Diffusion of DNA Molecules in Gel at High Electric Fields

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Abstract. Problems related to the gel electrophoretic migration of polymers can be investigated by models based on a Brownian-type ratchet where a particle can undergo a net transport on a potential energy surface that is externally driven to fluctuate between several distinct states. Here we describe the method of polymer transport and separation by means of the cellular automata technique. Numerical simulations of the polymer reptation in the model system allow us to understand the band-broadening processes in the gel electrophoresis experiments. They indicate also possible ways of fine-tuning the parameters in designing desired resolution of the experiments.

Key words: transport coefficients, ratchet systems, reptation model

Introduction

Reliable and fast separation of molecular fragments such as proteins and DNA is one of the most challenging tasks in contemporary biophysics. Based on seminal works of Smoluchowski [1] and Feynman [2] describing mechanisms of Brownian ratchets, several different models of rectifying nano-instruments have been proposed [3–5]. Their common feature is an action performed under the influence of nonequilibrium fluctuations and an asymmetry built into either the external potential or into the driving noise [3]. Effectively in all those systems, a net drift of moving particles can be observed, even though the net applied force is zero. Ratcheting systems were used in the description of the functioning and efficiency of molecular motors and nano-pumps crucial for life-activity in the biological world [3–5]. They were also proposed with application to separation experiments [5]. In particular, it was demonstrated [5] that a system with asymmetrical steric interactions may be used to separate molecules based solely on their internal entropy.

Gel electrophoresis (GE) is nowadays a major separation tool commonly used in molecular biology laboratories. It is an experimental technique to separate DNA molecules by length [6]. The first step is to prepare agarose gel of a given

concentration. However, the gel properties depend on many details, including the time of cooling of the gel, which in turn depends on the gel volume. As a consequence, results of the measurement of the velocity and bandwidth of DNA molecules of given length vary from one gel sample to another. The remedy is to compare the results with a reference sample, which contains molecules of known lengths. A lane with this reference is added to each gel sample, and the investigated lengths are identified by a comparison with the reference lengths [6, 7].

The mathematical formalism applied here is the technique of probabilistic cellular automata [8, 9], a common tool in simulations for statistical physics. Namely, we apply the model where the gel is just a lattice and each molecule is represented as a chain of so-called reptons [9]. There are three possible states of a chain link, when two neighboring reptons stay in the same lattice cell or in two adjacent cells. In the latter case, the link orientation can be parallel or antiparallel to the field. Then, each chain is represented as a chain of digits, 0 or ± 1 . In the *particle model* [9], turning points of the chain are represented by particles of two kinds, A and B. The probabilities of repton movements, that themselves depend on temperature and the intensity of the external electric field, are further translated to probabilities of particle movements. The precise formulation of the particle model follows the description presented elsewhere [9].

The main motivation for this work is experimentally documented nonlinear behavior of the DNA velocity at high electric fields, i.e. above 5 V/cm [10]. Under these conditions the diffusion coefficient D was discovered to increase abruptly with the field and with the molecular length. Variations of D are essential for establishing the resolving power of GE, as it directly influences the bandwidth. Understanding and analysis of this effect is therefore an intriguing problem and can be well reproduced and characterized in numerical simulations. (For recent reviews of theory and simulation of GE, see Refs. [7, 11, 12].) On the other hand, the observable nonlinear field dependence of the velocity of the model molecule migrating over the lattice mimicking the gel opens a possibility to investigate the nature of Brownian ratchets [3] relevant to GE. In this context, the problem of special interest is an entropic trapping that was claimed to convert simple steric ratchets into self-modulating ones, capable of size (mass) fractioning [13].

In the next section we analyze the meaning of the diffusion coefficient D in the presence of an electric field which varies in time. In two subsequent sections, results of the simulation are presented for constant and time-dependent fields, respectively. The last section is devoted to the discussion.

