DNA electrophoresis in dilute polymer solutions: A nonbinary mechanism

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The dynamical behavior of the neutral polymer (dextran, $M_w = 2 \times 10^6$) is investigated during DNA electrophoresis in a dilute solution. Using a fluorescence recovery after photobleaching setup, we measured the velocity of fluorescein-labeled dextran induced by the migration of the DNA. We found that each DNA molecule drags a large number of dextrans with it. We show that DNA-dextran interactions are not only binary but long range and indirect. We conclude that the DNA-dextran complex creates a hydrodynamic field that entrains polymers far from the DNA during electrophoresis.

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I. INTRODUCTION

Capillary electrophoresis has become a routine technique, allowing fast separation of nucleic acids. Thanks to the capillaries' small cross section [usually 50–200 μm internal diameter (ID) and 30 cm long] which allows fast heat evacuation, high electric fields up to 300 V cm^{-1} can be applied. To mimic gels, for which detailed models exist, semidilute polymer solutions are generally used since they yield the same kind of topology. A refinement of the biased reptation with fluctuation model [1] developed for gels [2], taking into account constraint release (the relaxation of the polymer matrix), was proposed to describe the mechanism in such polymer solutions. Since then, much experimental work has been done to improve the technique and determine which polymers to use [3]. However, finding the best conditions to optimize DNA fragment separation is rather empirical and more theoretical work is still needed.

Although high-resolution separation is mostly achieved in semidilute solutions of neutral polymers, Barron, Blanch, and Soane [4] reported in 1996 that they could separate double stranded DNA molecules in dilute and ultradilute solutions within a few minutes. They proposed that the separation mechanism is based on transient entanglement between the neutral polymer and the DNA. Thus, the longer the DNA molecule, the more polymers it drags. Hubert, Slater, and Viovy [5] developed a model based on this assumption that fits the experimental results fairly well for small DNA chains and low concentrations. Sunada and Blanch [6] stressed that most DNA-polymer interactions are collisions with no entanglement and proposed their own model, although the final equation they derive is not significantly different from the one proposed by Hubert, Blanch, and Soane.

Concerning the mechanism, it is commonly assumed that DNA is completely free draining during free solution electrophoresis. This hypothesis explains why the mobility $\mu = q/\xi$ is independent of the chain length L in free solution electrophoresis, since both q and ξ are proportional to L. Assuming that this property is maintained during DNA electrophoresis in dilute polymer solutions, interactions between the DNA and the polymer molecules should be direct, coming only from collisions and/or entanglements. Here, we show that the DNA-dextran interaction is not based solely on transient entanglements and collisions. We propose that the DNA-dextran complex creates a hydrodynamic field that drags many neutral polymers along.

In this work, we measure the dextran mobility and compare it to that of the DNA under the same conditions to gain more insight about the mechanisms in dilute solutions. The principle is as follows: By labeling DNA molecules, we were able to measure simultaneously the mobility and the diffusion coefficient of DNA fragments with a fluorescence recovery after photobleaching (FRAP) setup [7]. By labeling the neutral polymer (*and not the* DNA), we were able to measure the velocity at which they are dragged by the DNA during electrophoresis. Our results show that the large number of neutral polymers dragged is not compatible with the binary interaction.

II. MATERIAL AND METHODS

The setup we have used is the same as described by Tinland, Meistermann, and Weill [8]; two laser beams are crossed on the sample, creating a fringe pattern with a fringe spacing i. At $t = 0$, a high power pulse is generated that bleaches the dyes in the bright fringes and generates a sinusoidal concentration profile of fluorescence molecules. The amplitude of this pattern is detected by modulation of the illuminating fringe position using a piezoelectrically driven mirror and lock-in detection of the emerging fluorescence collected at the photomultiplier by an optical fiber. When an electric field E is applied, motion of the molecules yield a sinusoidal signal of period T. Additionally, under the conditions chosen, the signal decreases due to the molecules that are moving away from the probed region resulting in a Gaussian decay [9]. The FRAP signal can then be fitted to the expression:

$$I = I_o e^{-(t/\tau)^2} \sin\left(\frac{2\pi t}{T}\right) \tag{1}$$

from which we deduced the mobility of the labeled molecules, $\mu = V/E = i/(ET)$. Note that, for analogy with DNA mobility, we define the dextran mobility as $\mu = V/E$.

