THE MODEL OF DNA CHAIN MOTION IN PULSED FIELD GEL ELECTROPHORESIS

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Развита количественная модель, описывающая движение длинных цепей ДНК (дезоксирибонуклеиновой кислоты) в геле в сеансе нестационарного электрофореза. Основу модели составила концепция рептаций de Gennes. Показано, что вектор, соединяющий начало и конец цепи, можно представить в виде линейного прямоугольного фильтра (свертка с прямоугольным окном) с постоянной времени, равной времени обновления конфигурации цепи. Аномальное поведение подвижности и появление так называемого антирезонанса в периодических электрических полях объясняются реакцией фильтра на эти поля. Модель с удивительной точностью описывает как одномерный электрофорез с переменной полярностью электрического поля (FIGE), так и вариант электрофореза, когда вектор поля периодически меняет свое направление в пространстве на заданный угол (OFAGE).

Pulsed field electrophoresis is widely used for size separation of the long charged DNA (Deoxirybonucleic Acid) fragments. The discovery of size dependence of the DNA chain mobility in variable electric fields led to the development of such modern separation techniques as OFAGE (orthogonal field agarose gel electrophoresis) [1] and FIGE (field inverse gel electrophoresis) [2].

These techniques are using the mobility anomalies arising at certain relations between the field variation period and the DNA chain size. As opposed to the continuous field electrophoresis which allows to separate the relatively small molecules only, up to 50 kilo-base-pair (Kbp), the time- or space-variable electric fields separate chains of up to hundreds and thousands Kbp. Significant success has been achieved in the past fifteen years by complicated numerical calculations using statistical modeling techniques [3–5]. However, notwithstanding their utmost importance for the biological applications, the reasons behind the mobility anomalies and the form of the velocity (mobility) dependence on the molecule size and the field period are still lacking an adequate physical interpretation.

The present paper proposes a model describing with amazing accuracy the key experimental results on the non-stationary electrophoresis of the long DNA chains, including the "anti-resonance" (a sharp minimum in mobility) and the band inversion.

Let us consider the movement of a doublestrained charged DNA chain in an external electric field. The chain motion is determined by the external field and the thermal Brownian motion. The gel surrounding the polymer chain of the DNA molecule creates obstacles to its motion, thus the molecule can only move inside a "tube" formed by the gel pores. According to de Gennes [6], such motion occurs by reptation, interpreted as conformational fluctuations of the polymer chain on the scale inferior to the gel pore size.

Let us recall the main ideas of the reptation model [6]. The reptation mechanism describes the motion of defects on the form of loops of a freely linked polymer chain consisting of M monomers. Each defect contains a chain fragment of an average size b. New defects are created on the chain ends due to the thermal motion. The defect conservation law applies to the internal chain fragments, so that the continuity equation relates the defect concentration ρ to the defect flux J. The defect flux includes diffusion and migration, the latter dependent on the external field:

$$J_n = -Da^{-1}\frac{\partial \rho}{\partial n} + \mu a^{-1}\rho(\mathbf{b}_n\mathbf{f}). \tag{1}$$

Here, $\mathbf{f}$ is the external field force acting on a single monomer, D and μ are the respective diffusion and mobility coefficients, $\mathbf{b}_n$ is a vector of the length b tangential to the chain, a is the monomer length, n is the monomer number, increasing from the chain tail to the head. At zero external field, the defect concentration equals the equilibrium value $\overline{\rho}$. The steady-state defect distribution along the chain is achieved in the diffusion time $T_d \approx \dfrac{L^2}{\pi^2 D}$, where $L = Na$ being the effective chain size (chain length). Some of the monomers belong to the defects, thus the effective monomer number in the chain N will be lower than the total: $N = M(1 - \overline{\rho}b)$. The total chain length we denoted as $S = Ma$. The chain center of mass drift velocity U_d in the linear approximation with respect to the field is described by the equation:

$$\mathbf{U}_d = \frac{\mu\overline{\rho}b^2}{L^2 a}\mathbf{P}(\mathbf{f}\mathbf{P}). \tag{2}$$

Where the vector $\mathbf{P} = (a/b)\sum_{n=1}^{N}\mathbf{b}_n$ joins the two ends of the molecule. This expression is valid in the quasi-static case, with the dynamics of the $\mathbf{b}_n$ vector variations neglected. An expression similar to (2) is in the base of the known Biased Reptation Model, describing quite well the steady-state DNA electrophoresis in the constant electric field [7, 8].