The Diffusion Coefficient

In a standard approach [14], the diffusion coefficient D for GE is defined via the dispersion of the position

$$\sigma^2 \equiv \langle x^2 \rangle - \langle x \rangle^2 = 2Dt \quad (1)$$

where $\langle \cdot \cdot \cdot \rangle$ is an average over the ensemble of DNA molecules, x is the molecule position and t is time. Two warnings should be made here. First, as it follows directly from the experimental procedure, the initial bandwidth is finite; therefore, Equation (1) cannot be applied directly. Instead, we should measure D as the time derivative of $\sigma^2/2$. This is true for the measurement as well as for the simulation. In the latter case, the calculation of D can be started only when the simulated process is stationary, and to check this condition is far from trivial. Second, the relation between σ^2 and the squared bandwidth cannot be reduced to a multiplicative constant which follows, for example, from the assumption about the Gaussian character of the spatial density of the band [15]. Instead, a more sophisticated procedure is necessary [10] as a consequence of a threshold of the molecule density below which the band cannot be observed. However, for the purposes of the simulation this procedure can be omitted.

In the case when the applied electric field is strong and varies in time, the migration process is non-stationary and we should more precisely define the meaning of the diffusion coefficient. For this purpose we follow the original Einstein derivation (cf. [15]), based on the analysis of the random walk problem. At high driving fields, the GE process is characterized by the band broadening due to a local hooking of molecules by gel fibres [16, 17]. This process leads to the so-called geometration effect [7, 17], when the molecule conformation oscillates between a tight- and an open- structure elongated along the field direction. Let us introduce a probability r that a molecule, moving or not, will be hooked by a gel fibre at a given time step. At initial time, all molecules start from the same position. Their distribution in discrete space is $(1, 0, 0, 0, \dots)$. After one time step, all but r move: then the distribution is $(r, 1 - r, 0, 0, \dots)$. After two steps, it is $(r^2, 2r(1 - r), (1 - r)^2, 0, 0, \dots)$ and so on. After t time steps, the spatial distribution is just the binomial one

$$f(x, t) = \binom{t}{x} r^{t-x} (1 - r)^x \quad (2)$$

and we get $\sigma^2 = r(1 - r)$. Then, the diffusion coefficient $D = r(1 - r)/2$. This derivation is valid for any process, stationary or not. Note that the average velocity $v = (1 - r)$.

The probability r depends on the applied electric field and can vary in time. Then, the diffusion coefficient D also varies in time. In our simulations, D is estimated as a time average over the duration τ of the electric field oscillations, averaged in turn over not less than 500 molecules.

In this formulation, we can rewrite analytical expressions for the velocity v and the diffusion coefficient D . Let us assume that the electric field E is piecewise constant. The probability r depends on the dimensionless field intensity ε expressed by the ratio

$$\varepsilon = \frac{aqE}{kT} \quad (3)$$

where a is the gel cell size, q stands for the effective repton charge, k is Boltzmann's constant and T is the temperature. Let us apply a field of intensity ε_+ during the time interval t_+ and ε_- during the time t_- , with $t_+ + t_- = \tau$. The net velocity of the molecule is then given by the time average

$$v = \frac{[1 - r(\varepsilon_+)]t_+ - [1 - r(\varepsilon_-)]t_-}{t_+ + t_-} \quad (4)$$

and, similarly, the diffusion coefficient reads

$$D = \frac{r(\varepsilon_+)[1 - r(\varepsilon_+)]t_+ + r(\varepsilon_-)[1 - r(\varepsilon_-)]t_-}{2(t_+ + t_-)}. \quad (5)$$

These expressions are exact if the time intervals t_+ and t_- are much longer than the time of relaxation to the stationary state. This is not true for our simulation, as we see in Figure 1, where there is some deviation from the linear time dependence of σ^2 even within the time duration of the constant field pulses t_+ , t_- . Nevertheless, the algorithm of time averaging of the diffusion coefficient remains valid for any time dependences of the applied field.

