DNA electrophoresis in a monodisperse porous medium

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(Received 5 October 1999; revised manuscript received 1 March 2000)

Electrophoresis of DNA migrating in an ordered matrix is studied and compared with classical agarose gel electrophoresis. A well-defined migration medium is obtained by crystallization of monodisperse silica spheres. Electrophoretic mobility of DNA is measured with fluorescence recovery after photobleaching experiments. The main result is that, as it was the case for gel electrophoresis, diffusion and electrophoretic mobility of DNA in such a medium are well described with reptation theories.

PACS number(s): 87.15.−v, 82.45.+z, 83.10.Nn, 83.20.Fk

Separation techniques play a great role for the analysis and understanding of biological systems. Electrophoresis is one of the most important tools which had proved its usefulness in the Human Genome project. People have underlined the complexity of the gel structure but the role of the gel disorder in separation mechanisms has never been understood properly. Building monodisperse rigid matrices answer to the purpose of understanding the transport mechanisms of DNA in a well-defined porous medium focusing specifically on the influence of the pore size distribution on the separation properties. Molecular motion of individual DNA in ordered structures has been observed in a two-dimensional microlithographic array [1] and in a stabilized suspension of a hydrophobic ferrofluid [2] using fluorescence microscopy. These systems permit the study of the disentanglement mechanism of DNA hooked around obstacles [3–6]. In our paper the migration properties of double-stranded DNA are considered through quantitative measurements of electrophoretic mobility. Building a three-dimensional calibrated matrix allows us to investigate the influence of the geometry of the migration support by comparison with values obtained in agarose gel in similar experimental conditions. The matrix is built by packing spherical monodisperse nonporous silica beads. The silica beads were kindly provided by Micra Scientific, Inc. Measuring their diameter d by scanning electron microscopy gives a value of $(1.04\pm0.04)\ \mu$m. Their high modulus and their density (2.1 g/cm^3) enable us to make a rigid matrix by sedimentation. A dilute suspension of spheres (volume fraction $\Phi=0.004$) in water (pH=7) is packed under gravity in a homemade cell (Fig. 1). Water is then replaced by a solvent containing TBE (Tris Borate ethylenediamine tetra-acetic acid 10^{-2} M), a usual electrophoresis buffer, mixed with sucrose (1.83 M) in order to closely match the refractive index of the solvent (1.422) to that of the silica beads (1.45). The diffraction pattern of the 1 μm beads packed in the electrophoretic cell (1 mm wide thus around 1000 beads layers) turns in Bragg spots indicating the high order of this matrix (Fig. 2). The spots are a bit diffuse which indicates some defects in the arrangement. Calculating the interdistances between spots and supposing that the network is hexagonal, we deduced a 1 μm diameter in good agreement with the value determined in the direct space. Scanning electron micrographs (Fig. 3) confirm the ordered structure of this packing. However the matrix is not crystallized over the whole volume. Scanning the matrix with a focused laser beam shows monocrystalline regions (evidenced by quite well-defined Bragg spots) alternating randomly with polycrystalline regions revealed by several Bragg rings.

The transparency of the medium permits us to use an optical method to study the DNA migration. The mobility and the diffusion coefficient of YOYO-labeled DNA were measured by a fringe pattern fluorescence photobleaching technique [7]. The light beam of an etalon-stabilized monomode Ar laser (1 W at 488 nm) was split and the two beams were crossed in the electrophoretic cell, providing illumination in a deep interference fringe pattern. The fringe spacing $i=2\pi/q$ set by the crossing angle φ, $q=(4\pi/\lambda)\sin(\varphi/2)$, ranged from 3 to 60 μm, defining the diffusion distance. Fluorescence bleaching of the labeled polymers in the illuminated fringes was obtained by producing a 1 s full-intensity bleach pulse by means of a Pockels cell between nearly cross polarizers. Experiments were performed in the ordered regions of the sample and the existence of the diffraction pattern obtained with the FRAP laser beam was always checked before running experiments. We can easily verify that applying the electric field does not destroy the matrix arrangement by checking that this pattern is not al-

