Dielectrophoretic Trapping and Polarizability of DNA: The Role of Spatial Conformation

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Dielectrophoresis is a convenient tool for controlled manipulation of DNA with numerous applications, including DNA trapping, stretching, and separation. However, the mechanisms behind the dielectrophoretic properties of DNA are still under debate, and the role of conformation has not been addressed yet. Here, we quantify dielectrophoretic effects on DNA by determining its polarizability from microfluidic single molecule trapping experiments. We systematically study different DNA configurations (linear and supercoiled, 6–164 kbp) and demonstrate that the polarizability strongly depends on the specific conformation and size of the DNA molecules. The connection to its spatializability strongly depends on the specific conformation and size of the DNA molecules. The theory of polymer dynamics is established by measuring diffusion coefficients and from that the radii of gyration; details about the spatial extension is established by measuring diffusion coefficients. Our results imply a scaling of the polarizabilities and the diffusion coefficients. Considerable effort has been put into characterizing the relevant DNA property, i.e., its polarizability. For linear DNA, different from that of single DNA strands. Noting that the dielectrophoretic properties of such DNA motives differ from that of linear DNA, we demonstrate the separation of DNA topoisomers based on their dielectrophoretic properties, achieving baseline resolution within 210 s. Purified DNA samples of specific configuration may be of great importance for DNA nanosassembly or future DNA vaccines.

The understanding and handling of DNA in its different conformations, specifically linear and supercoiled (covalently closed circular (ccc)) DNA, are of great importance for molecular biology and biotechnology. Supercoiled DNA has, for example, become a major tool for gene transfer and expression and may in the near future supplement traditional vaccines. Furthermore, DNA has also been used for directed self-assembly of artificially designed 2D and 3D architectures, with promising applications in nanotechnology.

In soft matter theory, the structure and conformation of linear and supercoiled DNA is well studied and many conformational and structural properties can be adequately modeled. On the other hand, comparative experimental studies of different DNA topoisomers in solution with or without external fields are rare. This is the more surprising as there is huge general interest in electrokinetic manipulation of DNA with practical implications for electrophoresis and dielectrophoresis as analytical tools. Especially, dielectrophoresis, i.e., motion in an inhomogeneous electric field due to (induced) dipole moments, has proven to be a suitable method for manipulating and controlling DNA molecules. Applications range from trapping, immobilization, and stretching to separation and purification. Recently, Kuzyk et al. reported the trapping of DNA origami, noting that the dielectrophoretic properties of such DNA motives differ from that of single DNA strands.

Considerable effort has been put into characterizing the relevant DNA property, i.e., its polarizability. For linear DNA, the radius of gyration, \( R_g \), is determined by diffusion coefficients. Our results imply a scaling of the polarizability with a power-law scaling for the polarizabilities and the diffusion coefficients. Recently, Kuzyk et al. reported the trapping of DNA origami, noting that the dielectrophoretic properties of such DNA motives differ from that of single DNA strands.

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length, frequency, and ionic strength dependencies have been intensively investigated via ensemble methods such as transient electric birefringence, conductivity dispersion, or time domain reflectometry. The current state of knowledge concerning dielectric and dielectrophoretic properties of DNA has recently been reviewed, revealing the lack of a coherent systematic understanding of the underlying processes. For example, different power laws for the scaling behavior of the polarizability with molecular weight have been presented with exponents ranging from 1 to 2 and possibly saturating for long DNA strands. Lately, techniques have been introduced to access the polarizability at single molecule level by observing the escape of DNA molecules out of dielectrophoretic traps or by using electrostatic force microscopy. A seminal study of the acting forces and mechanisms involved in dielectrophoretic trapping has been contributed by Chou et al.15

Here, we extend our work on dielectrophoretic trapping and polarizability of linear DNA and examine the role of size, topology, and spatial conformation. The dielectrophoretic effects are quantified by deriving the DNA polarizability from their dwell times in the traps. We introduce an optimized way of automatically determining these trapping times. The so obtained polarizability data is combined with information about the spatial extension and configuration of DNA, which we extract from measuring diffusion coefficients and from AFM images. The practical relevance of the obtained insight in the DNA polarizabilities and their dependence on conformation is demonstrated by separating DNA topoisomers of equal length according to their conformation within 210 s.

