Periodic Behavior of DNA Molecules during Steady Field Gel Electrophoresis

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Introduction. Various studies have indicated that the dynamics of DNA molecules during gel electrophoresis are rather complicated even in the steady electric field.[1] Schwartz et al.[2] observed the motion of DNA by fluorescence microscopy[3] and reported that the DNA motion is characterized by cycles of elongation and contraction as the DNA hangs on obstacles and then slides off. Similar observations have been made by other researches[4,5] by using the same technique and also by computer simulation.[6,7] This cyclic motion has been conjectured to be related to the "resonance phenomena" found in non-steady electric field.[8-10] Despite such descriptive reports, however, there has been little quantitative analysis. This is perhaps due to the following reasons: (i) observation of DNA molecules over extended periods of time is difficult and taking enough data for statistical analysis is laborious and (ii) elimination of the boundary effects of electrodes and glass plates is not easily realized in the small cells used in microscopic observations.

We have improved the technique reported in the previous study[11] by a computer-controlled image processing technique and by using a specially designed cell for the observation. We carried out a statistical analysis for the motion of DNA under a steady field. Here we demonstrate that (i) DNA undergoes indeed a periodic motion and (ii) the periodicity of the oscillation is closely related to the "resonance" frequency in the nonstationary electric field.

Experimental Section. Bacteriophage T4 DNA (166 kbp, contour length 55 μm) was purchased from Nippon Gene. The samples were diluted with TBE buffer solution (45 mM Tris, 45 mM borate, 1 mM EDTA) and mixed with 1.0 wt% agarose gel. Ethidium bromide (EB), a fluorescent dye which specifically binds to DNA molecules, was added to visualize each DNA molecule. The final concentrations of DNA in nucleotide and EB were adjusted to be 0.9 μM; under this condition, the intercalated EB has a negligible effect on the static properties of DNA.[12]

The mixture was placed in a hand-made electrophoresis cell. In order to observe the DNA motion in wider space, a larger observation area of the cell than previously,[8] about 2×2 cm^2, was used. The thickness of the migration region was also made larger, about 100 μm, which is sufficient to eliminate boundary effects of the glass plates sandwiching the gel. The electric field was applied by a potentiostat through a pair of silver wire electrodes 40 mm part. To monitor the actual field strength in the observation area, we used another pair of electrodes (Ag–AgCl) 32 mm apart and controlled the potentiostat when it was needed.

The excitation wavelength was 520 nm. Individual DNA molecules were observed as fluorescent images. The images were recorded on videotapes with a high-sensitivity SIT camera and an image processor, Argus 10 (Hamamatsu Photonics). The recorded images were processed with a programmable image analysis system PIAS3 (PIAS). After elimination of the background noise, the digitized two-dimensional DNA image function $I(x,y,t)$, which takes 1 if the pixel (x,y) is brighter than a certain threshold or 0 if it is not, is obtained as a function of t. Here the x-axis is taken along the applied field. The two-dimensional position vector of the center-of-mass $\vec{R}(t) = (R_x(t),R_y(t))$ and the radius of gyration of $R_g(t)$ were then obtained by

$$R_x(t) = N^{-1}\int I(x,y,t)\, x\, dx\, dy \tag{1}$$

$$R_y(t) = N^{-1}\int I(x,y,t) y\, dx\, dy \tag{2}$$

$$R_g(t) = \frac{3}{2}N^{-1}\int I(x,y,t)[(x-R_x)^2 + (y-R_y)^2]\, dx\, dy \tag{3}$$

where $N = \int I(x,y,t)\, dx\, dy$ is the normalization constant. The factor $^3/_2$ in eq 3 appears under the assumption that, on average, DNA molecules extend in the vertical direction as well as in the focal plane.

Steady electric fields of 2, 4, 6, and 8 V/cm were applied, and the image of each DNA was traced for more than 5 s. The observation time is limited because the DNA molecule passes out of the observation videoframe which is about 40×40 μm^2, or out of focus due to vertical Brownian motion. The sampling interval was 0.1 s. For each condition, the observation was carried out for about 10 DNAs and the statistical average was taken for both time and different DNAs.

Results and Discussion. From the series of data of $\vec{R}(t)$, we calculated the center-of-mass velocity along the applied field $v_x(t)$ by

$$v_x(t) \equiv \frac{R_x\left(t+\frac{1}{2}\Delta t\right) - R_x\left(t-\frac{1}{2}\Delta t\right)}{\Delta t} \tag{4}$$

where Δt was taken to be 0.5 s. Table I shows the averages of v_x and R_g. The values of average migration velocity agree with the reported values in macroscale DNA electrophoresis; for example, Shikata and Kotaka[13] reported that $\bar{v}_x$ is about 4 μm/s under 7.5 V/cm for T4 DNA. This implies that our experimental conditions of the direct observation are very similar to the usual macroscale ones and that the artifacts due to the interaction between DNA and dye or the boundary effects of the glass surfaces are negligible.

Figure 1 shows an example of the time development of v_x and R_g. One can see a clear indication of periodic motion. Direct observation of the video image indicates that the periodicity is a result of the following cycle, as reported earlier.[4,5]

1. A DNA molecule has a spherical coil conformation.

2. When a part of the DNA is caught by an obstacle, the DNA starts to be stretched.

3. While the DNA is stretched, it gradually assumes a V-conformation with the apex fixed at the gel fiber. At this stage, the migration is slowest.

