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Electrophoresis of long DNA molecules in linear polyacrylamide solutions

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Abstract

Electrophoresis of long DNA (T4 DNA; 166 kb, S. pombe chromosomal DNA; 3–6 Mb) in linear polyacrylamide solutions was investigated by fluorescence microscopy and capillary electrophoresis. In the past studies on electrophoresis of long DNA in a polymer solution, it was reported that DNA migrates in 'U-shape conformation'. We found that at higher polymer concentrations, the shape of the migrating DNA changes from U shape to linear shape ('I-shape conformation'). In the migration mode with the I-shape conformation, the DNA moves with almost constant velocity and constant shape. However, the migration velocity does depend on the DNA size, and it is possible to separate DNAs under this I-shape motion. Actually, Mb-sized DNAs are well separated within 5 min in the region for the I-shape motion by means of capillary electrophoresis with a DC field. Considering that it takes 20 h to separate Mb-sized DNAs by standard pulsed-field gel electrophoresis (PFGE), this results will be useful for the separation of giant DNAs.

Keywords: Electrophoresis; Polymer solutions; DNA; Linear conformation; Separation of DNA; Fluorescence microscopy

1. Introduction

Electrophoresis of DNA is frequently used to separate and recover DNAs of various sizes. However, in gel electrophoresis under a steady field, the electrophoretic mobility of long DNAs (over about 30 kb) becomes independent of molecular size [1,2]. For separation of Mb-sized DNA, pulsed-field gel electrophoresis (PFGE) is currently the most powerful method [3]. However, PFGE has many problems in practical use for extremely large DNAs (more than 2 Mb), since they either fail to enter the gel, or are not separated well due to molecular trapping [4–6]. Turmel et al. [7] performed high-frequency modulated PFGE to achieve molecular detrapping and band narrowing. Viovy et al. [8] reported irreversible trapping of DNA during crossed-field gel electrophoresis. The upper limit of the separable size in PFGE is due to the molecular trapping. Duke et al. [9] reported that DNAs take a linear conformation

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under certain conditions using electrophoresis on microlithographic arrays, and discussed the possibility of solving the problem of molecular trapping by using such arrays.

On the other hand, during the last 10 years, capillary electrophoresis has developed into a powerful analytical method for separating small organic or inorganic ions, proteins and nucleic acids. During this process, it was discovered that the use of linear polymer solutions offers several advantages over gels for the separation of biopolymers [10]. Since then, several reports have described the separation of DNA fragments in a variety of linear polymer networks. DNAs of up to 23 kb have been separated using the following polymers: polyacrylamide [11,12], methyl cellulose [13], hydroxypropylmethyl cellulose [14], hydroxypropyl cellulose [15], and hydroxyethyl cellulose [16,17]. Long DNA fragments (around 20 kb) can be separated even in ultradilute solutions (0.00125% hydroxyethyl cellulose) [17]. Hubert et al. [21] presented a theory of DNA separation in ultradilute polymer solutions, based upon a model in which the DNA drags along the polymer molecules it encounters during migration. Using direct observation, Shi et al. [22] investigated the conformational dynamics of DNA in hydroxyethyl cellulose under a steady field and reported the formation and deformation of U-shaped DNA in linear polymer solutions. Carlsson et al. [23] reported the linear relationship between the migration velocity of DNA and electric field strength in 0.1% polyacrylamide solution. Recently, we demonstrated the separation of rather long DNAs of up to 166 kb in concentrated polymer solution (PA7%T0%C) under a steady field, and reported the linear conformation of DNA based on direct observation with fluorescence microscopy [24]. In addition to these studies, some authors reported that Mb-sized DNAs can be separated in linear polymer solutions. Guszczynski et al. [18] separated S. pombe chromosomal DNAs within 17 min in 0.9% polyacrylamide under a steady field. Sudor and Novotny [19] separated DNA as long as 1 Mb in 180 min in 0.4% acrylamide solutions under a pulsed field, while Kim and Morris [20] reported the separation of DNA as large as 1.6 Mb in ultradilute (0.002%–0.004%) mixed hydroxyethyl cellulose/polyethylene oxide solutions, in only 4 min under a pulsed field.

In this study, we reported on the dynamical behavior of long DNA in linear polyacrylamide solutions under a steady electric field, by direct observation with fluorescence microscopy. Based on the dynamics of individual DNA chains, we separated S. pombe chromosomal DNAs in linear polyacrylamide solutions by capillary electrophoresis under a steady field.

2. Materials and methods

2.1. Materials

Polyacrylamide (PA), available as a 10% aqueous solution from Tokyo Chemical Industry, was dissolved in 0.5 TBE buffer solution (45 mM Tris–borate, 1.25 mM EDTA) to a desired concentration. The molecular weight of this PA is 7×10^5–10×10^5, and the contour length of this PA is in the order of 1 μm, which is sufficiently long to be entangled with DNA chains [21]. The overlap concentration $C*$ was estimated to be ca. 0.7% (w/w) from the reflection point in the concentration dependency of the viscosity. The mesh size of 7% PA is believed to be around 20 Å [25]. It is to be noted that this size is extremely small compared with the pore size of 1% agarose gel, ca. 1000 Å [26], and also with the typical persistence length of double-stranded DNA, ca. 600 Å [27,28].