Note the tensor nature of the center of mass drift velocity. As follows from (2), drift velocity does not coincide with the field vector, which allows to describe the two-dimensional electrophoresis, when the field vector changes periodically by a given angle.

Let us now consider the DNA movement in variable fields. The problem is the simplest for the case of the constant field amplitude, with the polarity (FIGE) or angle of the field action (OFAGE) being a periodic function of time. These two techniques are used in the major part of the DNA large fragment electrophoretic experiments on agarose gel. We shall use the limit of low frequencies, considering that the field period T exceeds significantly the diffusion-limited defect equilibration time: $T \gg T_d$.

Considering the DNA doublestrained chain, the monomer size to be used should be the persistent length λ, while the defects would be the loops created by chain thermal fluctuations. The typical size b of these loops is quite large, being comparable to the gel pore size. The DNA

chain size S is measured in base pair units (bp). The force $\mathbf{f}$ acting upon a single monomer of the DNA chain is equal to $\mathbf{f} = q\mathbf{E}$, q being the charge per single persistent length, and $\mathbf{E}$ the electric field strength. The chain changes its configuration constantly in a variable field, thus the end-to-end vector $\mathbf{P}$ becomes time-dependent. To describe the chain motion we shall need an equation describing the rate of change of the vector $\mathbf{P}$ determined by the velocities of the head ($n = N$) and tail ($n = 1$) segments of the chain:

$$\frac{d\mathbf{P}}{dt} = \frac{d\mathbf{r}_N}{dt} - \frac{d\mathbf{r}_1}{dt}. \tag{3}$$

According to the reptation model, the n-th monomer velocity is given by

$$\frac{d\mathbf{r}_n}{dt} = J_n\mathbf{b}_n = -Da^{-1}\mathbf{b}_n\frac{\partial\rho}{\partial n} + \mu\rho qa^{-1}\mathbf{b}_n(\mathbf{b}_n\mathbf{E}). \tag{4}$$

The vector $\mathbf{b}_n$ fluctuates due to the chain thermal motion. For the internal chain segments, the averaged value $\langle\mathbf{b}_n\rangle$ is non-vanishing and coincides with the local direction of the gel tube. For the head segment, however, all the directions are equally probable, thus $\langle\mathbf{b}_N\rangle = 0$. Averaging the equation (4) in the assumption of the differences between the defect concentration and the respective equilibrium value $\overline{\rho}$ being small, we obtain for the average velocity of the head segment

$$\langle\frac{d\mathbf{r}_N}{dt}\rangle = \mu\overline{\rho}qa^{-1}\langle(b_{Nx})^2\rangle\mathbf{E} = \mu_N\mathbf{E}, \tag{5}$$

here b_{Nx} is the projection of the vector $\mathbf{b}_N$ on the field direction, and $\mu_N = \mu\overline{\rho}qa^{-1}\langle(b_{Nx})^2\rangle$ is the mobility coefficient of the chain head.

Thus, on average the head segment moves along the field direction with a constant mobility μ_N. This is an extremely important result allowing us to build a closed system of equations for the vector $\mathbf{P}$ and the drift velocity of the chain center of mass in the non-steady-state electrophoresis.

