Biased Sinusoidal Field Gel Electrophoresis for Large DNA Separation

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ABSTRACT: For size-dependent separation of DNAs, we propose a new electrophoretic method, a biased sinusoidal field gel electrophoresis (BSFGE), which utilizes a sinusoidal field of strength E_s and frequency f superposed on a steady bias field of strength E_b. The BSFGE was tested on double-stranded DNAs with molecular weight (M) ranging from 200 basepairs (bp) to 2 Mbp in agarose gel of concentration (C_{gel}) from 0.25 to 1.5 wt %. From the M dependence of the electrophoretic mobility (μ_s) under a low steady field, the features were classified into three regimes that are similar to Slater-Noolandi's regimes: the Ogston regime, I; the entanglement (without stretching) regime, II; and the entanglement (with stretching) regime, III. The BSFGE results exhibited three features depending on the conditions: Under a high bias with $E_b \geq E_s$ ($\geq$5.0 V cm^{-1}) and varying f, a significant maximum in μ was observed on all DNAs at an f_M independent of M but dependent on C_{gel} as $f_M \propto C_{gel}^7$. Under an intermediate bias with $E_b \leq 5.0$ V cm$^{-1} \leq E_s$; μ, starting from μ_s at high f, increases with decreasing f and reaches a plateau (μ_0) at low frequencies for DNAs of M smaller than 10 kbp; the transition frequency (f_T) was nearly independent of M, when M was large. The ratio $\mu_0\mu_s^{-1}$ did not depend on M in regimes I and III but increased with M in regime II. Under still lower bias, μ for DNAs with $M > 20$ kbp, again starting from μ_s at high f, reached a pronounced minimum at a frequency (f_p, pin-down frequency) specific to M and C_{gel} in such a way that $f_p \propto C_{gel}^{-1}M^{-1}$, before reaching the low-f plateau (μ_0).

Introduction

Although basic mechanisms of separation have not yet been fully understood, the pulse field gel electrophoresis (PFGE)[1-10] is now the most routinely used technique for size-dependent separation of native DNAs of size up to a few megabasepairs (Mbp). An interesting feature of the PFGE method is the so-called *antiresonance* phenomenon[5,8,10] in which an adequate choice of pulse timing called resonance time significantly reduces the electrophoretic mobility (μ) of a particular DNA or virtually pins down the DNA but allows other to migrate with reasonable rates.[5,9,10] The phenomenon was interpreted as that a DNA migrating through a gel while entangling with gel strands may assume two different states: an elongated state in which the DNA can migrate with a reasonable rate and a compact state in which it coils up and entangles heavily with the gel strands and thus can hardly migrate.[5-8,10] Field reversal with the resonance time presumably increases the chance of the particular DNA being in the compact state and retards its migration.

However, so far the pulse timing was chosen arbitrarily, and thus, choosing of optimum conditions was rather difficult. In most of PFGE methods, such as crossed field gel electrophoresis (CFGE)[1-4,8] and field inversion gel electrophoresis (FIGE),[5-10] one utilizes a rectangular pulse by changing the field direction with constant time intervals. Since a rectangular pulse is composed of many higher order frequency harmonics, the frequency dependence of the mobility (μ) of DNAs is rather ambiguous.

To solve this problem and to design optimal conditions for efficient size-dependent separation of large DNAs, we proposed a new version of gel electrophoresis[11] that utilizes a sinusoidal field of strength E_s and frequency f superposed on a steady bias field of strength E_b: the field is then defined as

$$E(t) = E_b + E_s \sin(2\pi ft) \quad (1)$$

We called this method **biased sinusoidal field gel electrophoresis** (BSFGE).[11] In this article, we report the features of the BSF method tested on double-stranded DNAs and discuss the dynamics of gel strands and DNA molecules under the biased sinusoidal field.

Experimental Section

Materials. Experiments were conducted on double-stranded DNAs with molecular weights of 200 bp (bp) to 2 Mbp. DNAs of 200-726 bp and those of 600-1.35 kbp were the restricted enzyme *Hin*dI and *Hae*III digests of ϕX174 phage DNA, respectively. DNAs of 2-23.1 kbp were also *Hin*dIII digests of λ phage DNA, and those of 48.5 and 166 kbp were, respectively, native λ and Td4C phage DNAs. These DNAs were purchased from Nippon Gene Co. Ltd. (Tokyo, Japan). DNAs of 230 kbp to 2 Mbp were chromosomal DNAs of a yeast (*Saccharomyces cervisiae*, strain YNN 295, Yeast DNA-PFGE Markers) purchased from Pharmacia LKB (Uppsala, Sweden).

Agarose (DNA/RNA separation grade, Pharmacia LKB) gels of size 15 cm × 15 cm and concentrations C_{gel} = 0.25, 0.5, 0.7, 1.0, and 1.5 wt % were used as the media. The buffer solution was 50 mM TBE, which consists of 50 mM [tris(hydroxymethyl)-amino]methane (tris base), 50 mM boric acid, and 1 mM ethylenediaminetetracetic acid (EDTA) disodium salt. Ethidium bromide was added at a concentration of 0.5 mg L^{-1} to the buffer to visualize the position of the DNA bands with a UV illuminator.

