Mobility Minima in Field-Inversion Gel Electrophoresis

TAKESHI KOBAYASHI, MASAO DOI,* YOSHIHIKO MAKINO,[1] AND MASASHI OGAWA[1]

Department of Physics, Faculty of Science, Tokyo Metropolitan University, Fukazawa 2-1-1, Setagaya-ku, Tokyo 158, Japan

Received March 30, 1989;
Revised Manuscript Received November 27, 1989

Introduction

Field-inversion gel electrophoresis (FIGE) was invented by Olson et al.[2] to separate DNA of high molecular weight. The method involves applying an electric field periodically in the forward direction for a time period t_1 and in the backward direction for a time period t_2. As t_1 is chosen to be longer than t_2, the DNA molecules are moved in the forward direction on the whole. Olson et al. showed that when t_1, t_2, and the field strength are appropriately chosen, the method can separate large DNA molecules (of a few hundred kilobase pairs) that cannot be separated by the conventional stationary field method.

Despite the utility, the basic mechanism of separation in FIGE is not well understood. It is generally believed that the motion of DNA in a gel is described by the reptation model.[3-5] which indeed can explain the observed behavior of stationary field gel electrophoresis.[6-8] However, analysis of the model in the case of FIGE is difficult, and the existing theory[9] seems still tentative and qualitative. Recently, several computer simulations of FIGE[10-13] have been done, but for real systems, only a few experimental results are available.[14,15] In this note we report our FIGE results and propose a possible mechanism for FIGE.

Experimental Section

The DNA samples used in the experiment are summarized in Table I. They are double-stranded DNA of contour length of a few tens of microns. As the persistence length of DNA is estimated to be 70 nm, these DNA samples can be regarded as flexible polymers. The gel is a 1 wt % agarose gel, the mean pore size of which is estimated to be 90 nm. The buffer is X05 TBE and its Debye length is estimated to be 1.5 nm. Since this is much smaller than the persistence length of DNA, the effect of the electrostatic repulsion on the DNA conformation will be negligible.

The instrument consists of a vertical gel electrophoresis unit (Type SE600, Hoeffer Scientific Instruments), a pulse controller, and a circulator. While the electrophoresis is carried out, the temperature of the gel is kept at 8 ± 1 °C by the circulator. By direct measurement of the electric potential in the gel, we confirmed that the electric field is constant throughout the gel.

The electrophoresis is done for both stationary and alternating pulse fields. In the latter case, the magnitude of the electric field of the forward pulse and that of the reverse pulse are set to be equal to each other. The pulse duration time of the forward field (t_1) and that of the reverse field (t_2) are varied, with their ratio t_1/t_2 fixed at 3.

After the electrophoresis is carried out, the DNAs in the gel are stained by ethidium bromide, a fluorescent dye, and photographs are taken under ultraviolet rays for the measurement of displacement of DNA. The electrophoretic mobility μ_E of DNA is calculated by

$$\mu_E = d/ftE \tag{1}$$

where d, t, and E stand for the displacement of DNA, total time for electrophoresis, and electric field strength, respectively, and $f \equiv (t_1 - t_2)/(t_1 + t_2)$ is a correction factor which is put so that eq 1 reduces to the mobility in the stationary field in the limit of $t_1/t_2 \to \infty$; f is equal to 1 for the stationary field and $^1/_2$ for our pulse field.

Table I
DNA Samples

	DNA fragment				
	T4dC	λ	MK-I-1	MK-I-2	MK-I-3
no. of base pairs × 10^{-3}	166.0	48.50	23.13	9.42	6.56
contour length/μm	56.4	16.5	7.86	3.20	2.23

Results and Discussion

Figure 1 shows typical FIGE results. The mobilities are plotted against the duration time of the forward field t_1.

When t_1 goes to infinity, the mobilities are almost independent of the molecular size of DNA, indicating that the separation of DNA by the stationary field is not possible. The mobility μ_E depends on the field strength E. The field dependence is much weaker than that predicted by the low-field-limit equation $\mu_E \propto E^2$. This indicates that the present experiment corresponds to the nonlinear regime,[4,5] where DNA molecules are considerably elongated along the field direction.

As t_1 decreases, the mobility has a sharp drop at a certain time t_1*, which we shall call the resonance time, following Olson.[2] The resonance time depends on the contour length of DNA and the field strength as shown in Figure 2. It is seen that the resonance time increases almost linearly with increasing contour length L. The slope is a function of E: the higher the field is, the smaller the resonance time becomes.