The tail segment should move with the same velocity value as the head segment, the chain length remaining constant within the framework of the reptation model. Note that the two chain ends are not equivalent. The chain has a special end – a head, because the defect distribution in an external field varies along the chain. The chain head always moves along the electric field, even when the field changes direction. Other segments, repeating the trajectory of the head segment, follow it as the thread after the needle, creating a configuration that depends essentially on the field dynamics. Thus, the molecule configuration depends primarily on the history of the head segment motion influenced by the external electric field. This history depends on the field values which acted on the head segment during the interval of the chain displacement over the distance equal to its full length, called the configuration renewal time T_R. This characteristic time should not be confused with the renewal time without field, as introduced by de Gennes [6]. The chain tail repeats the trajectory of the chain head, delayed in time by the renewal time. In particular, the motion of the tail segment at the instant t coincides with that of the head segment at the instant $t - T_R$. Thus we may state that the chain tail moves under the action of the time-shifted field $E(t - T_R)$, having the same mobility of the chain head:

$$\langle\frac{d\mathbf{r}_1}{dt}\rangle = \mu_N\mathbf{E}(t - T_R). \tag{6}$$

Using the expressions (5) and (6) for the variation velocity of the average vector $\langle\mathbf{P}\rangle$ we shall write instead of (3):

$$\frac{d\langle\mathbf{P}\rangle}{dt} = \langle\frac{d\mathbf{P}}{dt}\rangle = \mu_N\mathbf{E}(t) - \mu_N\mathbf{E}(t - T_R). \tag{7}$$

The chain renewal time depends on the effective chain size L and the field amplitude E. For the constant-amplitude variable fields the renewal time is constant: $T_R = L/(\mu_N E)$. Note that in the general case of the variable-amplitude electric field the renewal time becomes a function of time, making the solution more complex. For the constant renewal time we integrate (7), obtaining:

$$\langle \mathbf{P} \rangle = \mu_N \int\limits_{t-T_R}^{t} \mathbf{E}(t) dt. \tag{8}$$

Equations (2) and (8) provide complete description of the motion of a long DNA chain in constant-amplitude variable fields. In fact, these equations enable to explain qualitatively without any additional constructions the anomalies of the DNA mobility in variable electric fields, such as "anti-resonance" (the minimum in mobility) and the band inversion. Indeed, for a single dimension the equation (8) describes a filter [9], which receives at the input the electrical signal $E(t)$, producing at the output the current value of $\langle P \rangle$. For a constant renewal time, the expression (8) describes a simple rectangular filter (convolution with rectangular window) with a time constant equal to the renewal time T_R. The mobility of the chain center of mass in a single-dimensional case will be proportional to the square of the filter output. The frequency response of such a filter is proportional to $\sin(z)/z$, where $z = \omega T_R/2, \omega$ being the frequency of the field variations and T_R the filter time constant equal to the renewal time. The first drift velocity minimum in the periodic field should be observed in the first filter zero at $z = \pi$, corresponding to the field period equal to the renewal time T_R. The linear field period dependence of the "anti-resonance" condition upon the length, and its quasi-linear dependence on the field amplitude had been consistently noted experimentally for various DNA fragments [10, 11]. Note that our model predicts a whole series of the mobility minima, conditioned by the filter having not one but a series of zeros at higher frequencies.

In a similar way, we may interpret the anomalies of the drift velocity (mobility) for the OFAGE experiment. The appearance of mobility minima at certain relations between the angle of action, and the period of field variation may be explained in a very simple manner. In this case the vector equation (8) describes a set of two rectangular filters, one along the x axis and the other along the y axis of the Cartesian referential in the field vector plain.

Thus, the anomalous mobility behavior leading to appearance of the "anti-resonance" and band inversion in the periodic fields may be qualitatively described as the reaction of the rectangular filter (8) on such fields. Modulation of the vector $\langle \mathbf{P} \rangle$ explains also the periodic chain stretching and chain compression, frequently observed experimentally (see, for example [10]).

Any corrections to this qualitative description due to the chain fluctuations are only noticeable for short chains. Such corrections enable not only to describe the action of variable fields, but also the steady-state electrophoresis of the moderate-sized DNA chains.