Electrophoretic Technique. To apply a BSF (eq 1) of arbitrary E_b, E_s, and f, we utilized a high-speed and high-voltage bipolar power amplifier (4305, NF Co. Ltd., Yokohama, Japan) and a conventional function generator. A generated sinusoidal wave with small strength was amplified 100 times, and a given steady bias was added to the wave with the amplifier. The BSF was then applied to the gel through a pair of parallel electrodes consisting of platinum wires of 16-cm length and placed at 16-cm separation. The E_b and E_s values were 2.5, 5.0, and 7.5 V cm^{-1}, and f was varied from 0.001 Hz to 1 kHz.

A submerge-type electrophoretic system was used, and the buffer solution was kept at about 20 °C by circulating thermostated water. Usually several runs in a series of BSF experiments were conducted by using a single gel bed under given E_b and E_s with varying f. At the end, the steady field experiment was conducted with a given E_b (and E_s = 0) to collect the steady field mobility (μ_s) data as a control for the particular gel. Thus, the μ_s data for a given DNA might be slightly different (within a few percent) from one series to another, even when E_b and other conditions such as C_{gel} were the same between these series (cf., Figure 2A-D, for example). For DNAs shorter than 166 kbp, the BSF was applied without any preseparation. On the other hand, because it was not clear how DNAs of size larger than 200 kbp

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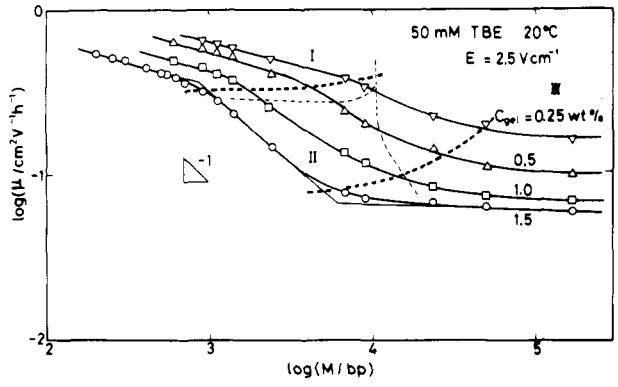

Figure 1. Dependence of electrophoretic mobility (μ) on molecular weight (M) for systems with gel concentrations (C_{gel}) of 0.25, 0.5, 0.7, 1.0, and 1.5 wt % and with 50 mM TBE buffer under the steady electric field of strength E = 2.5 V cm^{-1} at 20 °C. Thin dotted lines indicate the boundaries between regimes I (Ogston), II (reptation without stretching), and and III (reptation with stretching) for the systems quoted from the estimation[13] by Slater and Noolandi. The heavy dotted lines represent the boundaries redefined by us.

would respond to the BSF, we preseparated those DNAs in one direction by an already established CFGE method[5] using a gel of C_{gel} = 1 wt % cast in a commercially available hexagonal electrode system (a Pulsaphor system with a hexagonal electrode, Pharmacia LKB). Pulse periods of 70 s for 15 h and then 120 s for 12 h were used for this preseparation.[12] The BSF was then applied to the preseparated DNAs in the direction vertical to the preseparation field to achieve two-dimensional separation. We were thus able to determine the relation between the mobility (μ) and M for large-size chromosomal DNAs under BSFGE.

Results and Discussion

Mobility under Steady Electric Field. Slater and Noolandi[13] classified the electrophoretic behavior of DNAs under a steady field according to the molecular weight dependence of their mobility into the following three regimes: In regime I, which is called the Ogston regime,[14] small DNAs migrate without entangling with gel strands. In this regime, the mobility is almost independent of M or only weakly dependent on M. In regime II, called the entanglement without stretching regime, intermediate-length DNAs lightly entangle with and migrate through the gel network but their conformation is not affected much by the field so that they keep coiled conformation. In this regime, μ is proportional to M^{-1}, if the field strength is sufficiently low. In regime III, called the entanglement with stretching regime, large DNAs entangle rather heavily with gel strands and can hardly migrate so that their conformation is stretched along the field. In this regime, the dependence of μ on M is again little.

Figure 1 shows typical examples of log μ versus log M curves under a steady field of E = 2.5 V cm^{-1} in agarose gels with C_{gel} = 0.25–1.5 wt %. In the figure, the boundaries of Slater and Noolandi's (SN's) regimes[13] mentioned above are indicated with thin dotted lines. They simply defined regime II in such a way that in that regime the log μ versus log M curve possesses a slope of −1. Thus, in dilute gels of C_{gel} lower than 0.25 wt %, regime II does not appear and regime I directly changes to regime III as seen in Figure 1. However, this feature of the SN regime boundaries just does not make sense: In concentrated gels, DNAs change their regime with increasing M from the unentangled regime (I) through a lightly entangled regime (II) to the heavily entangled regime (III). This is why DNAs of intermediate size should run from regime I directly into regime III, skipping regime II in such a *dilute* gel in which the DNAs are less likely to entangle with gel strands.

We thus propose a different definition for the boundaries of these three regimes as follows. We postulate that in these regimes in a given gel μ should have different M dependences, and thus, we simply approximate the log μ versus log M curve with three straight lines. We then define the intersections between the straight lines as the crossover molecular weights between the regimes: For example in Figure 1, for the system with C_{gel} = 1.5 wt %, we draw such straight lines corresponding to the three regimes with thin lines. The thick dotted lines in Figure 1 thus indicate the boundaries redefined by us. Our crossover molecular weights (M_{cr}) between regimes I and II are close to SN's boundaries and are represented as $M_{cr} \propto C_{gel}^{-1}$, which fits SN's reptation picture[12] of DNA migration through the gel network. On the other hand, our other crossover molecular weights (M_{cr}') between the regimes II and III are entirely different from SN's boundaries. M_{cr}' also seem to be approximately proportional to C_{gel}^{-1} and about 7 times larger than M_{cr} for all the gels with different C_{gel} values. These features of our regime boundaries are obviously more likely and reasonable than SN's boundaries.