For direct observation of individual DNAs, T4 DNA (166 kb, Nippon Gene) solution, stained with the fluorescent dye YOYO-1 (Molecular Probes), and S. pombe chromosomal DNA (3.5 Mb, 4.6 Mb and 5.5 Mb, FMC and ATTO), stained with the fluorescent dye YO-PRO (Molecular Probes), were introduced into a PA solution containing 4% (v/v) 2-mercaptoethanol, 2.3 mg/ml glucose, 0.1 mg/ml glucose oxidase, and 0.018 mg/ml catalase [29].

2.2. Methods

2.2.1. Direct observation of the conformational dynamics of DNA

Fig. 1 shows a schematic diagram of the fluorescence microscopy apparatus used for the direct observation [30]. We placed an electrophoretic cell on top of the stage of a fluorescence microscope, the cell is shown in the figure as a broken-line box, the distance between the pair of monitor electrodes was

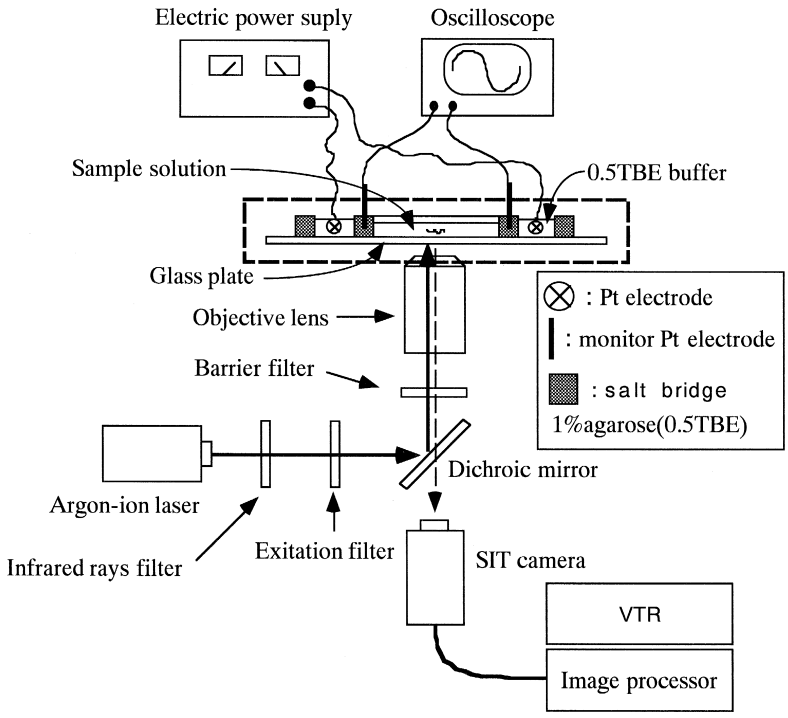


Fig. 1. Schematic diagram of a fluorescence microscope equipped with a miniature electrophoresis apparatus (shown in the box with a broken line).

20 mm, and the width of the aqueous layer was ca. 20 μm.

PA solution containing DNA/dye complex was put between the glass plates. The surface of the glass plate was coated with PA to prevent electro-osmotic flow [31]. Agarose gel (1% in 0.5 TBE buffer) was used for the salt bridge. The complex of DNA and dye was excited at 488 nm using an argon-ion laser (NEC, GLG3070). Fluorescence images were observed with a microscope (Carl Zeiss, Axiovert 135 TV) and recorded on VTR using an SIT camera and an image processor (Hamamatsu Photonics, ARGUS 20). The position of the center of mass and the size of the DNA during migration were evaluated using an image-processor (Library, HIMAWARY).

2.2.2. Capillary electrophoresis

Capillary electrophoretic separations were carried out using a Hewlett–Packard HP^{3D}CE capillary electrophoresis system. Fused-silica capillaries (360 μm o.d., 100 μm i.d.; Polymicro Technologies) with effective length of 8.5 cm and total length of 33.5 cm were used. PA10%T0%C in 0.5 TBE buffer was polymerized in the capillary [32]. The capillary was kept at room temperature for one day. By-products of polymerization were then removed by pre-electrophoresis. Each capillary was checked by making a current–voltage plot, before the use for separation of DNA fragments.

S. pombe chromosomal DNA was obtained as a 1% InCert agarose plug. The plug was cut into a piece of ca. 3 mm $\times$ 4 mm and melted at about 70°C for 5 min in water [18,22]. The DNA sample was then injected electrically (13 kV, 10 s) into the capillary. The DNA was detected at 260 nm.