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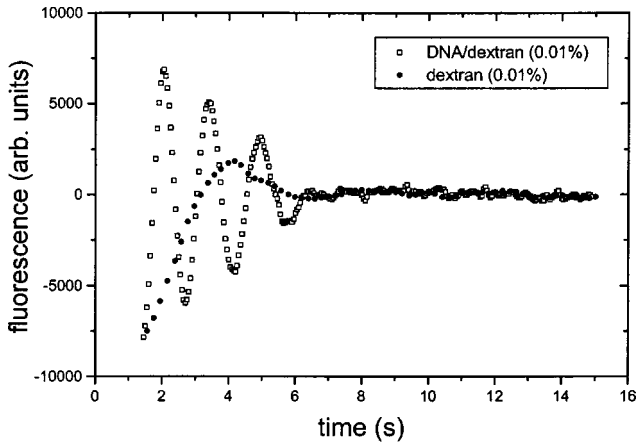

FIG. 1. Typical FRAP signals for pure dextran solution (0.01%) and DNA (5 μg ml^{-1})-dextran (0.01%) solution. Without DNA, the dextran moves due to the residual electric charge from the dye. When DNA is present, it induces momentum to dextran that then travels with a higher velocity.

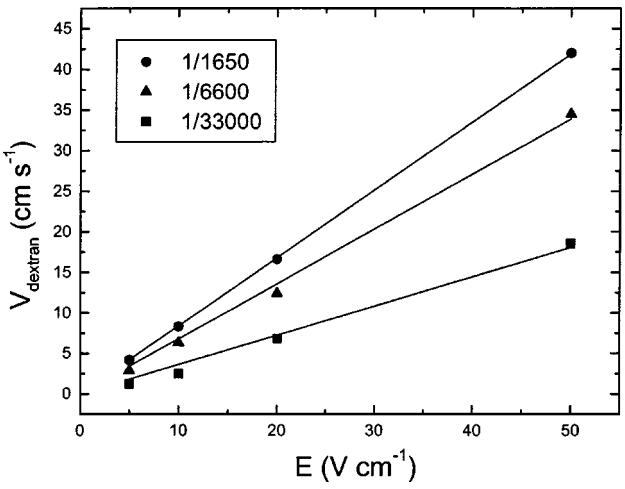

FIG. 2. Velocity of dextran dragged by DNA (5 μg ml^{-1}) as a function of the field E for several DNA/dextran ratios: 1/1650 ($C_{\text{dextran}}=0.05\%$), 1/6600 (0.2%), and 1/33 000 (1%). Linear fits yield the mobilities: 0.84 ± 0.12 (0.05%), 0.60 ± 0.08 (0.2%), and 0.36 ± 0.05 cm^2 V^{-1} s^{-1}.

The measurements were carried out in fused silica, square-section capillaries (Wales Apparatus Co.) with 0.5 mm ID and 100 mm length as described in Ref. [10]. The capillary walls were ''conditioned'' with a 1% w/w polydimethyl-acrylamide ($M_w=150\,000$, provided by Galin, ICS, Strasbourg) solution overnight in order to reduce the electro-osmotic flow (EOF). Under these conditions, the electrophoretic mobility of the DNA in free solution was measured to be $\mu_0=4.2\times10^{-4}$ cm^2 V^{-1} s^{-1} [10]. This is very close to the value given by Stellwagen, Gelfi, and Righetti, where they prevent EOF by polymerizing polyacrylamide on the capillary walls [11]. Thus, our coating procedure is very efficient and mostly eliminates the EOF.

The sieving matrix consists of dextran. This polysaccharide belongs to the same family as agarose. It was chosen because it is soluble and neutral and therefore has no trivial interaction with the DNA. We prepared our own fluorescein-labeled dextran with seven to eight dyes per chain as determined by fluorescence measurements. Dextran ($M_w=2\times10^6, C^*=1\%$) and isothiocyanatofluorescein were purchased from Sigma. Isothiocyanatofluorescein reacts with dextran in dimethyl sulfoxide at 95 °C. The reaction is catalyzed by dibutyltin dilaurate. The complete procedure is described in Ref. [12].

We purchased λ phage DNA [48 500 base pairs (bp)] from Biolabs. The 2100 bp fragment was restricted and linearized from the corresponding vector with the help of Jean-Marie Garnier (IGBMC, Strasbourg). DNA and dextran solutions were mixed to the desired concentrations in a 0.1 M tris-ethylenediamine tetra-acetic acid buffer at pH = 8.3.

Electric fields, ranging from 5 to 50 V cm^{-1}, were applied during the time of the experiment, which ranged from a few seconds to a minute. For these short times, no increase of the temperature in the capillary was detected. In this field range, all mobilities were found to be field independent as predicted by theoretical models. For one experiment, that is one cell, the error between the measurements is less than 5%, but for two experiments (after having removed the solution, rinsed the capillary, and coated it again) the measured mobility value can change by 15%.