Although we do not exhibit the data here, regime II became narrower with increasing field strength (E). This means that the proportionality constant between M_{cr}' and M_{cr} decreases with increasing E, reflecting that entangled DNA molecules tend to be more strongly stretched under a stronger field.

Behavior under Biased Sinusoidal Field. Dependence of μ on Frequency under Varying E_b and E_s. To examine the performance of the BSFGE method, we designed the following two series of experiments: In one series, the bias field strength (E_b) was kept at 7.5 V cm^{-1} and the sinusoidal field strength (E_s) was varied from 2.5 (a high-bias condition) to 12.5 V cm^{-1} (a low-bias condition), and in the other series, E_s was kept at 7.5 V cm^{-1} and E_b was varied from 7.5 (a comparable bias) to 2.5 V cm^{-1} (a very low bias). Under such bias conditions, we examined the frequency dependence of the mobility of several DNAs with different size M. Figure 2 shows typical results for the system with C_{gel} = 1.0 wt %.

The f dependence of μ exhibits the following features. Under E_b = 7.5 and the smallest sinusoidal field of E_s = 2.5 V cm^{-1} (a high bias condition: Figure 2A), the μ values of all DNAs exhibit no f dependence and coincide with the steady field values (μ_s; under E_s = 0; cf., Figure 1).

As E_s is increased up to 5.0 V cm^{-1} (an intermediate bias condition: Figure 2B), a pronounced maximum is found in each μ versus log f curve at a frequency (f_M) around 10 Hz for this gel with C_{gel} = 1.0 wt %, while the values of μ in the low- and high-f sides are identical with the steady field values (μ_s). f_M at the maximum μ is obviously independent of M but strongly dependent on C_{gel} ($f_M \propto C_{gel}^{7}$), as we shall see later in Figure 3.

When E_s is increased up to 7.5 V cm^{-1} (=E_b: a comparable bias condition: Figure 2C), for all DNAs the low-f values ($\mu_{f\to 0}$; =μ_0) increase but the high-f values ($\mu_{f\to\infty}$) remain essentially the same as the steady field values (μ_s). Thus, the μ maxima become obscured or even disappear as DNAs become longer than 6.56 kbp.

When E_s is further increased up to 12.5 V cm^{-1} (>E_b = 7.5 V cm^{-1}: a low bias condition), the low-f plateau values (μ_0) are enhanced more and more for all DNAs and the maxima found in the intermediate bias condition are completely obscured even for low-M DNAs (of M < 6.56 kbp), as seen in Figure 2D. When E_b is reduced as low as 2.5 V cm^{-1}, while E_s is kept at 7.5 V cm^{-1} (a very low bias condition: Figure 2E), the μ versus log f curves exhibit a