Let us calculate an average over the chain fluctuations of the chain center of mass drift velocity. We shall represent the vector $\mathbf{P} = (a/b)\sum \mathbf{b}_n$ as a sum of the regular value $\langle \mathbf{P} \rangle = (a/b)\sum \langle \mathbf{b}_n \rangle$, and the fluctuating component $\Delta \mathbf{P} = (a/b)\Delta \mathbf{b}_n$, where $\Delta \mathbf{b}_n = \mathbf{b}_n - \langle \mathbf{b}_n \rangle$. Presuming uncorrelated values of $\Delta \mathbf{b}_n$ and $\Delta \mathbf{b}_m$ for the different segments n and m, the average drift velocity of the center of mass may be represented by:

$$\langle \mathbf{U}_d \rangle = \frac{\mu \overline{\rho} q b^2}{L^2 a} \left[\langle \mathbf{P} \rangle (\mathbf{E} \langle \mathbf{P} \rangle) + \mathbf{E}(L/b) \langle (\Delta b_x)^2 \rangle \right]. \tag{9}$$

Here, the $\langle (\Delta b_x)^2 \rangle$ parameter represents the mean square of the fluctuations of the projection of the vector $\mathbf{b}_n$ referring to the internal chain segments upon a chosen direction. A correction

to the chain velocity (mobility) containing this parameter is proportional to $1/L$, decreasing for larger molecules. However, for the relatively low L values this term dominates, permitting to use our model to describe the electrophoresis of moderately long DNA fragments. The model parameters ρ, μ, b and $\langle(\Delta b_x)^2\rangle$ should be determined comparing the model to the experiment.

We have used expressions (8) and (9) do describe the mobility and velocity of the DNA molecules in periodic electric fields. The model produces analytical expressions for the drift velocity for the most frequently used rectangular field pulses. The final formulas, however, look like a computer program. Thus, it is easier to solve the problem by numerical modeling of the filter using a circular buffer. The main difference between the FIGE and the OFAGE simulations is that in the first case the vector $\langle\mathbf{P}\rangle$ has only one component, while in the second — two, hence requiring two circular buffers.

The calculations done using our model enabled to quantitatively interpret the most interesting experiments in the field inverse electrophoresis of the long DNA chains on the agarose gel.

As an example, Fig. 1 presents the calculated DNA mobility dependence on the period of the positive pulse of the electric field for a constant field amplitude and variable polarity: $E^+ = 5.3$ V/cm, $R_E = E^-/E^+ = 1$. The positive pulse duration t^+ is a factor of 3 larger that of the negative pulse t^-: $R_T = t^+/t^- = 3$. The calculation was done for four DNA fragment sizes, 350, 550 689 and 1000 Kbp, in a 1 % agarose gel in TAE buffer (tris-acetate etilen diamid tetra acetate (EDTA)), to be compared to the experimental results by Heller and Pohl [11]. Comparison of the calculated and the experimental results demonstrated an agreement surprising for this type of experiment, to within the experimental error. The difference is that the model predicts not one but a series of the mobility ms that the model predicts not one but a series of the mobiliinima for the small field periods, conditioned by the characteristic response of the rectangular filter to a pulsed signal. This range, however, is only represented by a single experimental point in [11].

Sabanayagam and Hozwarth [12] present a much more complete information for the small periods, although they used a 0.6 % agarose gel in tris-borate EDTA (TBE) buffer. Note that the main model parameter b and its moments depend essentially on the pore size and thus on the gel concentration. The pore size and the buffer solution composition may also influence the equilibrium defect concentration $\overline{\rho}$ and the mobility μ.

Fig. 2 presents the drift velocity dependence on the positive field pulse duration t^+ for four different values of the electric field strength of 2, 4, 6 and 10 V/cm for a 670 Kbp

