis with time-dependent fields (cross field, inverted field, etc.) have been developed for separating large DNA molecules [1]. In most cases, however, the experimental conditions are chosen only empirically, because the physical principles of gel electrophoresis are not fully understood in spite of large number of experimental and theoretical researches [2].

By studying dynamics of charged polymers in gel, it will become possible to optimize experimental conditions and even to develop new schemes for separation and analysis of large DNA. For this purpose, several simulational works have been reported so far. Shaffer II and Olivera de la Cruz [3] applied a Brownian dynamics technique to a two-dimensional Rouse chain (beads connected by entropic springs) on a plane of square lattice obstacles and pointed out that the chain dynamics are different from the dynamics predicted by a simple tube model [4, 5]. Deutsch and Madden [6] used a different model (beads connected by rods of constant length) and obtained similar results; later, they extended the model to the system with randomly-arranged obstacles [7]. Various other models were also developed to investigate anomalous dynamic behavior of DNA under pulsed fields [8-11], but the understanding of DNA dynamics in gel electrophoresis is still far from complete.

In this article, we describe a Brownian dynamics simulation of DNA in gel electrophoresis, and present the chain dynamics during the migration under constant electric fields. Although constant field electrophoresis is regarded as the simplest

of all gel electrophoresis techniques, we observed a rather complex and interesting behavior of DNA dynamics; the chains show a quasi-periodic conformational change under certain conditions. The frequency of this periodic motion is close to the frequency of anomalous mobility under field inversion electrophoresis, which suggests that the behavior of DNA in field inversion methods can be understood in terms of chain dynamics in constant fields.

SIMULATION METHOD

We adopt a simple "beads-rods" model; the DNA chain consists of beads jointed with rods of constant length. This is similar to Deutch's [6], but we include bending energy so that the elasticity of DNA is taken into account. The chain migrates on a plane where obstacles are randomly arranged, which corresponds to gel fibers. Each bead obeys the Langevin-type equation of motion. We neglect the inertia term so that the friction term, which is assumed to be proportional to the bead velocity, is always balanced with the sum of the electric force, the bending force, the constraint forces of the joint rods, and a random force representing the thermal fluctuations of the surrounding solvent.

The equation of motion for i-th bead is as follows:

$$\eta \dot{\mathbf{x}}_i = q\mathbf{E} + \mathbf{F}_i^{\text{bend,}} + \mathbf{F}_i^{\text{rod}} + \mathbf{F}_i^{\text{rand}}, \tag{1}$$

where η is the friction coefficient and q is the charge of each segment. $\mathbf{E}$ is the uniform electric field acting on the DNA. In this report, we are concerned about the constant field; results for time-dependent fields are to be reported elsewhere. The bending force $\mathbf{F}_i^{\text{bend}}$ is obtained by differentiating the bending energy U_i^{bend} in terms of the position $\mathbf{x}_i$. We assume a simple form for U_i^{bend}:

$$U_i^{\text{bend}} = 2k_B T \frac{l}{r_0}\left[1 + \frac{(\mathbf{x}_{i-1} - \mathbf{x}_i)\cdot(\mathbf{x}_{i+1} - \mathbf{x}_i)}{r_0^2}\right], \tag{2}$$

where k_B is the Boltzmann constant, T is the temperature, l is the persistent length, and r_0 is the length of the rods. In experiments, the persistent length of DNA is controllable by changing the environment (solvent, temperature, etc.) and can be a relevant parameter in electrophoresis. In this simulation, we choose $l = 2r_0$. To keep the rod length constant, a harmonic spring force is used:

$$\mathbf{F}_i^{\text{rod}} = k\left(1 - \frac{r_0}{|\mathbf{x}_{i-1} - \mathbf{x}_i|}\right)(\mathbf{x}_{i-1} - \mathbf{x}_i) + k\left(1 - \frac{r_0}{|\mathbf{x}_{i+1} - \mathbf{x}_i|}\right)(\mathbf{x}_{i+1} - \mathbf{x}_i), \tag{3}$$

where k is the spring constant; we allow 5% thermal fluctuations for the rod length. The random force $\mathbf{F}^{\text{rand}}$ is a Gaussian type and obey the fluctuation-dissipation theorem:

$$\langle \mathbf{F}_i^{\text{rand}} \rangle = 0,$$

$$\langle \mathbf{F}_i^{\text{rand}}(t)\cdot\mathbf{F}_j^{\text{rand}}(t')\rangle = \frac{2k_B T}{\eta}\delta_{ij}\delta(t - t'), \tag{4}$$

where $\langle \ldots \rangle$ represents the statistical average. The obstacles are hard circles of

finite size, and the DNA segments are not allowed to be inside the obstacles. The obstacle diameter is chosen to be twice the average rod length r_0 so that the chain can never cross the obstacles.