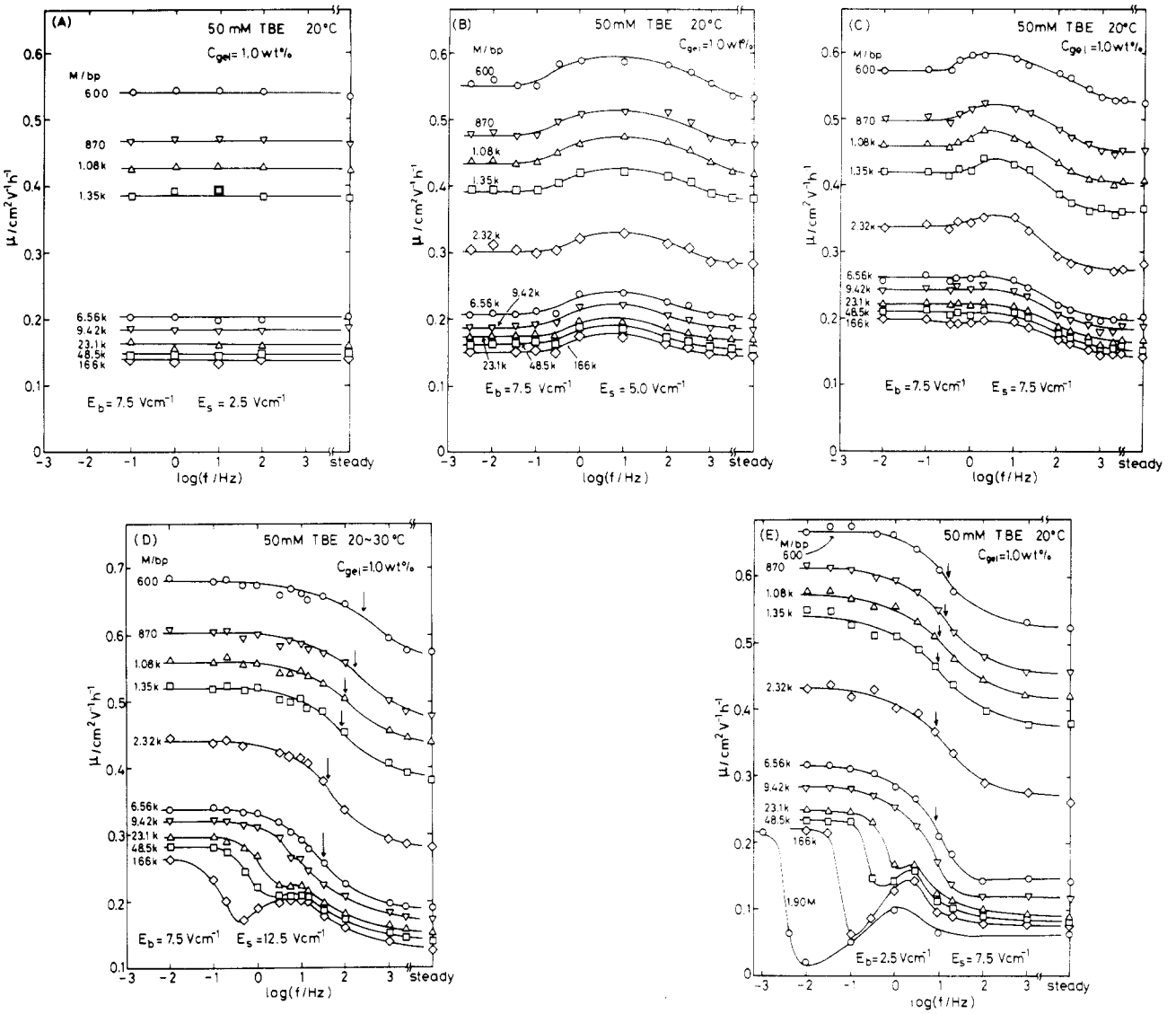

Figure 2. Dependence of mobility (μ) on frequency (f) under biased sinusoidal field (BSF) with several biased field strengths (E_b) and sinusoidal strengths (E_s) for the system with C_{gel} = 1.0 wt % at about 20 °C. (A) E_b = 7.5 and E_s = 2.5 V cm^{-1}, (B) E_b = 7.5 and E_s = 5.0 V cm^{-1}, (C) E_b = 7.5 and E_s = 7.5 V cm^{-1}, (D) E_b = 7.5 and E_s = 12.5 V cm^{-1}, and (E) E_b = 2.5 and E_s = 7.5 V cm^{-1}. Arrows in D and E show the frequency (f_T) of transitional increase in μ for each DNA. The measurements of the steady field mobility (μ_s) were repeated for each series, so that the μ_s data for the same DNA are somewhat different in panels A–D.

little more pronounced but essentially the same features as those seen in Figure 2D. The enhancement of μ in the low-f limit was not reported in any conventional PFGE methods. Interestingly, the $\mu_{f\to\infty}$ values still coincide with the steady field values, as opposed to conventional FIGE methods in which the μ values in the low-f (or long pulse timing) limit coincided with μ_s.[10] Here we define a *transition* frequency (f_T) at which the μ of each DNA assumes an intermediate value of μ_0 and μ_s. The f_T values are marked with arrows in Figure 2D and 2E.

A striking feature similar to the antiresonance phenomena already reported in conventional PFGE methods also emerges under the low bias conditions (cf., Figure 2D and 2E): For DNAs with M higher than 23.1 kbp, the μ versus log f curve exhibits a pronounced minimum at a f_p specific to each DNA. We call this frequency the pin-down frequency of the particular DNA. Essentially, the same features were found in the gels with different C_{gel} values. f_p is dependent on C_{gel} as well as on M of DNAs.

Maximum in μ under an Intermediate Bias Field. As seen in Figure 2B for BSFGE in the gel with C_{gel} = 1.0 wt % under the intermediate bias condition of E_b = 7.5 V cm^{-1} > E_s = 5.0 V cm^{-1}, each μ versus log f curve exhibits a peak at f_M independent of M. This trend was also found in gels with different C_{gel} values.

Figure 3 shows the f dependence of μ for DNA of 1.35 kbp in gels with different C_{gel}. We clearly see that f_M is strongly dependent on C_{gel} in such a way that $f_M \propto C_{gel}^7$, as the insert of the figure shows. We also note that the μ peak for 1.35 kbp DNA becomes broader and the peak height ($\Delta\mu_M$) smaller with increasing C_{gel}. For DNAs longer than 1.35 kbp, the peaks in μ become too small and cannot be found in gels of $C_{gel} \geq$ 1.5 wt %. However, even in C_{gel} = 1.5 wt % gel, a peak was still clearly observed even for DNAs with M < 500 bp.

Figure 4 shows $\Delta\mu_M$ versus log M plots obtained for gels with different C_{gel} values under E_b = 7.5 and E_s = 5.0 V cm^{-1}. Again the regime boundaries are shown with dotted lines: The thin lines are SN's boundaries and the heavy lines ours. $\Delta\mu_M$ is nearly constant for large-M DNAs in entanglement regimes II and III but increases with decreasing M for low-M DNAs found in Ogston regime I.