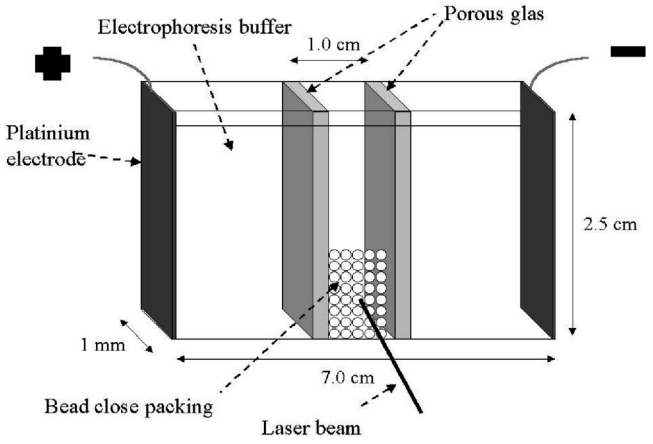

FIG. 1. The electrophoretic cell is made of two rectangular microscope slides with a 1 mm large spacer sandwiched between. Two platinum electrodes are positioned at both ends. The well is delimited by two porous glass walls inside which beads are packed. The cell is filled with the buffer to maintain pH and temperature constant.

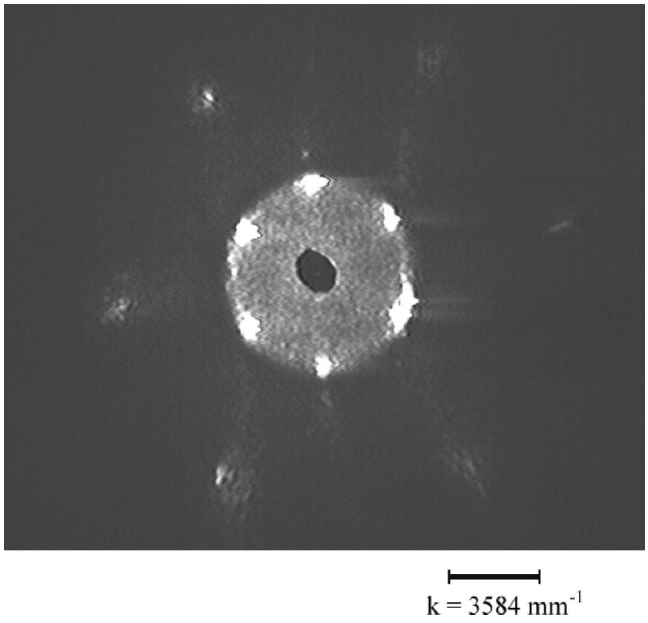

FIG. 2. Diffraction figure of the 1 μm beads packed in the electrophoretic cell (scaling bar expressed in the scattering wave vector $k=(4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle and λ is the laser wavelength).

tered. The electric field E was measured inside the silica well to take into account the drop of the applied voltage due to the dielectric constant of silica beads.

Two experimental signals are represented in Fig. 4. Without the field applied the signal is a simple monoexponential, where its characteristic time τ_1 gives the self-diffusion coefficient [Fig. 4(a)]. When the continuous electric field is applied, the ''sample'' pattern migrates through the optic pattern leading after phase detection to an alternating signal that vanishes because of diffusion under the influence of the electric field [Fig. 4(b)]. The time τ_2 of the oscillations gives access to the electrophoretic mobility: $\mu=i/E\tau_2$.