We choose r_0 as the unit length, k_BT as the unit energy, and $(\eta l^2)/(k_BT)$ as the unit time in this report. For the constant field electropeoresis, there essentially exist three relevant control parameters: the chain length N, the field strength Θ, and the obstacle density C. In our reduced unit, each parameter is

$$N = \text{the number of segments},$$

$$\Theta = \frac{qE}{k_BT},$$

and

$$C = \text{the number of obstacles per unit area.}$$

A typical experimental condition, e.g., the electric field $\simeq$ 10 V/cm, the persistent length $\simeq$ 1000 Å, the gel concentration $\simeq$ 1 g/cm^3, and the DNA length $\simeq$ 16 μm (λ-phage DNA), corresponds to $N = 320$, $\Theta = 0.64$, and $C = 0.25$. In this simulation, we examined the DNA dynarmcs in a wide range of control parameters: N = 10–500 (1000 for several cases), $\Theta = 0.3$, 0.5, 0.7, and $C = 0.04$, 0.16, 0.36.

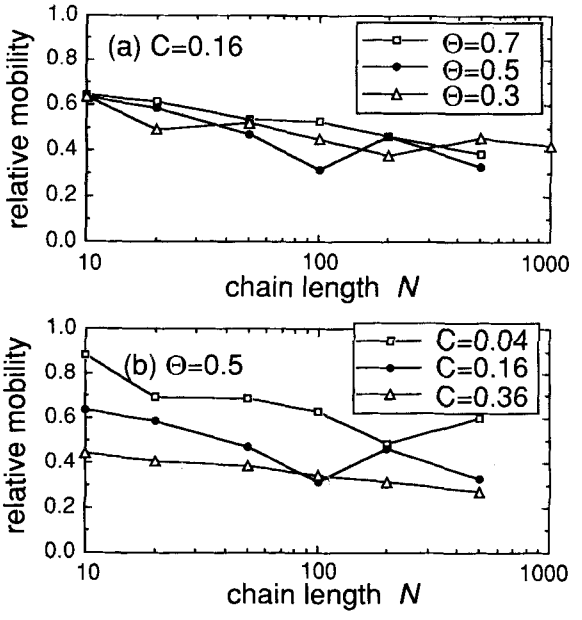


Figure 1 Mobility is plotted against the chain length for (a) constant obstacle density $C = 0.16$, and (b) a constant field strength $\Theta = 0.5$.

RESULTS AND DISCUSSION

The main concern about the electrophoresis experiments is the mobility μ, which is defined as the center-of-mass velocity divided by the field strength; constant μ implies that the velocity is proportional to the field strength. We show the simulational results in Figure 1, where the relative mobility (the calculated mobility divided by the mobility of a chain with no obstacles) is plotted as a function of the chain length N. In the range of simulated conditions, the mobility is not strongly dependent on the field strength [Figure 1(a)], but moderately depends on the obstacle density [Figure 1(b)].

Figure 2 is an example of the sequential snapshots of DNA conformation during the migration. It is often conjectured that large DNA chains have a stretched conformation under a constant field, which can explain the experimental results that the mobility is independent of the molecular weight for large DNAs. As one can see in Figure 2, however, chains take both stretched and shrunk conformations repeatedly during migration. Similar conformational change is also reported in other simulational works [3, 6] and direct observations with fluorescent microscope techniques [12, 13]. It is also important that, when the chain length is rather large as shown in Figure 2 ($N = 200$), each part of the chain shows an almost independent movement and causes the "hernia," or long doubled parts of the chain [6]. This hernia certainly has an important effect, but the theory of hernia dynamics is still to be developed.