The above-mentioned features for the appearance of μ maxima under high-to-comparable bias conditions suggest that the motion of gel strands rather than that of DNA molecules is an important factor. The enhancement in μ

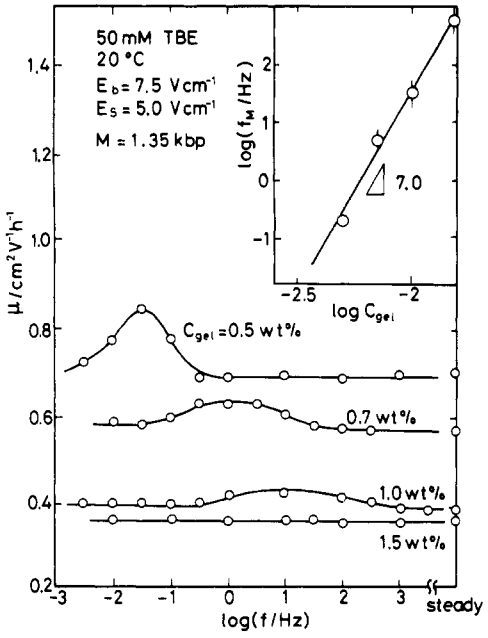

Figure 3. Dependence of mobility (μ) on frequency (f) for the DNA of 1.35 kbp under BSF of E_b = 7.5 and E_s = 5.0 V cm^{-1} in gels of various concentration (C_{gel}). An insert shows the relation between the frequency (f_M) corresponding to the maximum in μ curves and C_{gel} in the double-logarithmic scale.

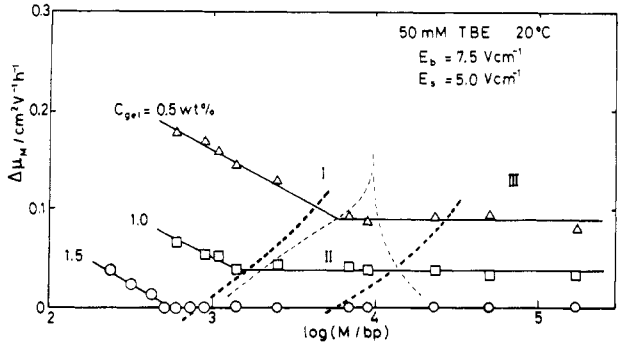

Figure 4. Relationship between increasing $\Delta\mu_M$ in μ curves and M of DNAs for systems with several C_{gel} values under BSF with E_b = 7.5 and E_s = 5.0 V cm^{-1}. Thin dotted lines indicate the boundaries between I and II and II and III quoted from the estimation[13] by Slater and Noolandi. Heavy dotted lines represent the boundaries redefined by us.

is dominant for low-M DNAs, which are supposed to *not* be entangling with gel strands.

In an ordinary polymer network or gel, the relaxation time (τ_{gel}) of gel strands was predicted to be proportional to the square of the molecular weight (M_x) between cross-links or to the mesh pore size (p) reflecting the mean-square length of the network strands, if the Mooney–Rouse model[15,16] is valid for network strands. According to Slater et al.,[13] the mesh pore size of agarose gels was estimated as

$$p \propto C_{gel}^{-0.65\sim(-0.7)} \quad (2)$$

Thus, the relaxation time of the gel network can be

$$\tau_{gel} \propto p^2 \propto C_{gel}^{-1.3\sim(-1.4)} \quad (3)$$

On the other hand, if f_M is related to τ_{gel} in such a way that $\tau_{gel} = 1/2\pi f_M$, we expect from the above model that $f_M \propto C_{gel}^{1.3-1.4}$. The observed f_M is certainly an increasing function of C_{gel}, but the observed power of 7 is far too large in comparison with 1.3–1.4 expected from the model (cf., Figure 3). This discrepancy implies that the above expectation is too naive: f_M is certainly related to the motion of gel strands but not in such a way as expected from the Mooney–Rouse picture.

In relation to the motion of gel strands, orientational relaxations of DNAs and gel strands under electrophoretic conditions were examined by birefringence and linear dichlorism (LD) measurements.[17-22] These experiments demonstrated that not only migrating DNAs but also gel strands exhibit orientational relaxation.[19-22] τ_{gel} for the orientational relaxation of agarose gel with C_{gel} = 1.0 wt % was estimated to be about 10 s from the data reported by Jonsson et al.[19] On the other hand, f_M of our system with C_{gel} = 1.0 wt % shown in Figure 2B suggests τ_M (=1/($2\pi f_M$)) to be 0.05 s. τ_M and τ_{gel} appear in entirely different ranges of time scales, and thus, these phenomena are again not directly related. Presumably, some rapid processes such as fluctuation of the pore size resulting from cooperative motions of gel strands forming narrow straits for DNA migration[23] might be resonant with the electric field at f_M (which is thus much higher than that expected from fluctuation of the average pores), and such processes might contribute to the maximum in μ.

Enhancement of μ at Low Frequencies under a Low Bias Field. Another feature to be noted in the BSFGE method is that under an intermediate-to-low bias condition of $E_b < 5.0$ V cm$^{-1} \le E_s$, the low-f μ_0 values for all DNAs are higher than those in the high-f or steady field (μ_s) values (cf., Figure 2C,D). For DNAs with $M < 10$ kbp, the μ value decreases monotonously but rather sharply from the low-f plateau value (μ_0) and, beyond a certain f_T, reaches μ_s. But for DNAs longer than 10 kbp, μ decreases with increasing f from μ_0 and passes a sharp minimum at f_p and then a small maximum before finally reaching μ_s. This sharp minimum in μ at f_p will be discussed later.

First we examine the behavior of f_T, which is indicated in Figure 2C,D with arrows. As seen in these figures, the f_T values for DNAs belonging to entanglement regimes II and III seem to be independent of M but shift a little toward the higher f side with decreasing M for DNAs in Ogston regime I.

