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Dancing DNA in Capillary Solution Electrophoresis

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Capillary electrophoresis is currently being studied extensively because of its promise as a technique for fast separation of DNA at high resolution.[1-9] Since the mobility of DNA alone in free solution is effectively independent of molecular weight,[10] a "sieving medium" such as a linear polymer has to be included. Although several separation mechanisms have been proposed,[5-7] the role of this medium has remained obscure. We report here the first direct observations using fluorescence microscopy revealing an oscillatory dynamics of DNA during capillary electrophoresis in dilute polymer solutions: as a result of entanglements with the polymer chains of the medium, the DNA molecules change rhythmically between extended and contracted configurations as they move through the solution. In contrast, at even lower polymer concentrations the DNA instead exhibits a rotatory, "waltzing" motion through the solution as a result of interplay of Brownian dynamics, interactions with the polymer chains and the electric field.

To investigate the interaction between DNA and a polymer medium during conditions of capillary electrophoresis, we used fluorescence microscopy to follow the migration of individual T2 DNA molecules (164 kbp) in dilute solutions of linear polyacrylamide (MW 18 × 10^6 or 5 × 10^6 denoted as PA18 and PA5, respectively). Upon application of an electric field along a layer of solution with a thickness of 110 μm, the migration of the YOYO stained DNA toward the anode can be studied in the middle of the layer, where electro-osmotic flow gradients due to wall charge are negligible. While the DNA chains according to the Ogston sieving model,[11] as well as in the absence of polymers,[12] take on average spherical random-coil shapes, they exhibit extreme deformations during electrophoretic migration through the polymer solution; three typical extended configurations denoted U, J, and I are shown in Figure 1. The U configuration indicates, by inference from the behavior of DNA in gel,[13-15] that the DNA molecule is roughly symmetrically hooked on one or several polymer chains. Two different scenarios can occur: either the whole U moves toward the anode (frequently seen in dilute polymer solutions), or it may stay fixed at the point where it was created (at high polymer concentration). In both cases the two arms of the U are seen to extend along the field and finally one of them wins a tug-of-war and pulls the other arm around the hooking point to form a J configuration, which turns into an I parallel with the field. The I thereafter contracts into a compact configuration from which two new arms soon begin to grow: a new U is created, and the cycle starts from the beginning.

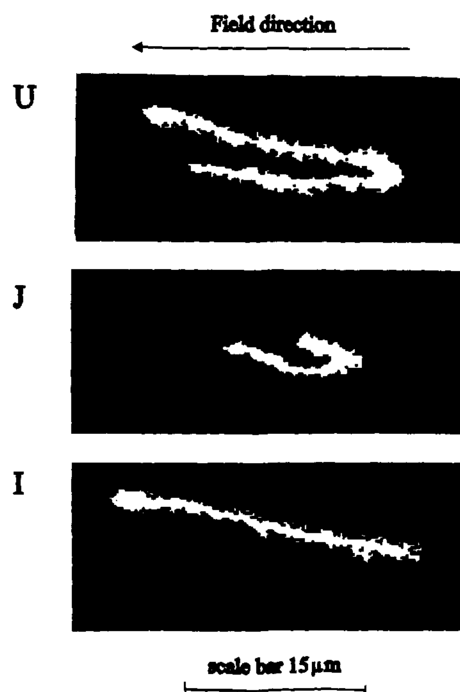

Figure 1. Three classes of extended DNA configurations observed by fluorescence microscopy during capillary electrophoresis: the U form, created by hooking on polymer chains, and the J and I forms, which are subsequently formed when the DNA gets released from polymer chains. Pictures (image-processed with Corel Draw) refer to T2 DNA migrating in 0.4% PA18, 6 V/cm. Other conditions as in ref 20.