3. Results

3.1. Conformational transition of long DNA in linear polymer solutions

3.1.1. U-shape motion

Fig. 2a shows typical fluorescence microscope images of a single T4 DNA in a PA 3% (w/w)

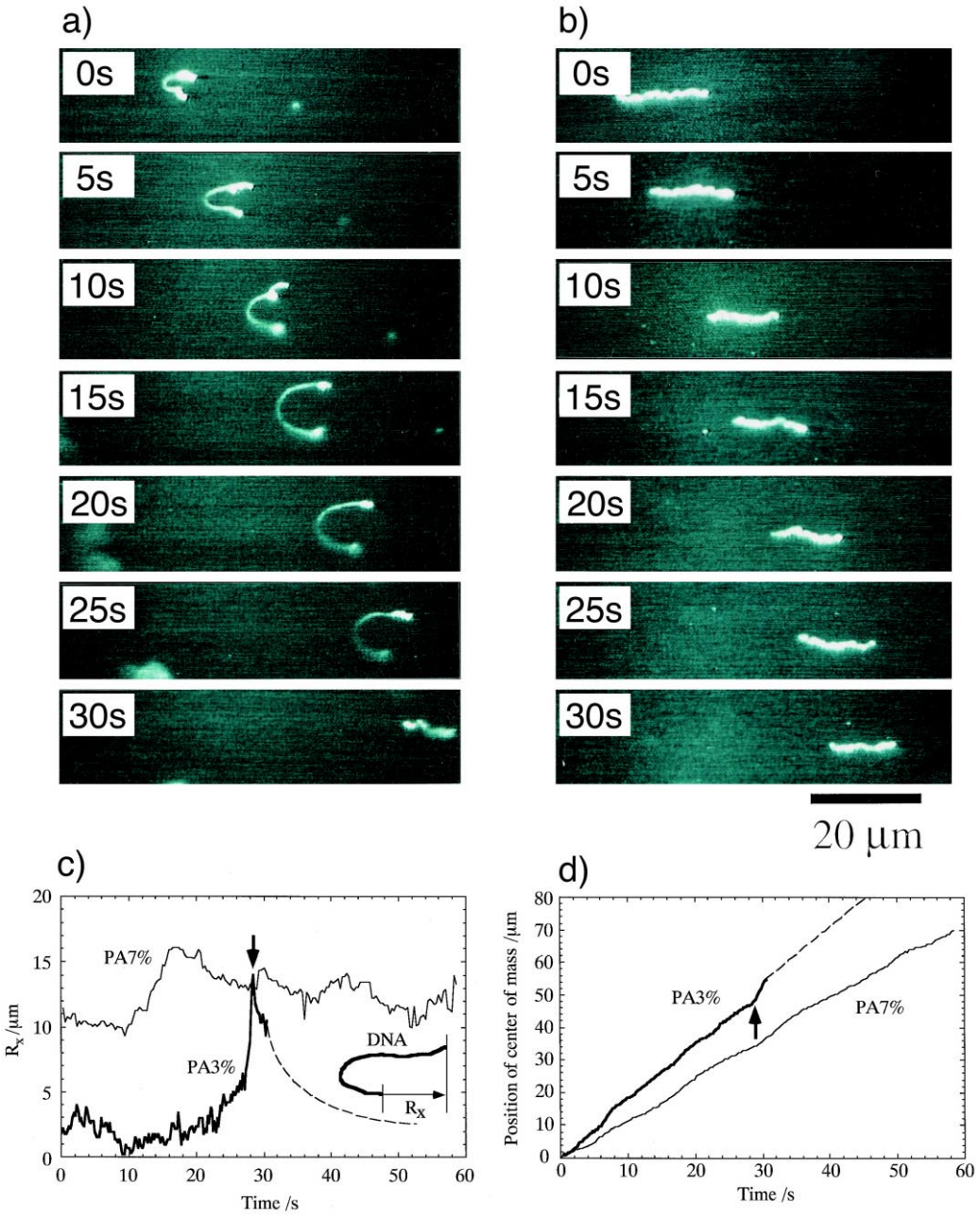


Fig. 2. Fluorescence images of the electrophoretic motion of T4 DNA in linear polyacrylamide solutions. (a) U-shape motion at 3% (w/w) PA. Photographs are taken at 5-s intervals (top to bottom). (b) I-shape motion at 7% (w/w) PA. Photographs are taken at 5-s intervals (top to bottom). We confirmed that the DNA chain retains the I shape during electrophoresis. (c) Time traces of the *x*-axis component R_x of end-to-end distance R, where the *x*-axis is parallel to the electric field. The thick line and thin line represent the U-shape motion in (a) and the I-shape motion in (b), respectively. The rather sharp maximum indicated by an arrow in the case of PA 3% corresponds to the abrupt increase in the migration velocity indicated by an arrow in (d), suggesting that the DNA chain is accelerated as a result of the release of the dragged polymers. (d) Time traces of the center of mass of T4 DNA in 3% (w/w) PA and 7% (w/w) PA. The electric field strength is 10 V/cm. The PA concentrations are 3% (w/w) and 7% (w/w). The broken lines in PA 3% (w/w) are the expected curves.

solution under a steady electric field of 10 V/cm, indicating the growing process and subsequent collapsing process of a characteristic U-shape. At this PA concentration, the characteristic U-shape or J-shape conformation is found. In this article, we call this mode of migration 'U-shape motion' and the PA concentration at which the U-shape motion is observed is referred to as the 'U-shape region'. The apex of the U-shape moves toward the positive electric potential in the polymer solution. This suggests that host-polymer chains would be dragged along by the migrating DNA [21,22], regardless of the overlap between the host polymers.

