

SHORT COMMUNICATIONS

Stretching of Long DNA under Alternating Current Electric Fields in a Concentrated Polymer Solution

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Long double-stranded DNA with the size over several kilo base pairs (kbp) exhibits random coil conformation in aqueous solutions.¹ Stretching long DNA is interesting from the both points of view of fundamental problems in polymer physics and applications to molecular biology. In the previous methods,^{2–4} DNA was stretched by some external forces supplied by optical tweezers, solvent flow and electric field, etc. In these methods, one or both ends were anchored on a certain object such as gel, glass plates and latex beads. On the other hand, Washizu *et al.*⁵ stretched DNA without anchoring it. They placed DNA in aqueous solution and applied a high-frequency alternating current (AC) field of *ca.* 1 MHz. This method has been applied to examine the sliding behavior of ribonucleic acid (RNA) polymerase along long DNA chain.⁶ In the present report, we will propose a new method to stretch long DNA by use of entanglement effect between DNA chain and host polymers. We placed DNA in a concentrated polymer solution and applied an AC field with the frequency of *ca.* 10 Hz. We found that the DNA is completely stretched out under proper condition.

EXPERIMENTAL

As a host polymer solution, linear polyacrylamide (PA) (MW = 7×10^5 – 10×10^5 , 10% in water, Tokyo Chemical Industry Co., Ltd.) was diluted with a TBE buffer solution (the final concentration: 45 mM Tris–borate, 1.25 mM ethylenediaminetetraacetic acid (EDTA), pH 8.3). The contour length of this PA is of the order of 1 μm , which implies that the PA has enough length to entangle with DNA chain. Overlapping threshold C^* (in 0.5 TBE buffer) of this PA was estimated to be *ca.* 0.7% from a viscosity measurement. T4 DNA (166 kbp, Nippon Gene) stained with a fluorescence dye, YOYO-1 (Molecular Probes, Inc.), was mixed with a PA solution (in 0.5 TBE buffer) including 4% (v/v) 2-mercaptoethanol, 2.3 mg ml⁻¹ glucose, 0.1 mg ml⁻¹ glucose oxidase, and 0.018 mg ml⁻¹ catalase,⁷ and this solution was injected between two glass plates. The glass plates were coated with PA in order to prevent electroosmotic flow.⁸ Thickness of the sample solution was adjusted to 25 μm with a spacer (Diafoil, Diafoil Hoechst Co., Ltd.). Other

experimental apparatus is similar to that in the previous work.⁹

RESULTS AND DISCUSSION

In our previous work,^{10–12} we reported that long DNA chain migrates through a concentrated PA solution with linear conformation under direct current (DC) electric fields. It has been noted that under DC electric fields the maximum length of the linear conformation was much shorter than the contour length of the DNA. In the case of T4 DNA, the natural contour length is 55 μm and the maximum length of DNA in the linear conformation was found to be *ca.* 16 μm in a 7% PA solution under a DC field of 45 V cm⁻¹. In this study, we report the stretching behavior of T4 DNA under AC electric fields.

Figure 1a shows the fluorescence images of stretching T4 DNA, where an AC field with the frequency of 10 Hz and with the amplitude of 100 V cm⁻¹ is applied at time 0 s. The solution is a 7% PA solution. Figure 1b shows the change in the maximum length (R_1) of the DNA. As shown in Figure 1b, R_1 increases gradually and reaches an asymptotic limit after *ca.* 60 s, where the value of R_1 fluctuates around 21 μm . This length is almost a half value of the natural contour length of T4 DNA. Note that this is not a completely stretched conformation of DNA. With closer inspection, we found that such a DNA is bent like a hair-pin, as drawn schematically in Figure 1b. We found that DNA is occasionally trapped in such a bent conformation. In order to avoid the DNA trapped in such a state, we gradually changed the frequency of the electric field from 0.1 to 6 Hz. As shown in Figure 2, the DNA can be fully stretched by this method. Moreover, after being stretched, DNAs remain in the fully-stretched state under the electric field of 6 Hz.