From video recordings on a large number of DNA migration experiments in solutions containing varied polymer concentrations was obtained a semiquantitive picture of the most probable scenarios and DNA configurations (Table 1, Figure 2). In 0.4% PA18 (contact concentration 0.3%) the behavior strongly resembles that observed for large DNA in agarose gel and may be characterized as *reptation*.[16] The DNA is hooked on a segment of a more or less entangled network, and its motion is confined in such a way that no significant fluctuations in directions transverse to the field are observed. At 0.1% PA18 concentration the same type of scenario occurs, but now the U configurations are frequently seen to move along the field. There is now also considerable transversal motion, and a slower and less regular pace of configurational alterations indicates that the DNA more seldomly encounters polymer chains in its way.

In the ultradilute polymer solution (0.003% PA18), the reptative behavior of DNA is replaced by a motion that may be described as "waltzing". The transversal motion is further enhanced compared to the higher polymer concentrations, and as a result of this freedom, when a U configuration encounters an obstacle (polymer) on its way along the field, it frequently performs a rotatory motion to "dance" around the obstacle. The scenario with oscillations between stretched and contracted configurations is observed at even lower concentrations of polymer; it is first at typically 0.0005% PA18 that the migration behavior becomes difficult to distinguish from that in a free solution. With the shorter polymer PA5 the need of higher concentration to get the same migrative behavior as with PA18 (Table 1) indicates that the critical parameter is the degree of polymer entanglement rather than the absolute polymer concentration.

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Table 1. Electrophoretic Migration Behavior of DNA at Different Polymer Concentrations and Lengths Demonstrating a Continuous Variation from Solution to Gel[a]

	free solution	0.1% PA5	0.4% PA5	1.0% PA5	0.003% PA18	0.1% PA18	0.4% PA18	1% agarose gel
extension[b]	0	++	+++	+++++	++	+++	+++++	++++++
alignment[c]	0	+	+++	++++	+	+++	+++++	++++++
periodicity[d]	0	0	++	+++++	0	++	+++++	++++++
reptation[e]	0	+	++	++++	+	+++	+++++	++++++
relaxation time[f]	nd[g]	0	++	+++	0	++	+++	++++++
Brownian motion[h]	++++++	+++++	++	+	+++++	+++	+	0
bunching[i]	0	+	++	+++++	+	++	+++++	++++++
width of U[j]	nd	++++++	+++	+	++++++	+++	0	0
velocity[k]	linear	nd	nd	nd	nd	linear	nd	nonlinear

[a] Electric field strength 6 V/cm. For comparison, results in free solution and in 1% agarose gel are included. The properties referred to are described by the scale 0 = minimum, ++++++ = maximum. [b] Estimated degree by which DNA is extended (cf. Figure 1). [c] Estimated average degree of orientation of extended configurations of DNA parallel to the field. [d] Regularity by which the shape of DNA changes between equivalent configurations. [e] Degree of tube constraints (reptation-like behavior). [f] Relaxation time for extended DNA to return to random coil upon removal of field. [g] Not determined. [h] Observed as shape fluctuations and transversal motions. [i] Accumulation of DNA segments at the front end of DNA as observed by local brightness of fluorescence. [j] Distance between the arms. [k] Functional dependence of velocity on the electric field strength. Free solution and gel from refs 21 and 19.

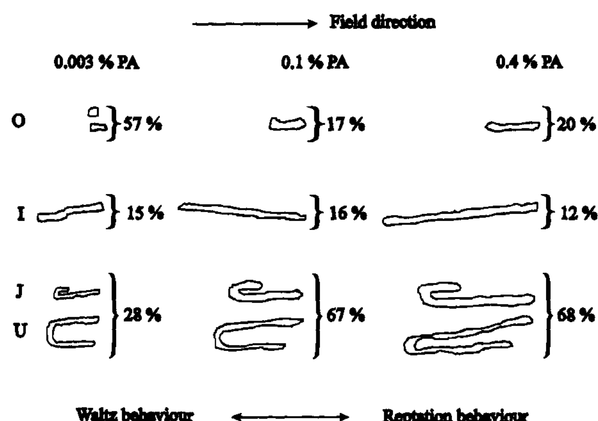
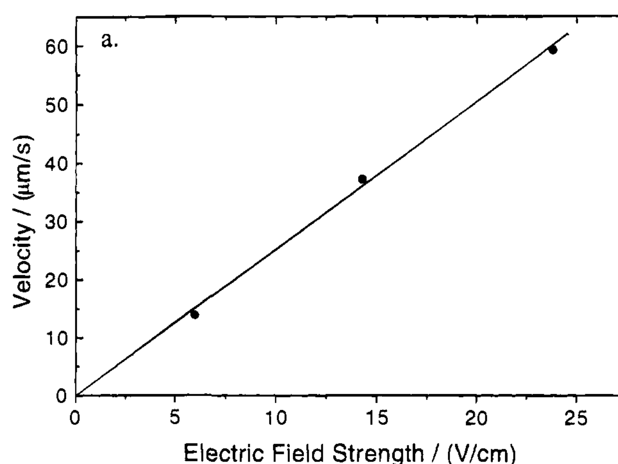