Fig. 2c shows the time course of R_x (x-component of the end-to-end distance of DNA, where the x-axis is parallel to the external electric field), indicating a rather sharp maximum (pointed in Fig. 2c by an arrow) in the case of PA 3%. As shown in Fig. 2d, the maximum R_x corresponds to an abrupt increase (pointed in Fig. 2d by an arrow) in the migration velocity V_x (x-component of the velocity of the center of mass of DNA), suggesting that the DNA chain is accelerated when it releases the polymers that is dragged along. However, as shown in Fig. 2d, the change in the migration velocity in PA 3% is not as remarkable as that reported elsewhere in agarose gel [30,33–35].

Fig. 3a shows that T4 DNA assumes transiently a linear conformation in the U-shape region at PA 3%. The arrows indicate the positions where the density of DNA segments is high. It can be noticed that these points with high segment density move accompanied by the translational motion of the entire DNA chain for a while (about 5 s), and then tend to collapse. The duration of the appearance of the linear conformation is quite short at this concentration.

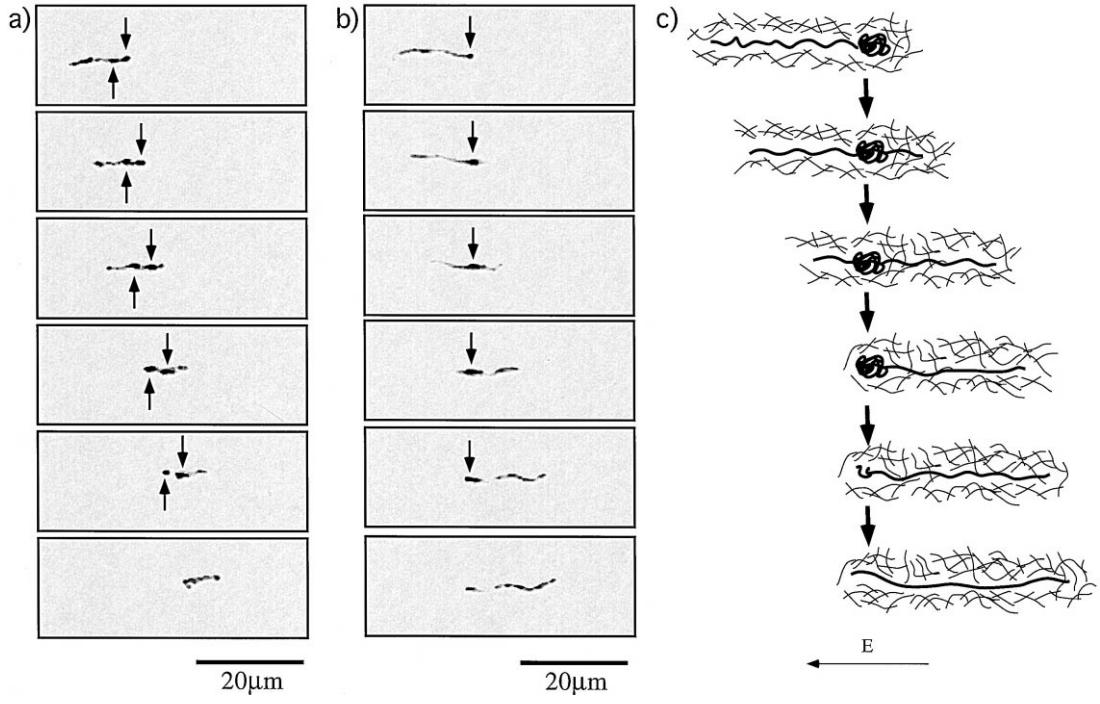


Fig. 3. Fluorescence images indicating the motion of high segment density regions on T4 DNA in PA 3% and PA 7% solutions. Photographs are taken at 0.5-s intervals (top to bottom) and inverted (positive-to-negative) to show segment density. The arrows indicate regions with high segment density. (a) In PA 3%, regions with high segment density move with translational motion of the entire DNA chain. The final image shows 'I shape' deformed by the migration of high segment density regions. After this state, DNA assumes an U shape. The electric field strength is 20 V/cm. (b) In PA 7%, regions with high segment density remain at the same positions, regardless of the translational motion of the entire DNA chain. The electric field strength is 40 V/cm. (c) Schematic diagram of regions with high segment density in PA 7%.

Let us summarize the main aspects of U-shape motion.

In the U-shape region (see, e.g., Fig. 5a), the conformation of a DNA chain exhibits marked temporal fluctuation during electrophoretic motion. The conformational behavior is characterized by the cycle of the following steps: (i) Whole DNA migrates in a somewhat shrunken conformation, or transiently in a linear conformation, (ii) host-polymer chains tend to concentrate around a certain position on the migrating DNA, as a result, the DNA starts to bend, hooking the host-polymer chains over, (iii) the characteristic U shape develops gradually, (iv) one arm of the U shape pulls another to give the J-shape conformation, and (v) the DNA chain finally releases the hanging polymers, which causes an increase in the velocity of the center of mass. Steps (i)–(v) are repeated. It is noted that the apex of the U shape moves from steps (i) to (v) in a manner similar to U-shape motion in dilute solution [22]. This is markedly different from the U or V shape of DNA formed during gel electrophoresis, although the patterns of the conformational change resemble each other [30,33–35]. In addition to this, the acceleration in the motion of center of mass at step (v) is smaller in more concentrated linear polymer solutions than it is in dilute polymer solutions and in gels.