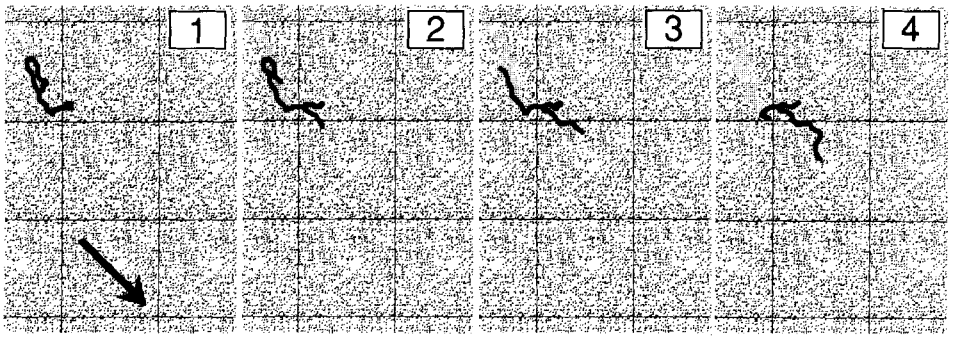
To see how the conformation change is correlated with the migration speed, we plot the migration distance of the center of mass [$d_x(t)$] and the field-parallel component of the end-to-end vector [$h_x(t)$] as functions of time (Figure 3). The time is expressed by the simulation step; with the choice of parameters in this simulation, 1000 steps are roughly corresponding to 0.1–1 sec. The chain motion is apparently intermittent. The velocity $v_x(t)$ [time derivative of $d_x(t)$] is very small when the chain has a shrunk conformation (i.e., it is hooked at obstacles). The chain gradually becomes stretched, and around the time of maximum h_x (or the chain is released from hooking) it moves most rapidly. This correlation between the chain conformation and its speed is in qualitative agreement with experimental observations [12, 13] and other simulational works [3, 6].

To investigate the chain conformation dynamics more closely, we calculate the autocorrelation function defined as

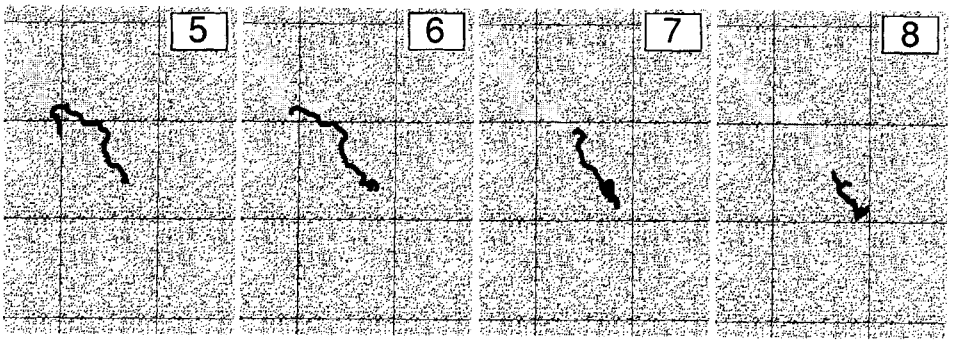
$$c(t) = \langle (w(t_0 + t) - \langle w \rangle)(w(t_0) - \langle w \rangle) \rangle_{t_0}, \tag{5}$$

where $\langle \ldots \rangle_{t_0}$ represents the ensemble average in terms of t_0. We choose three quantities, $v_x(t)$, $h_x(t)$, and $R_g(t)$ (radius of gyration) as the physical quantity $w(t)$.

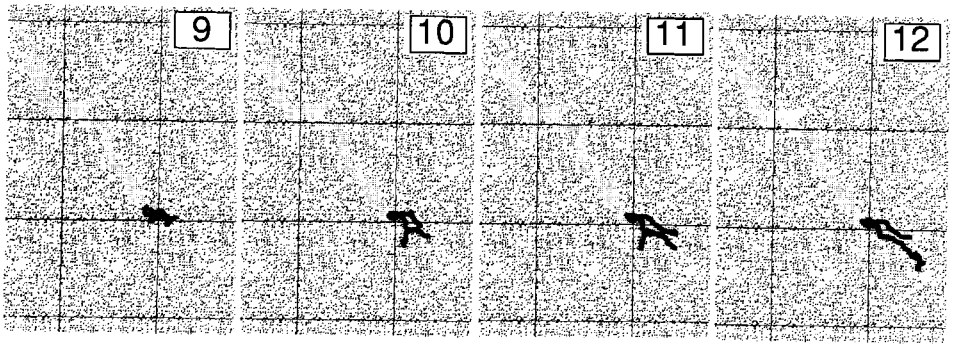
All of the three autocorrelation functions behave very similar to each other. Examples are shown in Figure 4. When the chain length is small or the obstacle density is low, the autocorrelation functions are monotonically dumped as a single exponential function of time [Figure 4(a), $N = 20$]; this implies that there is no clear periodicity in the inchworm motion. In the case of long chain in dense obstacles, however, the autocorrelation functions are oscillatory, as shown in Figure 4(b) ($N - 200$). This shows that the shrink-stretch motion is quasi-periodic, the frequency of which reasonably depends on both N, Θ, and C; the period τ is large for large N, small Θ, and large C. Analyses of τ is now under way.