III. RESULTS AND DISCUSSION

For an electric field of 50 V cm^{-1}, FRAP signals from a fluorescent dextran solution at a concentration $C=0.01\%$, and from the same solution but containing 48 500 bp DNA are shown in Fig. 1. Even when there is no DNA in the solution, fluoresceinated dextran exhibits a net velocity. This signal goes to zero very quickly (typically after one oscillation). From many measurements of this oscillation, we determine the average value $\mu=(0.25\pm0.15)\times10^{-4}$ cm^2 V^{-1} s^{-1}. In the presence of DNA, this mobility can be up to five times larger and decreases when the dextran concentration approaches the critical concentration C^*. Also, many oscillations can be seen and the exponential envelope is well defined. This is an evidence for the existence of a strong interaction between the DNA and the neutral polymer.

Does the fact that dextran moves even when no DNA is present indicates that there is a residual EOF dragging all polymer molecules? Using a pure DNA solution, we determined that the EOF is correctly cancelled. To investigate the direction of the motion of the dextran molecules, we carried out a fluorescent flow experiment. The capillary was half filled with a solution of DNA/fluorescent dextran on one side and DNA/nonfluorescent dextran on the other. The concentration profile of the interface was monitored using a laser beam. At a given position on the interface, we examined how the amount of fluorescence (that is, the number of fluorescent molecules) was changed when an electric field was applied. We found that fluorescent dextran molecules migrate towards the positive electrode. Moreover, the experiment was reversible (the fluorescent gradient would increase) when the polarity of the field was changed. However, the same experiment performed with fluoresceinated dextran alone showed

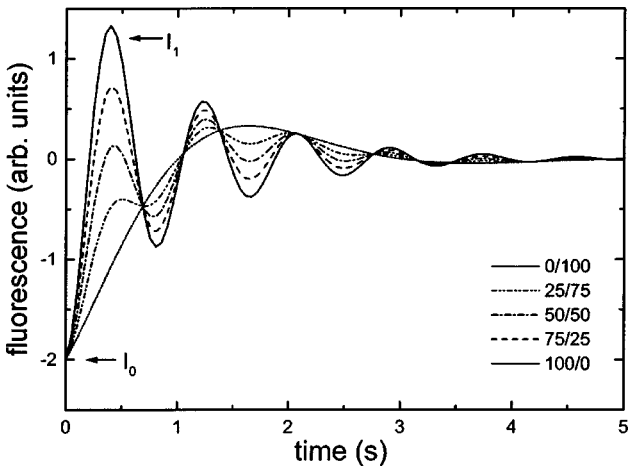

FIG. 3. Expected FRAP signals for differing dragged/nondragged dextran ratio. The time scales are experimental values.

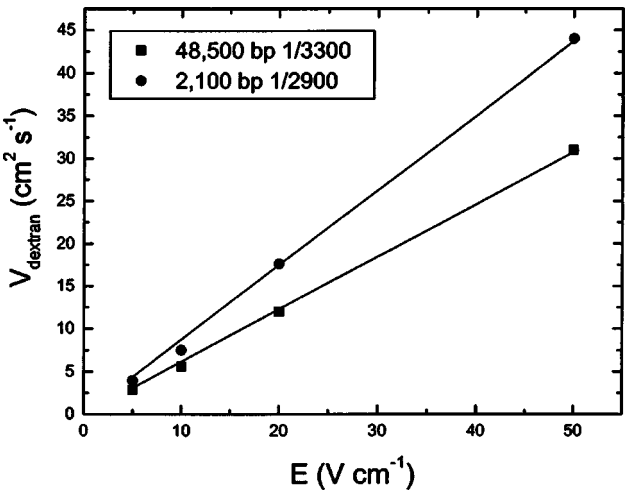

FIG. 4. Mobility of dextran ($C=0.1\%$) dragged by 48 500 bp and 2100 bp DNA chains as a function of E. The DNA concentrations were adjusted to give approximately the same DNA/dextran ratio. Linear fits yield 0.87 ± 0.13 (2100 bp) and 0.61 ± 0.9 cm^2 V^{-1} s^{-1} (48 500 bp).

that molecules were also moving toward the anode, but with a significantly lower velocity, in agreement with the FRAP measurement. The explanation is that fluorescein is negatively charged at this pH [13]. Since the dextran ($M_w=2\times10^6$) bears seven to eight dyes per molecule, it has a net charge that results in a low electrophoretic mobility.

Figure 2 illustrates the mobility of dextran measured as a function of the electric field strength. The 48 500 bp DNA concentration was kept constant, but the relative numbers of dextran molecules varied. In these experiments, the velocity is 1.5–3.5 times higher than that of dextran alone. We have observed it as five times higher (data not shown) in the presence of DNA. As expected, when there were more dextran molecules per DNA chains, the mobility decreased.