Figure 5A show a plot of log f_T versus log C_{gel} for DNAs with $M < 10$ kbp under a low bias condition of E_b = 2.5 V cm^{-1} and E_s = 7.5 V cm^{-1}. We see a rather simple power-law relationship:

$$f_T \propto C_{gel}^{-1.2\sim(-1.0)} \quad (4)$$

This C_{gel} dependence of f_T suggests that the enhancement in μ at low frequencies is somehow related to the motions of DNAs as well as of gel strands.

Figure 5B shows plots of the ratios of the low-f mobilities to the steady field mobilities ($\mu_0\mu_s^{-1}$) versus log M for DNAs in gels with varying C_{gel} under the same BSF condition shown in Figure 5A. The dotted lines again indicate SN's (the thin lines) and our (the heavy lines) regime boundaries. $\mu_0\mu_s^{-1}$ is nearly independent of M for DNAs either in Ogston regime I or in our regime III. In our regime II, however, enhancement in μ_0 becomes effective just as DNAs begin to entangle with gel strands. On the other hand, SN's boundary between regimes II and III appears not to be directly related to the changes in the $\mu_0\mu_s^{-1}$ versus log M curves. From these features, the boundaries redefined by us are likely to give much more reasonable classification than SN's boundaries.

The features of the M and C_{gel} dependences of $\mu_0\mu_s^{-1}$ in the entanglement regimes suggest that the increase in μ does not reflect the motion of the entire DNA molecule but presumably cooperative motions of some smaller portions of DNAs entangled with gel strands.

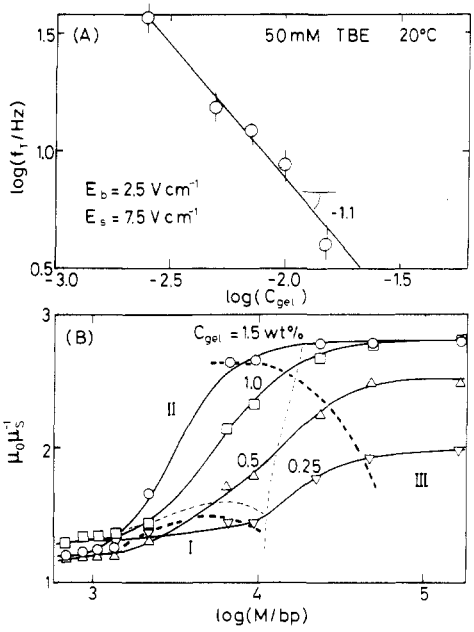

Figure 5. (A) Relationship between the frequency (f_T) of transitional increase in μ curves and the gel concentration (C_{gel}) for DNAs entangling with the gels under BSF with E_b = 2.5 and E_s = 7.5 V cm^{-1}. (B) Dependence of ratio $\mu_0\mu_s^{-1}$ of mobility (μ_0) in the low-frequency side to μ_s at the steady electric field on molecular weight (M) of DNAs under BSF with E_b = 2.5 and E_s = 7.5 V cm^{-1} in gels of various C_{gel} values.

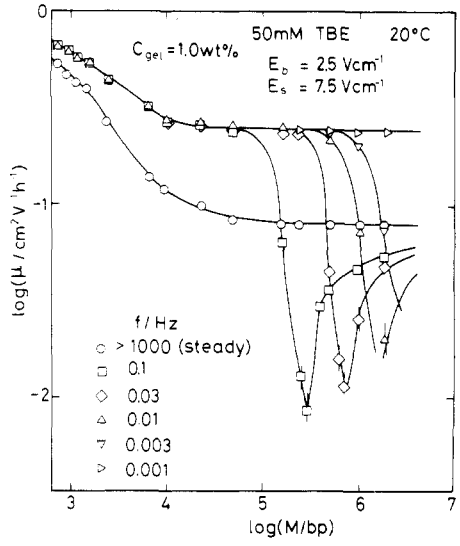

Figure 6. Relationship between the mobility (μ) and the molecular weight (M) of DNAs under BSF with E_b = 2.5 and E_s = 7.5 V cm^{-1} and with several frequencies (f) to 1000 Hz.

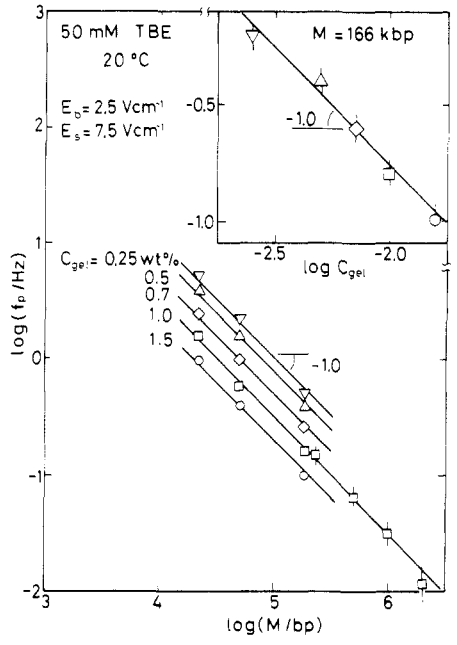

Figure 7. Relationship between the the pin-down frequency (f_p) and molecular weight (M) of DNAs under BSF with E_b = 2.5 and E_s = 7.5 V cm^{-1} in gels of various C_{gel} values. An insert shows the relation between f_p and C_{gel} for the DNA of 166 kbp under BSF with the same conditions as described in Figure 6.