(a)



(b)



(c)

Figure 2 Sequential snapshots of a DNA chain migrating under a constant field: chain length $N = 200$, field strength $\Theta = 0.3$, and obstacle density $C = 0.36$. The snapshots are taken every 500 steps. The direction of the external force is shown by the arrow. The small points represent the randomly arranged obstacles. The shadow is the trail of the chain.

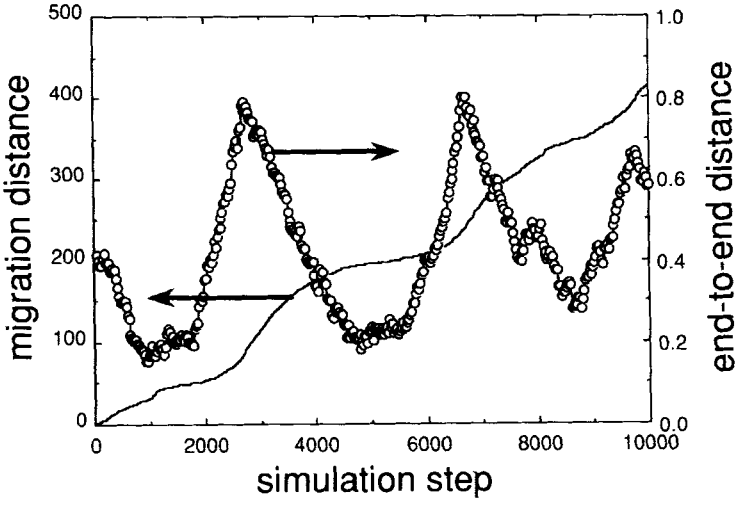


Figure 3 Migration distance and field-parallel component of the end-to-end vector (normalized by the chain length) are plotted against time (simulation step). The simulational conditions are the same as in Figure 2.

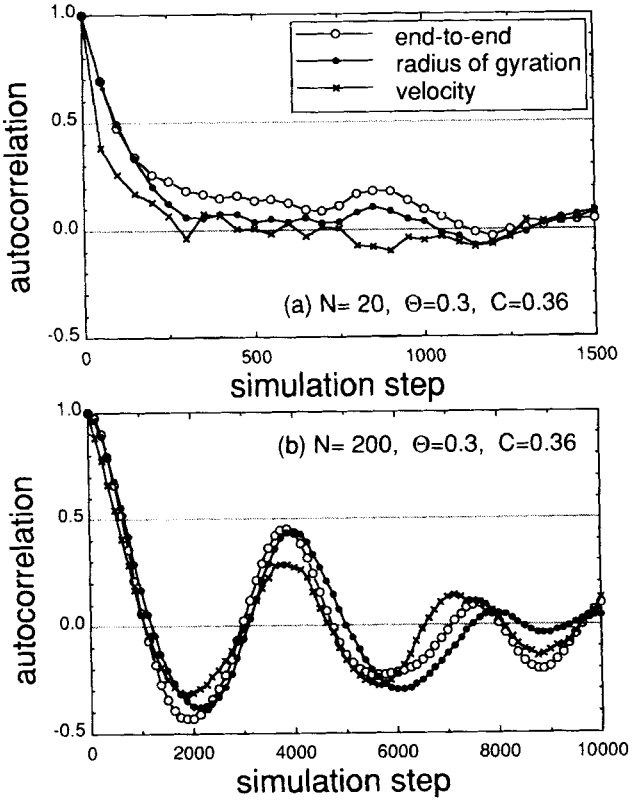


Figure 4 Three autocorrelations are plotted as functions of time (simulation step) for (a) a short chain $N = 20$ and (b) a long chain $N = 200$. Other simulational conditions are shown on the figure.