By inspection of Equation (4) one can easily deduce that, for observation of the ratchet process [3, 5] resulting in a non-zero net velocity for the zero net field $\varepsilon_+ t_+ + \varepsilon_- t_- = 0$, it is necessary to deal with a nonlinear $r(\varepsilon)$ dependence. Since at

Figure 1. The time dependence of the position-variance estimated in numerical simulations. Here the time period $\tau = 20000$ time steps.

Figure 2. The velocity v of molecules as dependent on the number N of reptons, for various ratios ε .

low electric fields the probability r can be approximated by a linear function, the ratcheting effect cannot be observable for weak fields.

Numerical Results

CONSTANT FIELD

In Figure 2, we show the results of our simulations presenting the velocity v of molecules as a function of the molecular length L , expressed in the number N of reptons ($L = Na$), for various electric fields. The time of numerical calculations increases abruptly with the molecular length. That explains why the values of L appear rather short when compared with the length of molecules for which the geometration effect can be observed [18]. However, the obtained results allow us to expect that the observed character of the curves, which appear to be constant with the molecule length, is preserved also for longer molecules.

In Figure 3, the numerical estimates of the diffusion coefficient D are displayed, presented as a function of the number of reptons N for various values of the ratio ε . For ε bigger than 0.35 the coefficient D increases with time. Formally, this could be equivalent to the case of an anomalous diffusion. However, with the limited time of simulations we cannot distinguish this effect from the case when the transient time before achieving the stationary state gets very long.

Summarizing the results for constant field presented in Figures 2 and 3, we see that the velocity v is constant with the molecule length, and the diffusion coefficient

Figure 3. The diffusion coefficient D as dependent on the number N of reptons, for various ratios ε .

D increases with the field and the molecule length. This allows us to state, that a qualitative agreement of the numerical simulations with the experimental data [7, 10] is obtained.

THE RATCHET SYSTEM

In Figure 4, the velocity v of the molecules as dependent on the applied field frequency $f = 1/\tau$ is presented for a particular choice of the ratios ε_+ , ε_- . The times t_+ , t_- are adjusted so as to get the averaged field equal to zero, which is the condition under which we were seeking the ratcheting effect in our model GE system. The obtained plot reveals the point where the velocity changes its sign. Depending on the space of parameters, $(f, N, \varepsilon_+, \varepsilon_-)$ many other similar inversion points can be detected. Note that when $\varepsilon_+ = -\varepsilon_-$, the condition $v = 0$ is fulfilled in a trivial way, from the up-down symmetry of the problem. Within the $v(f)$ plot we marked also the frequency at which the absolute value of the velocity displays a maximum. This frequency can be treated as an optimal one for the purposes of the particle separation in the GE process.

Figure 5 displays an analogous f -dependence of the diffusion coefficient D , and shows a sharp increase of this parameter with an increasing frequency of an applied driving field. On the other hand, we find that D increases with the applied field ε , when the ratchet condition is preserved. However, the registered f -dependent behavior of the diffusion coefficient is expected to be in force also when

Figure 4. The velocity v as dependent on the frequency f of the applied field.

Figure 5. The diffusion coefficient D as dependent on the frequency f of the applied field.

the conditions for observing the ratchet effect are not fulfilled. In more general cases of the molecular separation in varying electric fields [19], the observable reduction of the diffusion coefficient becomes responsible for a band narrowing, which increases the resolution of the experiment.

Conclusions

The paper presents an efficient GE modeling algorithm which uses the one-dimensional representation of various configurations of the polymer molecules composed of repton units represented by one of the set of numbers (0 or ± 1). Within the adopted scheme, the geometration process should be understood not as “hanging” [16], but rather as oscillations of the molecule length along the tube occupied by the chain.

The results of our simulations indicate that for pulsating fields, even at zero net applied force, a net drift of particles can be observed. For a given set of parameters, there exists also a particular value of the pulse frequency at which the maximum value of the absolute molecular velocity can be detected. The applied field-frequency and the intensity of pulses serve as possible tuning parameters whose modification may optimize the rectification process and increase resolution of bands in the separation experiments [20, 21].

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