The free solution electrophoretic mobility of DNA in the TBE/sucrose solvent is $\mu_0=1.9\times10^{-5}$ cm^2/V s. We have directly measured this value with our setup on various DNA fragments. As expected we found that μ_0 is independent of

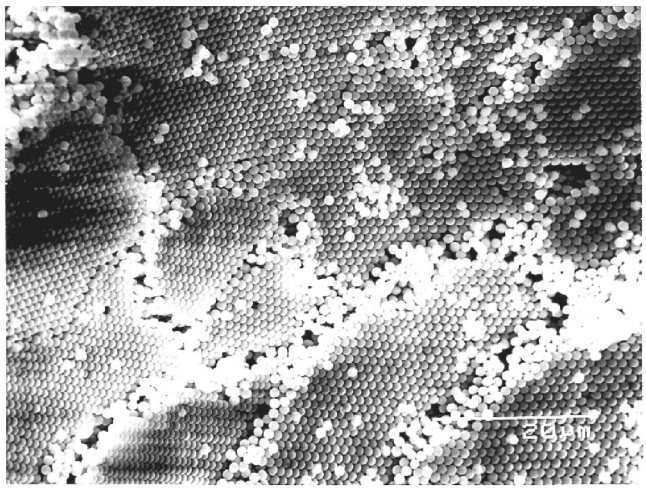

FIG. 3. Scanning electron micrograph image of 1-μm silica beads packing.

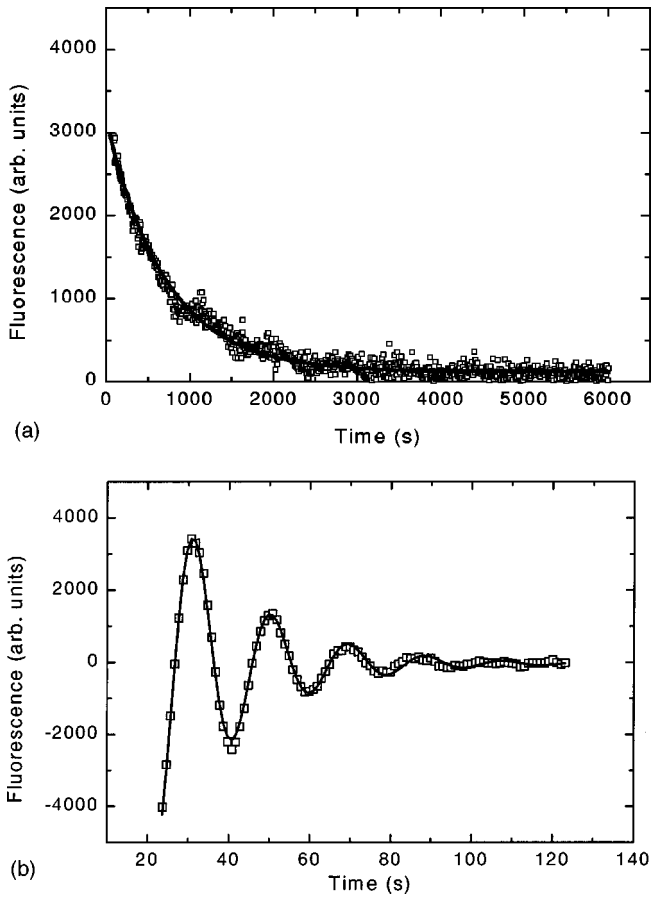

FIG. 4. (a) Typical fit (continuous line) and monoexponential decay (open squares) obtained for 5721-base pairs DNA in 1 μm beads matrix (scattering vector $q=5399\,\mathrm{cm}^{-1}$). (b) Fit (continuous line) and damped sinusoidal signal from the FRAP experiment (open squares) of a 5721-base pairs DNA in 1 μm beads matrix (field strength $E=31\,\mathrm{V\,cm}^{-1}$, scattering vector $q=5399\,\mathrm{cm}^{-1}$).

the DNA length since flexible polyelectrolyte chains act as free draining for electrophoretic transport in solution. We have also measured the self-diffusion coefficient in a solution of TBE/sucrose. For both sets of data (free-solution mobility and diffusion coefficient), we checked that their ratios with the values measured in pure TBE were equal to the