It must be emphasized that, in Eq. (1), the velocity derived from period T is *the average velocity of all the molecules that have a net velocity (i.e., not those moving by diffusion only)*. Thus, the problem can be simplified by assuming that any given dextran molecule is either dragged by the DNA molecules and moves constantly at the velocity i/T, or not dragged (and, in this case, only *diffuses*).

Figure 3 shows the expected shape of the experimental signal for various ratios of nondragged/dragged dextran. The expression was

$$I=I_o e^{-(t/\tau)^2}\left[f\sin\left(\frac{2\pi t}{T_1}\right)+(1-f)\sin\left(\frac{2\pi t}{T_2}\right)\right], \qquad (2)$$

where T_1 is the time of oscillations for nondragged dextrans (which have a low mobility due to the slight charge of fluorescein) and T_2 for the dragged ones. f is the fraction of nondragged molecules. Curves were drawn for various nondragged/dragged ratios and experimentally measured time constants. We found the boundary limits: when the dextran is alone (100% nondragged/0% dragged) the decreasing exponential presents a small bump coming from the displacement of the slightly charged dextran that was used to estimate the residual mobility of dextran without DNA; the signal becomes more and more sinusoidal as the nondragged/dragged ratio decreases. Consequently, since the time scales are very similar, we estimate roughly the fraction of dragged dextran from the ratio of the signal amplitudes I_1/I_0, neglecting the slight decrease of I_1 due to thermal diffusion. This fraction ranges from 50–80 % in all our experiments [14], showing that at least 50% of the dextran is moving at this higher velocity. This is quite surprising since the number of dextran molecules per DNA ranges from hundreds to thousands.

Considering the relative number of dextrans and DNA chains existing in the solution (Table I), this means that one DNA can impart momentum to several thousands of dextran molecules. Under the same conditions, the electrophoretic mobility of DNA was found to decrease only by 10% from its value in free solution. Thus there is an apparent contradiction between the observation that many dextran molecules are dragged by the DNA, whereas the mobility of the DNA is

TABLE I. DNA (48 500 bp, 5 μg ml^{-1}) and dextran concentrations (0.05%) in number and relative numbers of dextran per DNA chain. In the case with hydrodynamic interactions, the number of dextran molecules was calculated using the results from Ref. [13].

Dextran concentration (molecules/nm^3)	DNA concentration (molecules/nm^3)	Dextrans/ DNA chains (no hydrodynamic)	Dextrans/ DNA chains (hydrodynamic field)	Dextrans inside a DNA chain
1.5×10^{-7}	9.2×10^{-11}	1650	5000	110

only slightly affected. Many dextran molecules are entrained, but only a few directly through the entanglement-collision mechanism. Instead, collective effects are observed, involving a large number of neutral polymers per DNA chain. Since the direct entanglement-collision interactions cannot explain the observed behavior, we propose to consider hydrodynamic interactions as an explanation.

Assuming that the DNA is *non-free-draining* as in a diffusion experiment, a very naive calculation [15] suggests a hydrodynamic field acting over several micrometers, which would drive a number of dextran molecules comparable to the values in our experiments (Table I). Thus, one DNA molecule would be able to impart momentum to many neutral polymers. A more accurate calculation is called for, but this rough estimate serves to emphasize the potential importance of the hydrodynamic field. The presence of the neutral polymers makes the DNA-dextran complex a composite ''hard'' sphere yielding a hydrodynamic field.

Long, Viovy, and Ajdari [16] showed that, when electric and nonelectric forces are acting simultaneously on a polyelectrolyte, the flow is the superposition of a Stokes part avoiding the interior of the sphere and an electro-osmotic part that freely flows through the sphere. In this situation, the nonelectric force would initially be due to some of the numerous dextran molecules (Table I) inside that DNA sphere. They will eventually escape only to be replaced by other molecules that the DNA encounters.

Surprisingly, the velocity of dextran in the presence of the smaller DNA molecules was found to be higher than in the presence of the longer DNA molecules (Fig. 4), although concentrations were adjusted to give approximately the same DNA/dextran ratio. The result is counterintuitive, but highly reproducible. The explanation is still unclear. It might be that some dextran molecules never interact with the hydrodynamic field created by the smaller DNA chains. Accordingly, the ratio I_1/I_0 was found to be closer to 50% with 2100 bp (and $\sim$75% with 48 500 bp). Thus, fewer molecules would be dragged at a higher velocity.

Although it is likely that only polymers colliding with the DNA molecules are relevant for the separation, a global description of the mechanism must take into account the long-range interactions we report. These observations constitute stimulating results that need to be more deeply investigated.

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