3.1.2. I-shape motion

Fig. 2b shows electrophoretic motion of T4 DNA in a more concentrated PA solution of 7% (w/w) under 10 V/cm. The figure indicates that a long DNA chain moves with linear I-shape conformation, and never exchanges its leading and trailing end. Therefore, we call this type of migration 'I-shape motion' and the PA concentration at which the U-shape motion is never observed is referred to as the 'I-shape region'. As shown in Fig. 2c and d, in the I-shape motion, the time-dependent changes in R_x and in the position of the center of mass are simpler and more stable than those in the U-shape motion. The U shape does not appear at this concentration, even at the lowest electric field of our experimental conditions, 10 V/cm.

In Fig. 3b, note that the region of high segment density does not move, regardless of the translational motion of the DNA chain itself. In Fig. 3c, this situation is shown schematically: the PA network entangled around DNA stays at a certain position because of the strong overlap between the PA chains themselves. Larger obstacles than the mesh size of the PA network, 20, cannot move through the network.

Fig. 4a shows the effects of electric field strength on the end-to-end distance in the I-shape motion. When the field is higher than 25 V/cm, the average end-to-end distance $\langle R_x \rangle$ becomes independent of the field strength reaching about 16 μm. Since the

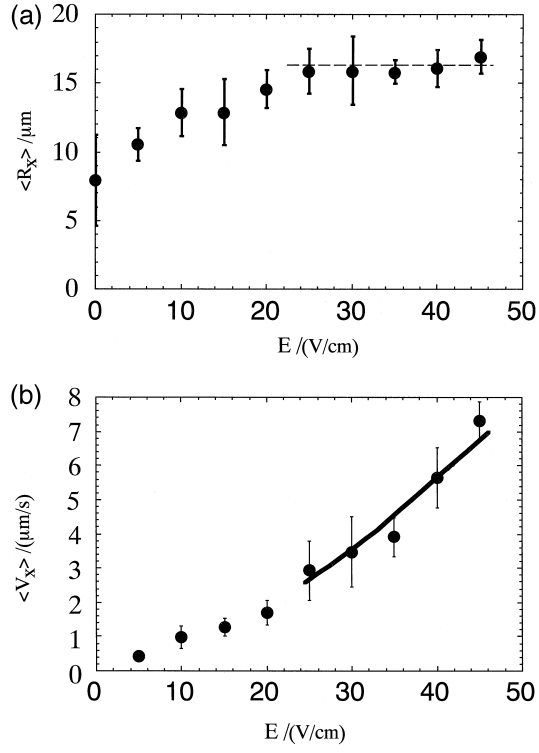


Fig. 4. Effects of the electric field on the size and migration velocity of T4 DNA in the I-shape motion. (a) Effect of the electric field on the average end-to-end distance $\langle R_x \rangle$. With an increase in the electric field, $\langle R_x \rangle$ tends to approach an asymptotic value, ca. 16 μm, which is much shorter than the natural contour length of T4 DNA (55 μm) [36]. This indicates that the DNA chain is not fully stretched in the I shape when the PA concentration is 7% (w/w). (b) Effect of the electric field on the average migration velocity $\langle V_x \rangle$. The fitted curve is calculated by least squares method in the region greater than 25 V/cm where $\langle R_x \rangle$ becomes independent of the electric field, E. In this region, the average migration velocity $\langle V_x \rangle$ scales to $E^{1.6}$. Whereas, in the region smaller than 25 V/cm, the migration velocity scales to $E^{1.0}$. The averages were calculated for approximately 20 molecules and the time-averages for about 7 s were calculated for each molecule.

natural contour length of T4 DNA is about 55 μm [36], this indicates that the DNA chain is not fully stretched even under the strongest electric field of our experimental conditions, 45 V/cm. In relation to such experimental behavior, it has been reported that long DNAs can get fully stretched in suitable conditions under an AC electric field but they cannot under a DC field [37]. As will be shown in Section 3.2, we have found that the separation of long DNAs is attained with capillary electrophoresis when $\langle R_x \rangle$ is independent of the field strength.

Fig. 4b shows the effects of the electric field strength on the average migration velocity $\langle V_x \rangle$. The fitted curve in the figure is calculated by the least squares method for the region where $\langle R_x \rangle$ becomes independent of the electric field E, i.e., over 25 V/cm. In this region, the average migration velocity scales to $E^{1.6}$. Whereas, below 25 V/cm, the average migration velocity scales to $E^{1.0}$.

3.1.3. Transition region

Generation of the I-shape motion mainly depends on the PA concentration, and is independent of the electric field strength. Fig. 5a shows the regions of both U-shape motion and I-shape motion in T4 DNA. The broken line around PA 5% indicates the transition region. Below PA 4.5%, DNA chains exhibit the typical U-shape behavior, as shown in Fig. 2a. Around PA 4.5%, the shrunken J shape appears without the formation of the complete U shape [22].