We conjecture that the stretching is due to entanglement effect between host polymers and DNA, based on the following two experiments.

First, we never observed the stretching in the solution of low molecular weight solvent such as saccharose solution. Figure 3 shows the conformation of DNA in 58% saccharose solution under an AC field of 6 Hz; the solution is quit viscous (72.3 cP). The DNA takes a

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random coil conformation. In contrast to this, with a 3% PA solution, which has almost the same viscosity as the 58% saccharose solution, we observed that DNAs are stretched. However, in dilute PA concentration regions under 1%, we do not observe the stretching.

Second, we found that the frequency is essential to obtain the fully-stretched conformation. In a 7% PA solution, we have noticed that DNA is never stretched

under the frequency lower than 3 Hz, as shown in Figure 4a. Shi *et al.*¹³ reported the similar observation which DNA takes the intermediate size between full extension and random coil under a low frequency AC field in hydroxyethyl cellulose solutions. On the other hand, when the frequency was increased from 6 to 100 Hz in a 7% PA, the stretched DNA tended to be relaxed as shown in Figure 4b. Full extension can be obtained only in the frequency region around 10 Hz. In addition to this, segregation of DNA molecules was never observed in PA solutions.¹⁴

In the case of a 7% PA solution, the mesh size of entanglement networks is *ca.* 20 Å,¹⁵ which is the same order of magnitude as the diameter of DNA. Thus, it is expected that DNA is enclosed by the tube of entangled PA with a diameter of *ca.* 20 Å (see Figures 5a and 5b). Although the segments of DNA can easily move through this tube, they can hardly move out from this tube because of topological restriction. However, in order to be stretched, the DNA has to escape from the old tube enclosing the DNA in a random coil state, which has many bending points.

Figure 5a schematically explains the mechanism of stretching. We consider a situation that the DNA is bent at a point as shown at the top of Figures 5a and 5b. Figure 5a shows what happens when the electric field is applied towards the apex of the bent DNA. In this case, the two tails of the DNA move with almost the same speed, and consequently, the end-to-end distance L_1 of the DNA after a time interval is almost the same as the original end-to-end distance L_0 . On the other hand, if the electric force is applied in the opposite direction, the movement of the two tails competes with each other. The DNA is then stretched and the end-to-end length L_2 becomes longer than the original length L_0 . Therefore, if the above two processes are repeated periodically, the DNA is gradually stretched.

If the above-mentioned effect on the stretching is correct, we can expect that the stretching will be achieved, for the case of much longer DNA, for example Mbp-sized DNA with contour length on the order of mm. With this method, it may become possible to visualize restriction