In relation to the motion of entangled DNA molecules, Akerman and Jonsson carried out linear dichroism (LD) measurements on migrating DNAs of 40–166 kbp under CFGE through a gel of C_{gel} = 1.0 wt % in a buffer close to our systems. They showed that orientational response of DNAs at a pulse period shorter than 0.1 s (=10 Hz) was independent of M and coincided with that under a steady electric field.[24] They suggested that the critical time period of 0.1 s corresponds to the time scale of orientational motion of chain ends of DNAs dangling from gel network, and they estimated the size of the dangling ends to be about 4 kbp, independent of M of the whole DNA molecules. They further concluded that this value is in good agreement with that estimated from a biased reptation model.[24-31]

Interestingly, this time period of 0.1 s just corresponds to τ_T (=$(2\pi f_T)^{-1}$) of our BSFGE results carried out in a gel with the same C_{gel}. Thus, at first sight we guessed that the increase in μ at f_T might reflect the similar dynamics suggested by Akerman and Jonsson.[24] However, their idea of the motions of dangling chain ends is obviously not sufficient to understand our present observation in that even DNAs shorter than 4 kbp also exhibit enhancement in μ at the same f_T ($\simeq$10 Hz) as the DNAs longer than 4 kbp (cf., Figure 2D and 2E). In BSFGE, application of a low-f and high-E_s sinusoidal field on DNAs just entangling with gel strands appears to tend them to disentangle and accelerate their migration under the low-E_b bias field.

Minimum in μ under a Low Bias Field. In Figure 2D and 2E, we see that each of the μ versus log f curves of DNAs longer than 23.1 kbp subjected to low bias BSFGE exhibits a sharp minimum at a f_p specific to each DNA. As seen in the figures, the minimum at f_p is deeper for larger DNAs. In fact, DNAs longer than 200 kbp (yeast chromosomal DNAs) hardly migrate at the f_p (=0.01 Hz for 1.90 Mbp DNA in C_{gel} = 1.0 wt %, for example), but at a still lower and/or higher f, the DNA can migrate.

The data in Figure 2D are replotted in Figure 6 in the form of log μ versus log M at various frequencies from 1 mHz to 1 kHz and over ($f \to \infty$). We see that at a particular f_p, a particular DNA, for example, at f_p = 0.1 Hz, 166 kbp DNA, hardly migrates but all other DNAs migrate at reasonable rates. Utilizing this feature, we were able to separate two rather large-size DNAs but with a small difference in M.[32]

Figure 7 shows the molecular weight dependence of f_p in the gels with several C_{gel} values ranging from 1.5 to 0.25 wt %. An interesting finding is that, irrespective of C_{gel}, f_p is inversely proportional to M of DNAs, and for a given DNA, f_p is inversely proportional to C_{gel}. f_p is then given by

$$f_p \propto C_{gel}^{-1} M^{-1} \quad (5)$$

This result suggests that using a highly diluted gel we should be able to separate a DNA of much larger M, say 10 Mbp, by pinning it down at a reasonable frequency, say 0.01 Hz, which is readily acessible.

In entanglement regimes II and III, the molecular weight between entanglements of migrating DNAs may be roughly estimated as $M_e \propto C_{gel}^{-1}$. Equation 5 thus suggests that f_p is inversely proportional to the number of entanglement points in the migrating DNA molecules.

The minima of μ similar to those found in our systems have also been observed in CFGE[1-4,8,32] and in FIGE.[5-10,24] The phenomenon was called an antiresonance phenomenon.[5,8,10] Akerman and Jonsson[24] reported that, under CFGE, μ and the anisotropy detected by LD of a DNA exhibit such a minimum at a particular pulse period, which is roughly proportional to M. They concluded that, at this pulse period, called the resonant time, the DNA assumes a less anisotropic, nearly *coiled* conformation, and thus the minimum in μ can be ascribed to the higher friction coefficient of the coiled DNA molecules at the given pulse period than that of the same DNA molecules in stretched and anisotropic conformation at other pulse periods.[24] The pinning-down phenomena found in the present study should also have resulted from a similar mechanism: At f_p, the particular DNA molecules assume coiled conformation on the average so that they can hardly migrate.

Adolf proposed a biased reptation model for the dynamics of an entangled macromolecule in a gel under a strong steady electric field.[34] He showed that the M dependence of the apparent relaxation time for such a macromolecule of large M to renew its conformation asymtotically approaches $\tau \propto L/v$ ($\propto E^{-2}M$), where L is the contour length and v is the curvilinear velocity of the migrating chain under field E.[34]

Heller and Pohl investigated the performance of FIGE by widely varying the pulse time and the field strength,[9] and they also found that the μ of DNA exhibited a profound minimum at a particular pulse time dependent on M. Their relationship between μ and M under a field strength of 5.3 V cm^{-1} and the ratio of the forward to backward pulse of 3 shown in Figure 4 of ref 9 is interestingly very similar to our Figure 6. The mechanism of the pin-down phenomenon in our BSFGE must be essentially similar to that in FIGE.