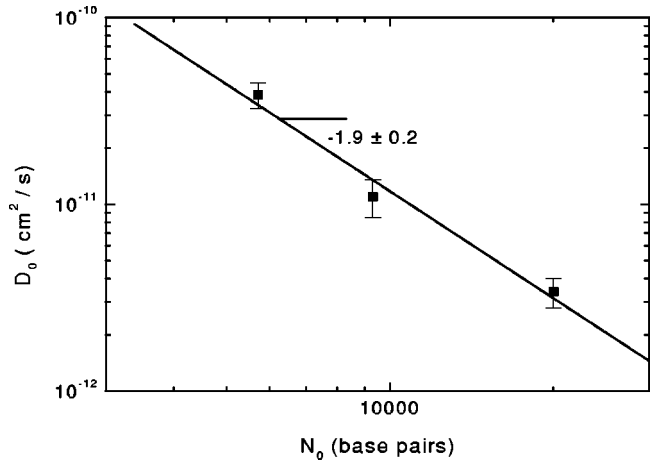

FIG. 5. Self-diffusion coefficient in 1-μm beads matrix as a function of DNA base pairs.

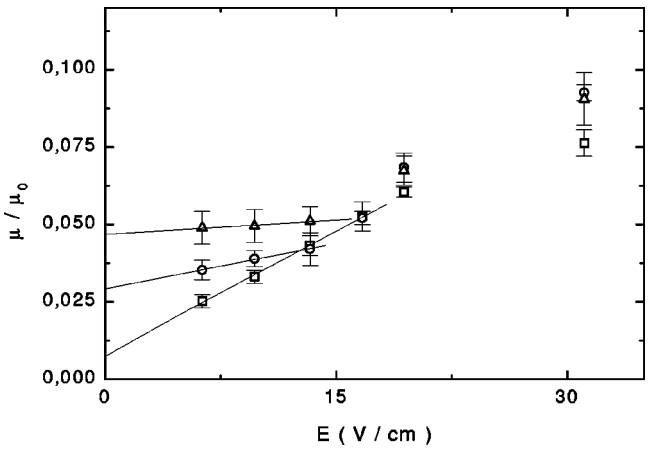

FIG. 6. Field dependence of the ratio of electrophoretic mobility in 1-μm beads matrix over mobility in the solvent for various DNA lengths in base pairs: 5721 (△), 9300 (○), 48 500 (□). Linear extrapolation to zero field (continous lines) used to evaluate $\mu_{E\to 0}$.

vicosity ratio (15.5 ± 0.5). In other words the solution properties of DNA fragments are not affected by the presence of sucrose.

Figure 5 shows the variation of the measured self-diffusion coefficients in the calibrated matrix (D_0) as a function of the DNA length expressed in base pairs (N_0). The slope is -1.9, close to the -2 prediction for the diffusion of polymer molecules in a network [8,9]. This indicates that these DNA molecules move by reptation in this medium.

The biased reptation model with fluctuations (BRF) has been developed to describe the mobility dependence with the polymer length, the average pore size, and the electric field [10–12]. The chain is constrained to move into a virtual tube of length Na where N is the number of pores occupied by the DNA chains and a the average pore size. The electrophoretic mobility is given by the expression:

$$\mu=\mu_0\left[\frac{1}{3N}+f(a,E)\right]. \tag{1}$$

For weak electric fields the first term in Eq. (1) is predominant, the mobility of the molecules is length dependent, and the separation is possible. When increasing the electric field,

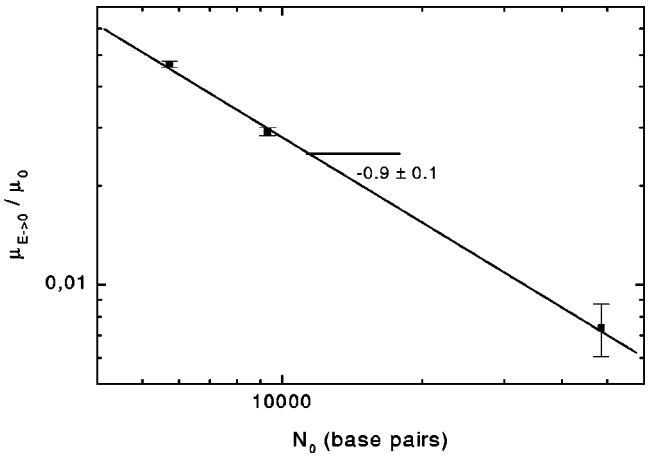

FIG. 7. Log-log plot of the mobility ratio $\mu_{E\to 0}/\mu_0$ as a function of DNA length.