4. When the DNA is fully stretched, the longer arm of the V-conformation starts to pull the other arm, making the shorter arm even shorter.

5. When the shorter arm disappears, the DNA is released from the gel fibers and starts to shrink rapidly toward the spherical conformation. During this process, the migration velocity is at a maximum.

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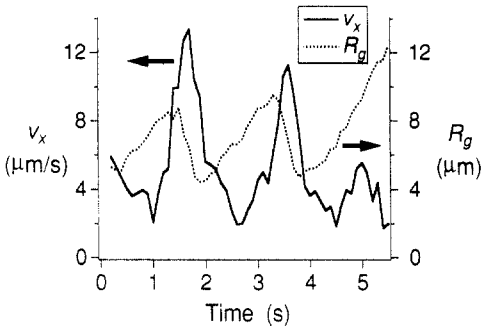

Figure 1. An example of the time development of v_x and R_g of T4 DNA during steady field gel electrophoresis. The field strength is 8 V/cm.

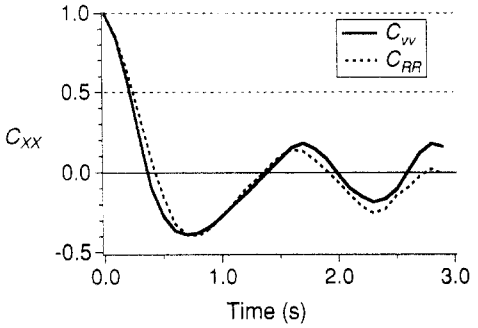

Figure 2. Ensemble average of autocorrelation functions of v_x and R_g.

Table I. Center-of-Mass Velocity v_x and Radius of Gyration R_g of T4 DNA under Various Field Strengths E

E (V/cm)	$\overline{v_x}$ (μm/s)	$\overline{R_g}$ (μm)
2.0	0.84 ± 0.26	3.23 ± 0.66
4.0	2.70 ± 0.43	3.27 ± 0.75
6.0	4.23 ± 0.71	3.68 ± 0.97
8.0	6.88 ± 0.97	4.77 ± 1.53

To investigate the behavior quantitatively, we calculated the velocity autocorrelation function $C_{vv}(t)$ defined by

$$C_{vv}(t) \equiv \frac{\int (v_x(t+t') - \overline{v_x})(v_x(t') - \overline{v_x})\,dt'}{\int (v_x(t') - \overline{v_x})^2\,dt'} \simeq \frac{\sum_i (v_x(t_i + t) - \overline{v_x})(v_x(t_i) - \overline{v_x})}{\sum_i (v_x(t_i) - \overline{v_x})^2} \quad (5)$$

and $C_{RR}(t)$, the autocorrelation function of R_g. As shown in Figure 2, both functions show a clear oscillation, with the same period of oscillation.

To extract the characteristic time of oscillation, we obtained the time t_0 at which the autocorrelation function C_{RR} first becomes zero and defined the "periodicity of oscillation" τ by

$$\tau \equiv 4t_0 \quad (6)$$

Figure 3 shows the periodicity τ as a function of the electric field. The period decreases with the increase of the field strength; this corresponds to the observation that conformational change of DNA molecules is swifter under a stronger field.

It is interesting to note that the oscillation is closely related to the phenomenon of mobility minimum (or antiresonance) under alternating electric fields. Doi et al.[14] reported that in field inversion gel electrophoresis the mobility is minimized when the periodicity is ~3 s for the field strength E = 15.4 V/cm and ~8 s for E = 7.72 V/cm. Shikata and Kotaka[10] also carried out a gel electrophoresis for a biased-sinusoidal field $E(t) = E_b + E_s \sin(\omega t)$ and reported that the mobility takes a local minimum when the periodicity $T = 2\pi/\omega$ is about 2.5 s for E_b = 7.5 V/cm, E_s = 12.5 V/cm and 8 s for E_b = 2.5 V/cm, E_s = 7.5 V/cm. Since the oscillation period observed under the steady field is close to the alternation period of the mobility minimum with about the same field dependence, we conjecture that the two periods have an intimate relation.

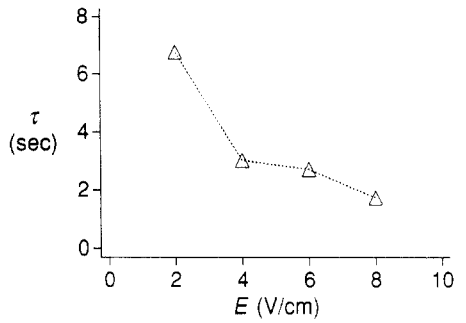

Figure 3. Oscillation period τ estimated from the autocorrelation function of R_g plotted against the field strength E.

The periodic motion is remarkable, considering that we apply a steady electric field and that the gel matrix is random. A similar oscillatory behavior in the autocorrelation functions $C_{vv}(t)$ and $C_{RR}(t)$ under a steady field has been recently observed also by Brownian dynamics computer simulation.[15] The origin of this oscillatory behavior is not fully understood. At this stage, we think that it is a kind of sustained oscillation. A model which describes the motion is now being developed.

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