Recently, Doi et al.[10] also reported that in their FIGE experiments the resonant time is proportional to the tube disengagement time $\tau_d = L/V_{cont}$, where V_{cont} is the velocity of DNA of contour length L migrating through the gel network along its contour under a steady continuous field. V_{cont} is nearly independent of L or M in regime III as shown in Figure 1. They conjectured on the basis of his biased reptation model that a fraction of DNA molecules in compactly coiled conformation is sharply increased when the time period of field inversion was comparable to τ_d.[10]

More recently Zimm developed a new model for FIGE of DNAs.[23] He described the gel with a chain of open space, "lakes", connected by narrow "straits", and he allowed DNA loops to overflow out from one lake through a strait to another lake under a strong electric field. His simulations on FIGE showed a very pronounced antiresonance phenomenon and semiquantitative agreement with experiments of Doi et al.[10] that the resonant time was nearly proportional to time τ_d.[23] He then concluded that the antiresonance occurred when the inversion cycle of the electric field was just matching the timing for a DNA of periodic formation and dissipation of Λ shape conformations with two arms both pulled with high tension in the direction of the field near its vertex.[23]

Adolf's[34] and Zimm's[23] predictions described above explain at least qualitatively some of the present results: the M dependence of τ_p ($=1/2\pi f_p$) in eq 5 and the bias field (E_b) dependence of τ_p seen in Figure 2D,E. These results suggest that τ_p might correspond to the relaxation time for DNA molecules to renew their conformation or the so-called biased reptation time[35] of DNA in a gel under a BSF condition different from the reptation time in a gel under no sinusoidal electric field.

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References and Notes

(1) Schwartz, D. C.; Saffran, W.; Welsh, J.; Haas, R.; Goldengeg, M.; Cantor, C. R. *Qunat. Biol.* **1983**, *47*, 189.
(2) Schwartz, D. C.; Cantor, C. R. *Cell* **1984**, *37*, 67.
(3) Chu, G.; Vollrath, D.; Davis, R. *Science* **1986**, *234*, 1582.
(4) Gardiner, K. W.; Laas, W.; Paterson, D. *Somatic Cell Molec. Genet.* **1986**, *12*, 185.
(5) Carle, G. F.; Frank, M.; Olson, M. V. *Science* **1986**, *232*, 65.
(6) Landel, M.; Noolandi, J.; Turmel, C.; Rousseau, J.; Slater, G. W. *Proc. Natl. Acad. Sci. U.S.A* **1987**, *84*, 8011.
(7) Turmel, C.; Lalande, M. *Nucleic Acid Res.* **1988**, *16*, 4727.
(8) Viovy, J. L. *Electrophoresis* **1989**, *10*, 429.
(9) Heller, C.; Pohl, F. M. *Nucleic Acid Res.* **1989**, *17*, 5989.
(10) Kobayashi, T.; Doi, M.; Makino, Y.; Ogawa, M. *Macromolecules* **1990**, *22*, 4480.
(11) Shikata, T.; Kotaka, T. *Biopolymers* **1991**, *31*, 253.
(12) Data sheet of Yeast DNA-PFGE Markers, Pharmacia LKB.
(13) Slater, G. C.; Rousseau, J.; Noolandi, J. *Biopolymers* **1988**, *27*, 509.
(14) Ogston, A. G. *Trans. Faraday Soc.* **1958**, *54*, 1754.
(15) Ferry, J. D. *Viscoelastic Properties of Polymers*, 3rd ed.; John Wiley & Sons: New York, 1980; Chapter 10.
(16) Moony, M. *J. Polym. Sci.* **1959**, *34*, 599.
(17) Chu, B.; Xu, R.; Wang, Z. *Biopolymers* **1988**, *27*, 2005.
(18) Akerman, B.; Jonsson, M.; Norden, B.; Lalande, M. *Biopolymers* **1989**, *28*, 1541.
(19) Jonnson, M.; Akerman, B.; Norden, B. *Biopolymers* **1988**, *27*, 381.
(20) Sturm, J.; Weill, G. *Phys. Rev. Lett.* **1989**, *62*, 1484.
(21) Stellwagen, N. *Biomol. Struct. Dyn.* **1985**, *3*, 299.
(22) Parus, S. J.; Shick, R. A.; Matsumura, M.; Morris, M. D. *Anal. Chem.* **1988**, *60*, 1635.
(23) Zimm, B. H. submitted to *J. Chem. Phys.* **1990**.
(24) Akerman, B.; Jonnson, M. *J. Phys. Chem.* **1990**, *94*, 3828.
(25) Lumpkin, O. J.; Zimm, B. H. *Biopolymers* **1982**, *21*, 2315.
(26) Lumpkin, O. J.; Dejardin, P.; Zimm, B. H. *Biopolymers* **1985**, *24*, 1573.
(27) Slater, G. W.; Noolandi, J. *Biopolymers* **1985**, *24*, 2181.
(28) Slater, G. W.; Noolandi, J. *Phys. Rev. Lett.* **1987**, *26*, 1579.
(29) Slater, G. W.; Rousseau, J.; Noolandi, J. *Biopolymers* **1987**, *26*, 863.
(30) Noolandi, J.; Rousseau, J.; Slater, G. W.; Turmel, C.; Lalande, M. *Phys. Rev. Lett.* **1987**, *58*, 2428.
(31) Slater, G. W.; Noolandi, J. *Electrophoresis* **1989**, *10*, 413.
(32) Shikata, T.; Kotaka, T. Unpublished data.
(33) Baase, W.; Moore, D. P.; Schellman, J. A. *Biophys. J.* **1988**, *53*, 408.
(34) Adolf, D. *Macromolecules* **1987**, *20*, 116.
(35) Doi, M.; Edwards, S. F. *The Theory of Polymer Dynamics*; Oxford University Press: Oxford, 1989.