TABLE I. Number of pores occupied by the DNA chain and pore sizes estimated from mobility values extrapolated to zero field for DNA of differing lengths (in base pairs) migrating in a silica beads matrix.

DNA length N_0	Number of pores N	Pore size a (mm)
5721	7.1	165
9300	11.4	166
48 500	45.0	191

molecules start to be oriented, the mobility varies linearly with E (instead of E^2 predicted in the biased reptation model) and molecules are no longer separated. The molecular orientation is responsible for the loss of separability. Figure 6 shows the variation of the mobility as a function of the electric field for various DNA lengths. The observed behavior is in agreement with the BRF model. For $E<10$ V/cm and for short DNA's the mobility is independent of the field. For higher fields the linear dependence with the field and the loss of separability are observed. Figure 7 shows that the variation of mobilities extrapolated to zero field ($\mu_{E\to 0}$) over mobility in free solution (μ_0) with DNA length close to theoretical predictions ($N_0^{-0.9}$ instead of N_0^{-1}). The mobilities values can be affected by electroendosmosis (EEO) since the silica is charged at pH$=8.4$. However this will neither change the $\mu\propto N_0^{-0.9}$ and the $D_0\propto N_0^{-1.9}$ behaviors at zero field (EEO becomes null) nor the field linearity of μ at higher fields. According to these values of mobilities we can estimate the mean number of occupied pores $N=\mu_0/3\mu_{E\to 0}$ (Table I). Assuming that chains are Gaussian at low fields, one can write: $Na^2=N_kb^2$, where b is the length of a Kuhn segment and N_k the number of Kuhn segments in one chain. This allows us to evaluate the pore size a for the different probing DNA's (Table I). They are in good agreement with the geometric estimation assuming hexagonal packing of rigid spheres; the pore space can be viewed as a spherical volume (diameter$=(\sqrt{3}/\sqrt{2}-1)d=225$ nm) from which are attached cylindrical canals (diameter$=(2/\sqrt{3}-1)d=155$ nm). The value of a does not depend on the length of the probing DNA as it is the case in gels (see Table II). Gels present a wide pore size distribution and there are some evidences that larger DNA's choose larger pores. Therefore this fact constitutes a further evidence of the well-ordered character of these calibrated matrices. Using the Einstein relation between the mobility and the diffusion coeffi-

TABLE II. Number of pores occupied by the DNA chain and pore sizes estimated from mobility values extrapolated to zero field for various DNA lengths (in base pairs) migrating in agarose gels at concentrations 0.7% (w/v) and 2%(w/v) (data from Ref. [13]).

DNA length N_0	Number of pores N		Pore size a (nm)	
	0.7% agarose	2% agarose	0.7% agarose	2% agarose
5386		6.7		165
10 900	1.6	14.0	475	163
48 500	4.2	19.3	624	292

cient, we can calculate the base pair effective charge q_{eff} from the equation $q_{eff}=kT\mu_{E\to 0}/N_0D_0$. The values obtained in silica matrix ($e/9$ and $e/7$ for, respectively, 5721 and 9300-DNA base pairs) are similar to the one obtained in agarose gels [13,14].

Our study shows that rigid matrices packed with silica beads are suitable for electrophoresis of DNA. The behavior of DNA is similar to that observed in a gel in the sense that the biased reptation theories allow us to quantitatively describe the field and the DNA length dependence in such media. The linearity of the mobilities with the field shows that the BRF model is the most suitable. Away from the fundamental interest of such well-defined matrix, one possible promising development comes from the fact that the packing of hard spheres leads to rigid matrices and thus it should be possible to separate DNA larger than the current limit (40 kilo base pairs) in constant field electrophoresis using larger spheres, that is to say using larger pores (using 5 μm beads should allow DNA separation until approximatively 160 kilo base pairs). This is not possible in gels because when the gel concentration is decreased to obtain larger pores, they become mechanically too weak.

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