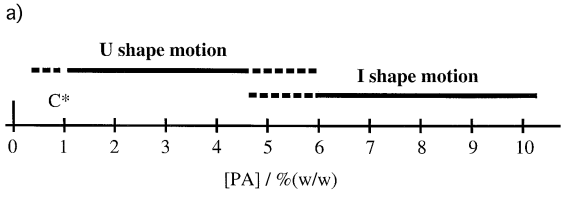
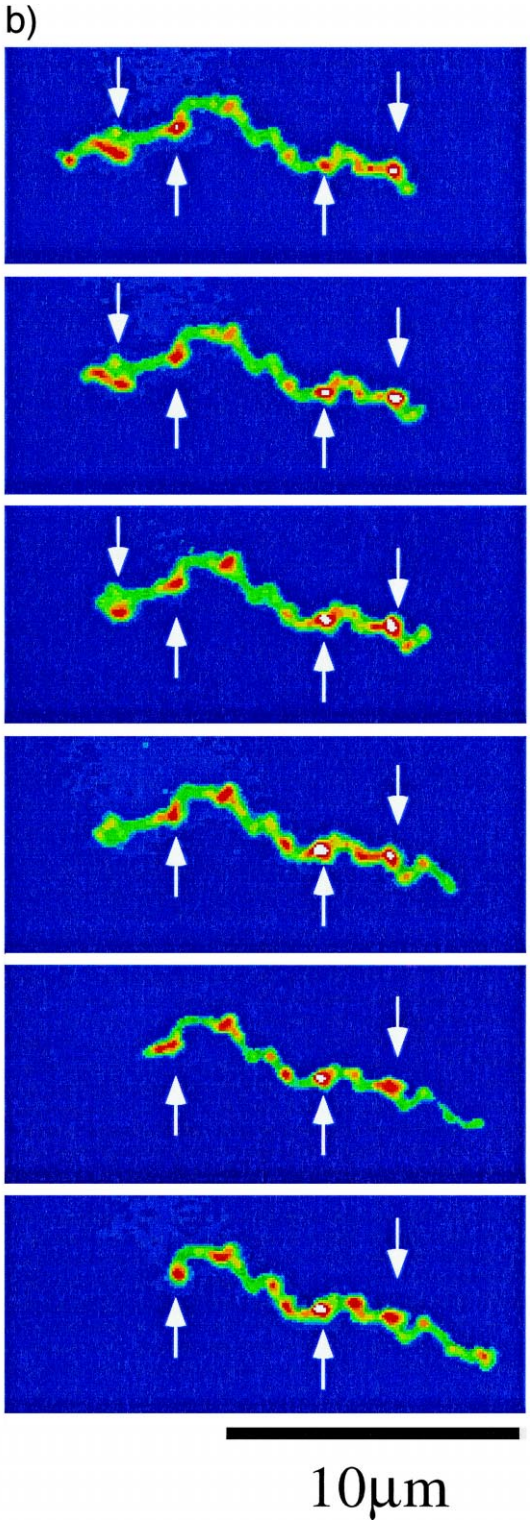


Fig. 5. Observation of T4 DNA around the transition region between the U-shape motion and the I-shape motion. (a) Diagram of the U-shape motion and the I-shape motion. $C*$ is the entanglement threshold, and is estimated to be ca. 0.7%. Broken lines indicate a 'transition region'. Around 5% PA (w/w), the I shape has significant spatial fluctuation of segment density as shown in (b). However, in this region, the U shape is never observed and a J shape is rarely observed. (b) I-shape motion in the transition region. It's spatial fluctuation of segment density is very large. Photographs are taken at 1-s intervals (top to bottom). The arrows indicate the regions with high segment density. These regions remain at the same positions, regardless of the translational motion of the entire DNA chain.



Around PA 5%, individual DNAs exhibit the I-shape motion. As shown in Fig. 5b, significant spatial fluctuation of the segment density is observed under this condition. The regions with high segment density are fixed at certain positions in the PA solution, regardless of the translational motion of the entire chain. This implies that in the I-shape region, PA chains remain at fixed positions despite the fact of being pushed by DNA segments, and indicates strong entanglement among PA polymers. Therefore, we suggest that the degree of the entanglement of PA chains determines the migration mode of T4 DNA. Near PA 7%, DNA chains exhibit the I-shape motion. However, fragmented DNAs with contour lengths shorter than about 5 μm show the U-shape motion under the I-shape conditions for DNAs with contour lengths of 55 μm (data not shown). In addition, the I-shape motion of elongated long DNA is observed in other concentrated polymer solutions such as methyl cellulose and hydroxyethyl cellulose (data not shown).

3.2. Capillary electrophoresis

As we reported previously [24], separation of DNAs by size can be achieved in the I-shape region by means of capillary electrophoresis. Since long DNA is never trapped with cross-links in linear polymer solutions, electrophoresis in linear polymer solutions is expected to be useful for the separation of Mb-sized DNA. Therefore, we performed capillary electrophoresis of Mb-sized DNAs in the U-shape region and the I-shape region.