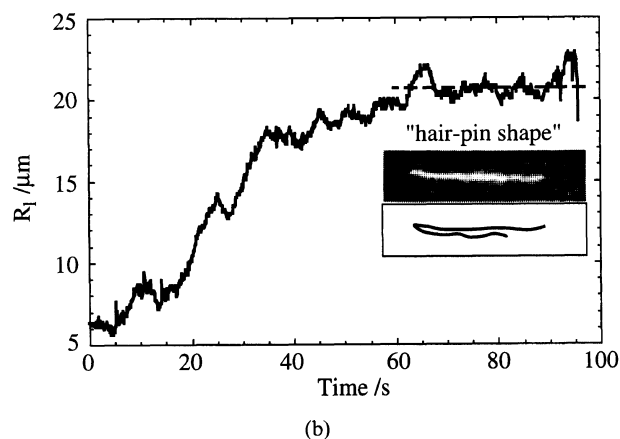
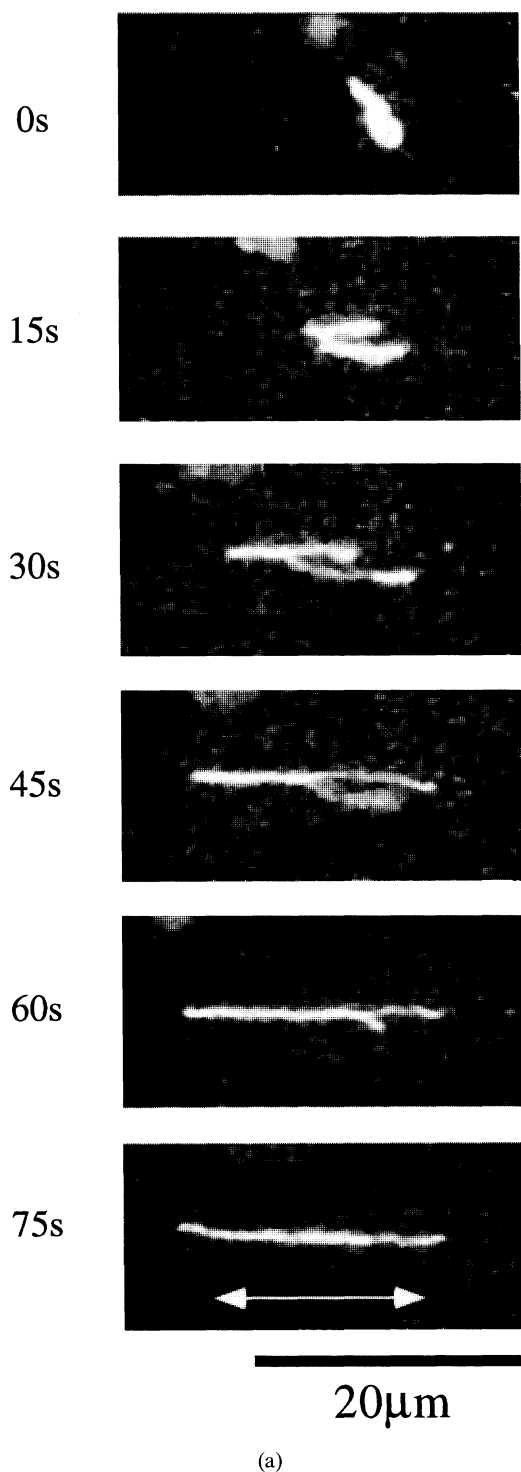


Figure 1. Visualization of the stretching process of a T4 DNA in a concentrated PA solution under AC electric field. a: Fluorescence images of a T4 DNA under AC field: Photographs are arranged at 15-s intervals (top to bottom). Electric field with 10 Hz was switched on at 0 s to random coiled dsDNA. b: Increase of the maximum length R_1 of DNA after the application of the AC electric field. R_1 increases asymptotically to *ca.* 21 μm , which correspond to a bent conformation. Although T4 DNA has a native contour length of 55 μm , it is somewhat extended by intercalation of fluorescence dye, YOYO-1. PA concentration is 7% (w/w) in 0.5 TBE buffer. Electric field is 10 Hz in frequency, 100 V cm^{-1} in amplitude. A white arrow in the photograph indicates electric field direction.

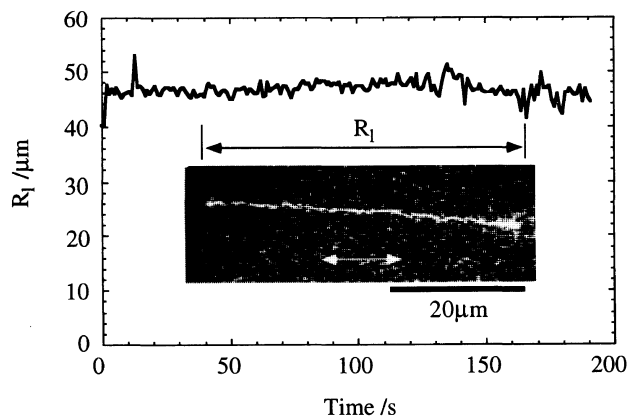


Figure 2. With the application of AC electric field with 6 Hz, T4 DNA is fully stretched. In this experiment, in order to avoid the trapping in a bent conformation, the frequency of electric field was changed from 0.1 Hz to 6 Hz. The fully-stretched conformation is maintained as long as the application of the electric field.