To understand the physical significance of the resonance time, we plotted t_1* against the disengagement time in the continuous field $t_{dis} = L/V_{cont}$ in figure 3, where V_{cont} is the electrophoretic velocity in the continuous field. It is seen that t_1* and t_{dis} are proportional to each other:

$$t_1^* = At_{dis} = A\frac{L}{V_{cont}} \tag{2}$$

The proportionality constant A is 0.31 and is independent of the electric field (at least in the regime of the experiments we have done). The constant A may depend on t_1/t_2, but the dependence has not been studied.

Various interpretations can be made for eq 2. Our tentative interpretation is the following.

Recent computer simulations[7,10-12] and a theory[16] have shown that DNA migrating through a gel takes two distinctive states: the elongated state, in which the end-to-end distance h of the DNA is comparable to the contour length L, and the compact state, in which $h \ll L$. For most of the time, the DNA is in the elongated state, but occasionally it falls in the compact state. If a DNA falls in the compact state, it stays there for a relatively long time, during which it migrates very slowly since the mobility of DNA is proportional to h_x^2, where h_x is the component of the end-to-end vector of DNA in the direction of the electric field. For simplicity we shall assume that the DNA can be in either the elongated or compact state, with mobilities μ_e and μ_c, respectively; then the overall mobility is written as

$$\mu_E = (1 - \phi_c)\mu_e + \phi_c\mu_c = (1 - \phi_c)\mu_e \quad (\text{for } \mu_c \ll \mu_e) \tag{3}$$

where ϕ_c is the fraction of DNA in the compact state.

The time necessary for the DNA to recover from the compact state to the elongated state can be estimated by eq 14 in ref 16. If one neglects the random force, the

0024-9297/90/2223-4480$02.50/0

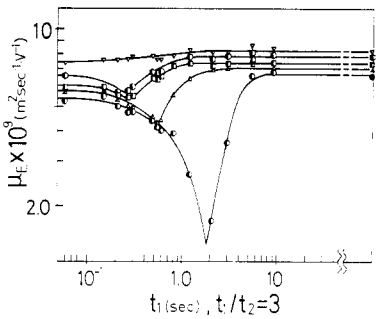

Figure 1. Electrophoretic mobilities under field-inversion plotted against the pulse duration time t_1 of the forward field. The field strength is E = 15.4 V/cm. The five curves correspond to DNA of different number of base pairs: from top to bottom, MK-I-3 (6.56 kbp), MK-I-2 (9.42 kbp), MK-I-1 (23.13 kbp), λ (48.50 kbp), and T4dC (166.0 kbp).

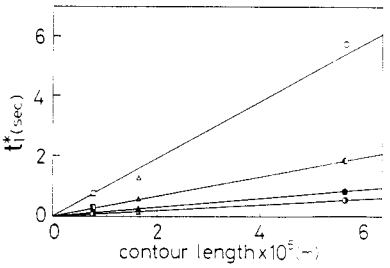

Figure 2. Resonance time t_1* of DNA plotted against its contour length. The four lines correspond to different field strengths: from top to bottom, 7.72, 15.4, 30.7, and 38.3 V/cm.

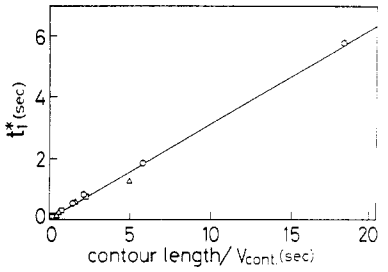

Figure 3. Resonance time t_1* plotted against the disengagement time of the stationary field gel electrophoresis. The symbols represent results for different fields.

equation is written as

$$\frac{dh_x}{dt} = \frac{V_{cont}}{LP(E)}\left[1 - \frac{h_x}{LP(E)}\right]h_x \qquad (4)$$

where $P(E) = \langle \cos\theta \rangle$ is a constant that depends on E and takes a value between 0 and 1. From eq 4 it is easy to show that the characteristic time of h_x is $LP(E)/V_{cont}$. Since $P(E)$ is of the order of unity in the present experiment where the nonlinear effect is large, the time necessary for the compact DNA to regain the elongated conformation will be approximately equal to $L/V_{cont} = t_{dis}$.

With this background, we conjecture that the mobility minima appear by the following reason.