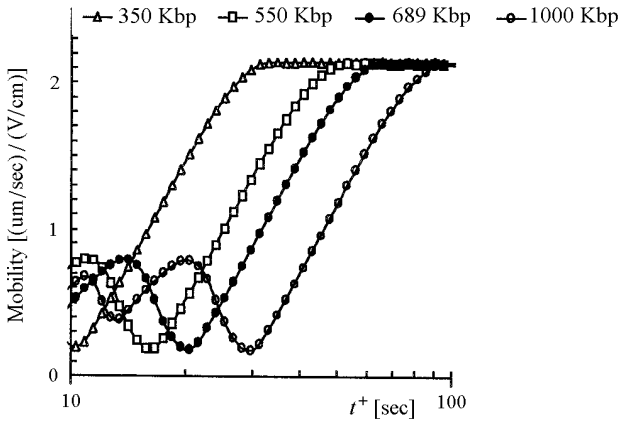

Figure 1

DNA fragment. The model calculations were done for the FIGE experiment, with the positive pulse duration exceeding the negative pulse duration by a factor of 3, $R_T = t^+/t^- = 3$, and with equal amplitudes, $R_E = 1$. These parameters and notations correspond to the experimental data in [12]. Comparison of the experimental results with the model demonstrates a qualitative agreement, at least in the range of the periods close to, or greater than that of the main minimum. As regards the range of the smaller periods, the cited experimental results have a strong scatter of the data points there, confirming indirectly the existence of additional minima predicted by our model. Although the smaller period range is never used for separations, producing excessively diffuse electrophoretic patterns and poor size separation of DNA bands, the question of the existence of additional mobility minima is of interest for the model verification, requiring future studies. Note that our model in this range is limited to the periods larger than the time necessary to achieve the equilibrium of defect distribution by defect diffusion.

Fig. 3 shows the modeling results of the two-dimensional OFAGE method, for three different field action angles. The zero angle corresponds to a constant field, where all the DNA fragments are moving with a velocity virtually independent on the fragment size, and there is no size separation of the DNA. As we may note, the fastest drift velocity variation in function on the size S is obtained at 120 degrees. In fact, this is the most frequently used angle in the DNA separations. The field period T was of 50 sec. The appearance of minima in this DNA fragment size range at smaller field action periods is similar to the results shown in Fig. 1 based on the FIGE experiment modeling.

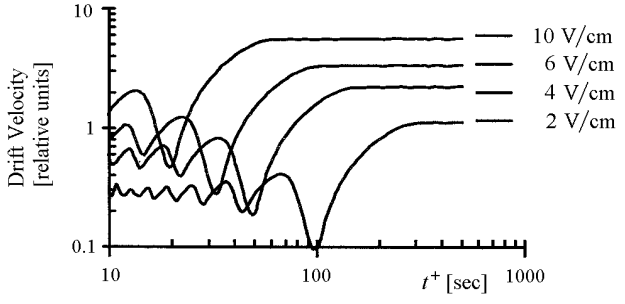

Figure 2

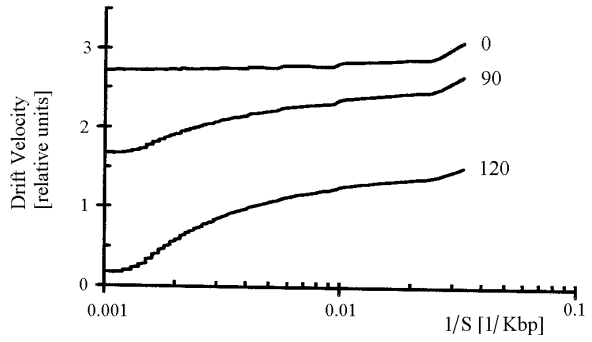

Figure 3

In conclusion, we shall formulate the main results of this work. The reptation concept by de Gennes was used to show that the head segment of the chain has, on average, a constant

mobility in an external electric field. For an inextensible chain the values of the end-to-end vector may be calculated as a response of a certain filter, with a time constant equal to the chain renewal time. The renewal time is constant in a constant-amplitude variable field, thus the respective filter is a simple rectangular filter. The frequency response of such a filter is known and has a series of zeroes for the field frequencies integer multiples of the chain renewal frequency.

The chain center of mass mobility is proportional to the square of the end-to-end vector, the velocity minimum lies close to the first zero of the filter, corresponding to the chain renewal time.

Comparison to the experiment has shown that the model enables to obtain both qualitative and quantitative description of the DNA chain anomalous mobility in one- and two-dimensional electrophoretic experiments using pulsed electric fields.

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