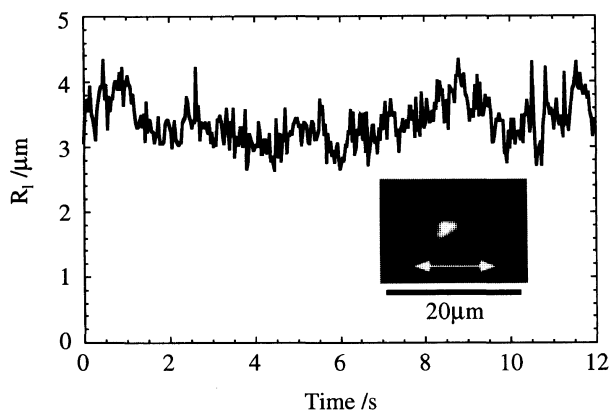


Figure 3. Time-trace of R_1 in a T4 DNA chain under an AC electric field of 6 Hz in 0.5 TBE buffer solution containing 58% (w/w) saccharose. Other experimental conditions are the same as in Figure 2.

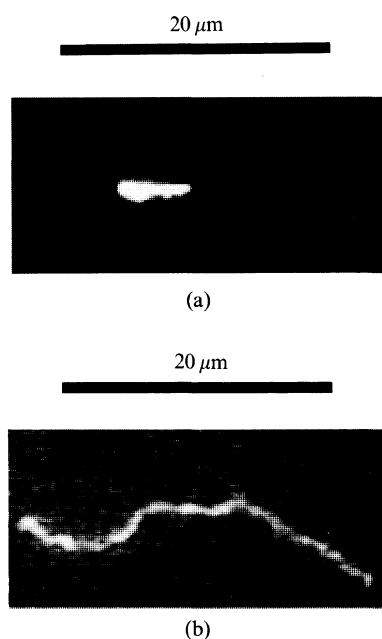


Figure 4. T4 DNA in a 7% PA solution with AC fields. a: Frequency of electric field is 1 Hz, indicating that DNA chain remains unstretched state. The size of DNA oscillates with the frequency of the external field. b: Frequency is changed from 6 Hz to 100 Hz. Stretched DNA tends to be relaxed.

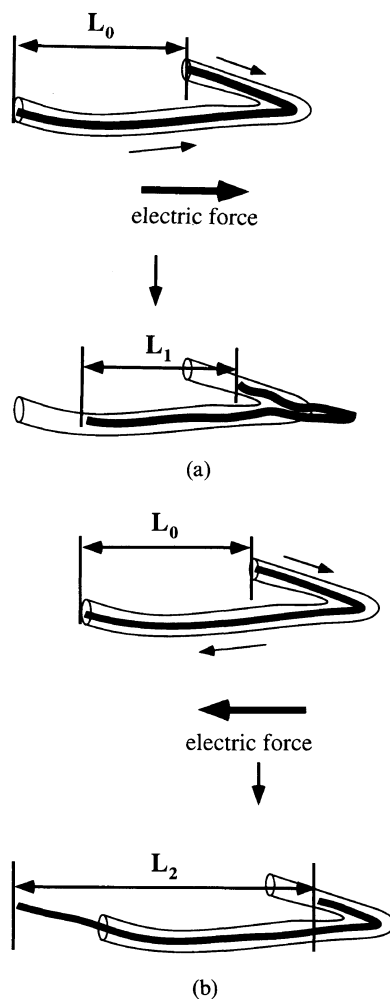


Figure 5. Schematic explanation of the stretching process from the bent conformation. DNA is entrapped within the tube surrounded by entangled PA. The bent conformation with single apex is illustrated as a simple example. a: On the occasion that the electric field is supplied to the bending head, the end-to-end distance keeps almost the same length during the electrophoretic translational motion ($L_0 \approx L_1$). b: When the electric field is switching to the opposite direction, the end-to-end distance increases during the electrophoretic motion ($L_0 < L_2$).

maps of long DNAs. In agarose gel, Schwartz *et al.*¹⁶ have already performed such an experiment. The method reported in the present article will be useful for the manipulation of individual giant DNAs.

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