Figure 2. Estimated occurrence of compact (O) and extended configurations (I, J, and U) of T2 DNA during electrophoresis (6 V/cm) at three concentrations of PA18. Differences in shapes are indicated schematically. DNA configurations recorded every 3 s and statistics based on 250 observations for each polymer concentration.

While a nonlinear dependence of the migration velocity of DNA on the electric field strength is observed for DNA reptating through gel,[17-19] an approximately linear dependence is seen in the semidilute polymer solution (Figure 3a), indicating that the total friction is independent of field strength. There is no indication of any abrupt transition between dilute polymer solution and the gel; the mobility gradually decreases with the polymer concentration (Figure 3b), and the value of the highest concentration is similar to that in 1% agarose gel.

The observed dynamic interplays of DNA with the polymer chains of the medium suggest mechanisms that could explain separation by capillary electrophoresis at both high and low polymer concentrations. Enhancement of separation,[2,5] and even separation of Mbp DNA,[2] by capillary electrophoresis in a PA solution under pulsed-field conditions has been reported. For T2 DNA in 0.1% PA18 during field-inversion electrophoresis using 50−100 ms pulse times, we found that extended configurations were more or less suppressed so that the DNA was migrating preferentially in a coil-like configuration. This behavior is similar to the one observed in field-inversion electrophoresis in gel,[14] where more efficient reptation of extended configurations of larger DNA is believed to be the reason for poor separation at constant fields.[17,18] We thus infer



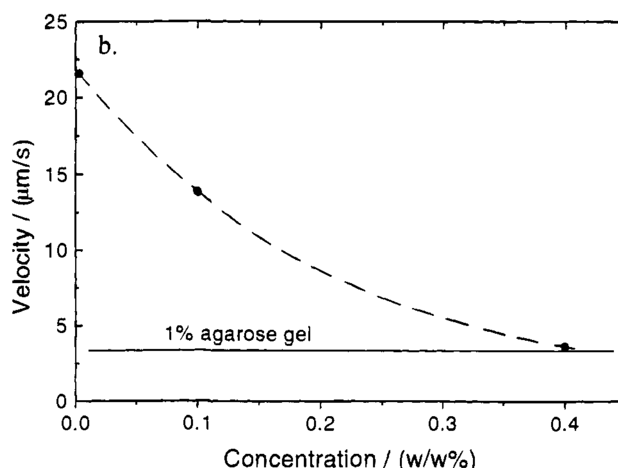


Figure 3. (a) Velocity of T2 DNA in 0.1% PA18 solution as a function of electric field strength. Each point is an average from approximately 50 observations. (b) Dependence of velocity of T2 DNA (6 V/cm) on concentration of PA18. (For comparison, velocity of equally stained T2 DNA in 1% agarose gel is shown.)

that the improved separation by pulsed fields, also in polymer solution, is a result of suppressed reptation. At low polymer concentrations neither the reptation nor the Ogston sieving model can appropriately describe the migration behavior, but instead a "waltzing" motion is observed. Since Brownian dynamics can be expected to disfavor disentanglement of larger DNA molecules compared to smaller ones, larger DNA will "dance" more slowly around the obstacles, and we have here a possible separation mechanism.

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