Fig. 6a shows the results of capillary electrophoresis on T4 dc + T4 dc/Bgl I digest (upper) and on S. pombe chromosomal DNA (lower) in the I-shape region under a constant DC field, together with the results with S. pombe chromosomal DNA in the U-shape region (inset). Note that the separation of S. pombe is achieved in several minutes. This speed is comparable to that reported earlier using more dilute solution [18]. However, the present separation technique in the I-shape region gives a much

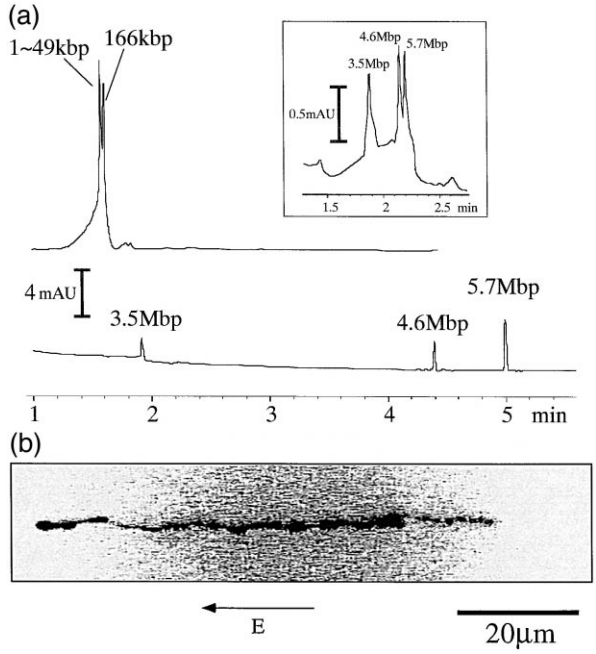


Fig. 6. Electrophoretic separation of S. pombe chromosomal DNA. (a) Capillary electropherogram of T4dc + T4dc/Bgl I digest (upper) and S. pombe chromosomal DNA (lower). The inset electropherogram shows the separation of S. pombe chromosomal DNA in 1% PA (similar to the conditions reported by Guszcynski et al. [18]). The electric field strength was 400 V/cm. (b) A fluorescence microgram, indicating that the I-shape motion is generated for Mb-sized S. pombe chromosomal DNA stained by YO-PRO in PA10%T0%C under an electric field of 10 V/cm.

better resolution. The resolutions in the separation of the three components of S. pombe are $R_{12} = 2$ and $R_{23} = 1$ in PA 1%, and $R_{12} = 69$ and $R_{23} = 20$ in PA 10%, respectively, where $R_{ij} = 2(t_j - t_i)/(W_j + W_i)$ $R_{ij} = 2(t_j - t_i)/(w_j + w_i)$; t_i and t_j are migration times, and w_i and w_j are the bandwidths of each component. In addition, the baseline in electrophoresis is more stable at a high polymer concentration.

Fig. 6b shows the I-shape motion of Mb-sized DNA in a concentrated PA solution. The I-shape of Mb-sized DNA has many number of 'nodes', (regions with high segment density). The number and size of the 'nodes' are expected to be dependent on the natural contour length of DNA and the strength of the external field. Here, it may be important to notice that the sizes of the 'nodes' are much larger than the mesh size of PA.

4. Discussion

4.1. Formation of U shape

In low-molecular-weight viscous solvents, such as saccharose or glycerol, DNA never forms U shape or I shape under electrophoresis. However, in polymer solutions, even in ultradilute solutions, DNA can assume an U-shape conformation. This suggests that entanglement plays a very important role under a strong external field. In this subsection, we discuss the mechanism of the formation of the U shape.

Hubert et al. [21] represented the mean lifetime of interaction between DNA and polymer in ultradilute solutions as:

$$\tau = \left(\frac{1}{\tau_p} + \frac{1}{\tau_{DNA}} \right)^{-1}$$

where τ_p is the time required for the polymer to disengage from the DNA (see Fig. 7a), and τ_{DNA} is the time required for the DNA to disengage from the polymer (see Fig. 7b). To explain the formation of the U shape, we introduce the notion that the lifetime of interaction between DNA and polymer varies along the DNA chain. Fig. 7c shows that the polymers that are dragged near both ends of DNA can escape from the DNA with a shorter lifetime than τ_p and τ_{DNA}. Thus, the apparent friction coefficients of DNA are smaller near the ends of the DNA. Consequently, the U shape is repeatedly reproduced because the ends tend to migrate faster than the central region.

4.2. Transition from U-shape motion to I-shape motion

In Section 3.1.1, where the motion of U shape was discussed, we mentioned that dragged PA chains migrate with DNA, due to the movement of the apex