The field reversal presumably increases the chance for the DNA to be trapped in the compact conformation. Indeed recent computer simulation[13] has shown that when the field is reversed, the average mobility of DNA drops for a certain time. Various mechanisms are conceivable:[13] (i) additional kinks are introduced at the chain ends or (ii) the stretched chain snaps back upon field reversal. Thus

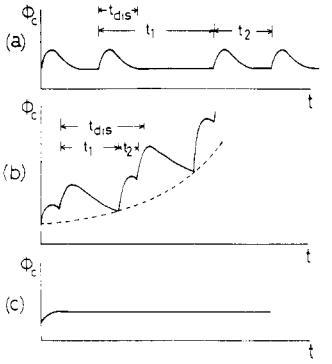

Figure 4. Time evolution of the fraction of the compact conformation in the FIGE: (a) $t_1 \gg t_{dis}$; (b) $t_1 \cong t_{dis}$; (c) $t_1 \ll t_{dis}$.

if $t_1 \gg t_{dis}$, $\phi_c(t)$ will change as shown in Figure 4a. When the field is reversed, $\phi_c(t)$ will become larger than the steady-state value but then will relax to the steady-state value after a time t_{dis}. If $t_1 \gg t_{dis}$, the effects of each field inversion are independent of each other, so that the overall mobility is reduced only by a factor $[1 - t_{dis}/(t_1 + t_2)]$, which is close to 1 for $t_{dis} \ll t_1$.

If $t_1 \cong t_{dis}$, ϕ_c will behave as shown in Figure 4b. In this case, the field is inverted before the effect of the previous inversion dies out, so that after one cycle of FIGE, $\phi_c(t)$ will become larger than the value before the cycle; i.e., $\phi_c(t + t_1 + t_2)/\phi_c(t) > 1$. Thus if the cycle is repeated many times, $\phi_c(t)$ increases exponentially with time untill it levels off. This will cause a large reduction of the mobility.

If $t_1 \ll t_{dis}$, the exponential growth of ϕ_c will not occur since the DNA will move essentially in the same tube during a cycle of the field reversal. In this case, $\phi_c(t)$ will behave as shown in Figure 4c, and there will be no significant reduction of the mobility.

In the above explanation, the tube length fluctuation that has been accounted for in recent computer simulations[10-13] and theory[9] is not considered explicitly. The fluctuation may be important, but our experimental result seems to be explained without considering the fluctuation: the mobility minima will appear as long as the field reversal creates compact conformations that last for the time t_{dis}. If this is the case, the minima will appear in the computer simulation for the simple biased reptation model.

References and Notes

(1) Fuji Photo Film Asaka Laboratory, Sensui 3-11-46, Asaka-shi, Saitama 351, Japan.
(2) Carle, G. F.; Frank, M.; Olson, M. V. *Science* **1986**, *232*, 65.
(3) de Gennes, P.-G. *J. Chem. Phys.* **1971**, *55*, 572.
(4) Lumpkin, O. J.; Dejardin, P.; Zimm, B. H. *Biopolymers* **1985**, *24*, 1573.
(5) Slater, G. W.; Noolandi, J. *Phys. Rev. Lett.* **1985**, *55*, 1579.
(6) Hervet, H.; Bean, C. P. *Biopolymers* **1987**, *26*, 727.
(7) Noolandi, J.; Rousseau, J.; Slater, G. W.; Turmel, C.; Lalande, M. *Phys. Rev. Lett.* **1987**, *58*, 2428.
(8) Slater, G. W.; Noolandi, J. Proceedings of ACS Symposium, Canada, 1988.
(9) Viovy, J. L. *Phys. Rev. Lett.* **1988**, *60*, 855.
(10) Deutsch, J. M. *Science* **1988**, *240*, 922.
(11) Zimm, B. H. *Phys. Rev. Lett.* **1988**, *61*, 2965.
(12) Duke, T. A. J., to be published.
(13) Noolandi, J.; Lsater, G. W.; Lim, H. A.; Viovy, J. L. *Science* **1989**, *243*, 1456.
(14) Bostock, C. J. *Nucleic Acids Res.* **1988**, *16*, 4239.
(15) Holzwarth, G.; Mckee, C. B.; Steiger, S.; Crater, G. *Nucleic Acids Res.* **1987**, *15*, 10031.
(16) Doi, M.; Kobayashi, T.; Makino, Y.; Ogawa, M.; Slater, G. W.; Noolandi, J. *Phys. Rev. Lett.* **1988**, *61*, 1893.