There are several experimental studies concerning the "antiresonance"; the mobility shows a minimum at a certain frequency of the applied pulse field [14, 15] and the sinusoidal field [16]. For example, Shikata and Kotaka [16] reported that the resonant frequency, or "pin-down frequency", is 1–2 sec for λ-phage DNA under the bias field of 2.5 V/cm and the sinusoidal one of 7.5 V/cm, and suggested that the antiresonance phenomena correspond to coupling of the chain relaxation time and the applied field frequency. Although our simulation is done under constant fields, the observed oscillation period is close to the experimentally-observed antiresonance time. We can speculate that the mechanism of chain conformation change is same both in steady-field and in pulse-field (or sinusoidal-field) gel electrophoreses. Recently, similar quasi-periodic motion under constant fields has been experimentally confirmed by our group [13].

It might seem strange that the system of a constant driving force (**E**) with white noises ($\mathbf{F}^{\mathrm{rand}}$) in a random medium shows such an apparent periodic behavior. Theoretical explanation will be possible from the view point of chain elongation and relaxation, and also it will contribute much to the understanding the physical principles behind time-dependent-field gel electrophoreses.

Acknowledgements

We are grateful to Prof. K. Yoshikawa, Dr. K. Minagawa, Ms. Y. Matsuzawa, and Mr. Y. Masubuchi for providing their experimental results and helpful discussion. We wish to thank the Computer Center, Institute for Molecular Science, Okazaki National Research Institutes, for allowing us to use their computer facilities. This work has been financially supported by Research Foundation for the Electrotechnology of Chubu (No. R-02138) and the Ministry of Education, Science and Culture, Japan (Grant-in-Aid for Scientific Research, Nos. 04750011 and 04NP0401).

References

[1] B. Birren and E. Lai, *Pulsed Field Gel Electrophoresis*, Academic Press London, 1993, and references therein.

[2] B. Nordén, D. Elvingson, M. Jonsson, and B. Åkerman, "Microscopic behaviour of DNA during electrophoresis: electrophoretic orientation", *Quart. Rev. Bioshys.*, **24**, 103 (1991), and references therein.

[3] E.O. Shaffer II and M. Olivera de la Cruz, "Dynamics of Gel Electrophoresis", *Macromolecules*, **22**, 1351 (1989).

[4] P.G. de Gennes, "Reptation of a polymer chain in the presence of fixed obstacles", *J. Chem. Phys.*, **55**, 575 (1971).

[5] O.J. Lumpkin and B.H. Zimm, "Mobility of DNA in gel electrophoresis", *Biopolymers*, **21**, 2315 (1982).

[6] J.M. Deutsch, "Theoretical studies of DNA during gel electrophoresis", *Science*, **240**, 922 (1988); J.M. Deutsch and T.L. Madden, "Theoretical studies of DNA during gel electrophoresis", *J. Chem. Phys.*, **90**, 2476 (1989).

[7] T.L. Madden and J.M. Deutsch, "Theoretical studies of DNA during orthogonal field alternating gel electrophoresis", *J. Chem. Phys.*, **94**, 1584 (1991).

[8] J.L. Viovy, "Molecular mechanism of field-inversion electrophoresis", *Phys. Rev. Lett.*, **60**, 855 (1988).

[9] T.A.J. Duke, "Tube model of field-inversion electrophoresis"; *Phys. Rev. Lett.*, **62**, 2877 (1989).

[10] H.A. Lim, G.W. Slater, and J. Noolandi, "A model of the DNA transient orientation overshoot during gel electrophoresis", *J. Chem. Phys.*, **92**, 709 (1990).

[11] B.H. Zimm, "Lakes-straits model of field-inversion gel electrophoresis of DNA", *J. Chem. Phys.*, **94**, 2187 (1991).

[12] C. Bustamante, "Direct observation and manipulation of single DNA molecules using fluorescence microscopy", *Ann. Rev. Bioshys. Chem.*, **20**, 415 (1991), and references therein.
[13] Y. Masubuchi, H. Oana, K. Ono, M. Matsumoto, M. Doi, K. Minagawa, Y. Matsuzawa, and K. Yoshikawa, "Periodic behavior of DNA molecules during steady field gel electrophoresis", *Macromolecules*, in press.
[14] G.F. Carle, M. Frank, and M.V. Olson, "Electrophoretic separations of large DNA molecules by periodic inversion of the electric field", *Science*, **232**, 65 (1986).
[15] T. Kobayashi, M. Doi, Y. Makino, M. Ogawa, "Mobility minima in field inversion gel electrophoresis", *Macromolecules*, **23**, 4480 (1990).
[16] T. Shikata and T. Kotaka, "Biased sinusoidal field gel electrophoresis for size dependent DNA separation", *Biopolymers*, **31**, 253 (1991).