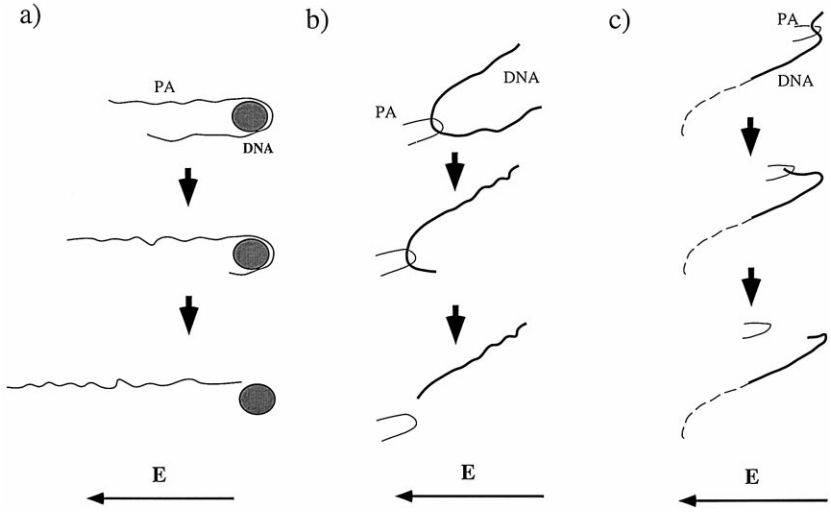


Fig. 7. Schematic representation of the time-dependent changes in the interaction between the PA network and DNA. (a) Disengaging of PA chain from DNA [21]. (b) Disengaging of DNA from PA chain [21]. (c) Escaping of PA chain from near the end of DNA.

and the high segment density regions of the DNA in the U-shape region. On the other hand, in Section 3.1.2, where we discussed the I-shape motion, we noted that PA chains cannot migrate with DNA because of the strong overlap between PA chains themselves. The high segment density regions along DNA do not move in the I-shape region, regardless of the translational motion of the entire DNA chain. Therefore, we suggest that the transition from the U-shape motion to the I-shape motion is induced by a change in the degree of entanglement in the PA network. If this speculation is correct, it indicates that the PA concentration of the transition from U-shape region to I-shape region might be related to the polymerization degree of PA chain.

An important open question for the future research is whether the I-shape motion is characteristic in concentrated linear polymer solutions, or not. Until now, tube like motion such as the I-shape motion has never been reported in any gel matrix. To make clear this point, it is necessary to perform experiments using cross-linked acrylamide gel with the same mesh size that we use in this article.

4.3. Separation of long DNA in I-shape motion

At present, we have no conclusive idea about separation mechanism in the I-shape motion. It seems that current theories cannot predict the possibility of separation of long DNAs with linear conformation [38–40]. Contrary to our study on the I-shape motion, most of the past experiments [10,18], have never been performed under extreme conditions (large DNAs (3.5 Mb), such as highly concentrated linear polymer solutions (PA 10%), and high voltage (400 V/cm)). In order to develop the theory of the I-shape motion, systematic experimental studies together with numerical simulations are necessary. For the convenience of further theoretical interpretation, we would like to point out some experimental aspects on the I-shape motion in relation to electrophoretic separation.

In our previous work, the separation of T4 dc (166 kb) + T4 dc/Bgl I digest (1.26 kb–49.31 kb) was performed under 75 V/cm [24]. In contrast to this, as shown in Fig. 6a, T4 dc/Bgl I digest (1.26 kb–49.31 kb) cannot be separated under 400 V/cm, which is suitable for the separation of S. pombe chromosomal DNAs (3.5 Mb–5.7 Mb). Further more, complete separation of λ-*Hin*dIII digest (125 bp–23.13 kb) is attained in the U-shape region but not in the I-shape region [41]. Consequently, electrophoresis in the I-shape region is suitable for separation of long DNA sample with large size differences, such as chromosomal DNAs. Moreover, high electric fields result in better separation of large DNAs in concentrated linear polymer solutions. Low electric fields yield band broadening and no distinguished signals (data not shown).

As shown in Fig. 6b, the migrating size of Mb-sized DNA is compatible to the inner diameter of capillary used. Thus, the capillary wall effect may be significant in case of large DNA like S. pombe and may affect the resolution in the separation process. In order to investigate this wall effect, it is necessary to perform capillary electrophoresis using more narrow capillary tubes [42].

Although we examined the current–voltage plot of capillary before each experiment of electrophoresis, the degree of separation was dependent on the capillary tubes used. Then, efforts on the optimization of the experimental conditions are expected, including the parameters of the precoating of the inner wall, polymerization reaction, and so on.

4.4. Possibility of macroscopic electrophoresis

As has been described in Section 4.3, we have succeeded in the separation of Mb-sized DNAs with high speed and high resolution in capillary electrophoresis. Although this result implies the possibility of high speed separation for more large-sized DNAs, it is rather difficult to recover large amounts of DNA by conventional capillary electrophoresis. However, it is also difficult to separate extremely long DNA of more than 2 Mb by pulsed-field gel electrophoresis (PFGE), in spite of the usefulness of PFGE to separate and recover long DNAs. For example, irreversible trapping of long DNA in gel fibers causes the migration velocity to become almost zero or causes bands to be broad [7,8]. In contrast, in the case of electrophoresis in concentrated linear polymer solutions, DNA motion becomes simple and stable. As shown in Fig. 6b, long DNA even with Mb-size exhibits a simple I-shape motion, and trapping of Mb-sized DNA has not been

encountered in the I-shape region. In relation to this, under 120° crossed-fields electrophoresis in concentrated linear polymer solutions, it has been reported that DNA shows simple orientation [43].

Consequently, we suggest that electrophoresis in the I-shape region offers great potential for the separation and recovery of Mb-sized DNA.

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