**MICROFLUIDIC CHIP DESIGN FOR DIELECTROPHORETIC TRAPPING**

Dielectrophoresis (DEP) refers to the movement of a particle caused by polarization effects in a nonuniform electric field. Such fields are typically created either with electrode arrays integrated into the microfluidic system or by including nonconducting constrictions into the microchannel design (electrodeless or insulating dielectrophoresis). The system used here is based on insulating dielectrophoresis as sketched in Figure 1. Such a design allows easy chip production by replica molding, reduces interfering electrochemical effects within the channel, and creates field configurations not changing over the channel height. Upon application of a voltage signal $U(t) = U_{DC} + U_{AC}\sin(\omega t)$, the array of posts creates an inhomogeneous electric field $\vec{E}$. (See also Figure 1.) The potential energy of a polarizable particle or molecule due to DEP can be written as

$$W_{DEP} = -\frac{1}{2}\alpha \vec{E}^2$$ (1)

This phenomenological description assigns an effective polarizability $\alpha$ to the molecule in solution, which quantifies the microscopic polarization process by an effective induced dipole moment $\vec{p} = \alpha \vec{E}$. The polarizability $\alpha$ is assumed to be real valued, so that polarization effects are isotropic and dissipative processes are neglected. It is dependent on the frequency of the electric field, $\alpha = \alpha(\omega)$.

In general, eq 1 has contributions from both DC and AC electric fields. The DC contributions, however, are negligible,
because we use \( U_{\text{DC}} \ll U_{\text{AC}} \) in our experiments, and the DC and AC polarizabilities are of comparable magnitude at the low AC frequency applied (\( \omega = 60 \text{ Hz} \)). Further, a time-averaged, quasi-static description can be adopted in eq 1, if the electric field strength \( E \) is taken to represent the rms (root-mean-square) value of the AC field strength.\(^{42,43}\) The latter is proportional to \( U_{\text{AC}} \), and we conclude that \( W_{\text{DEP}} \propto \alpha U_{\text{AC}}^2 \), independent of \( U_{\text{DC}} \). On the other hand, the net electrophoretic drift induced by the voltage \( U(t) \) depends only on the DC electric field, since the AC component averages out. (The contribution of electrorotation can be neglected, as it is largely suppressed;\(^{44}\) see Experimental Section.) Consequently, the DNA molecules are effectively residing in a (quasi-static) dielectrophoretic energy landscape, whose “strength” is controlled by \( U_{\text{AC}} \) and which can be (independently) “tilted” with electrophoretic forces by applying \( U_{\text{DC}} \).

**QUANTIFYING DIELECTROPHORETIC TRAPPING**

The effective polarizability in eq 1 is used to quantify the observed DEP effects. In the following, we explain how it can be derived from the DNA motion in a microfluidic post array like the one presented in Figure 1.\(^{21}\)

For not too large electrophoretic forces, we observe DNA trapping by DEP in the high-field regions in the constrictions (gaps) between two adjacent posts (see Figure 1).\(^{15,21}\) corresponding to \( \alpha > 0 \) (positive dielectrophoresis). Escapes from these traps are driven by the ambient thermal noise and, thus, give rise to a distribution of escape times with a mean escape (or trapping) time \( \tau \). As a central step in our analysis, we assume that \( \tau \) can be related to the potential barrier \( \Delta W_{\text{DEP}} \) characterizing the “depth” of the dielectrophoretic trap according to

\[
\tau \propto \exp(\Delta W_{\text{DEP}}/kT)
\]

with the thermal energy \( kT \) at temperature \( T \) (\( k \) is Boltzmann’s constant). The electrophoretic contribution to the energy barrier, which actually has to be surmounted in order to escape from the trap, is taken into account in the proportionality constant. This approach constitutes a very good approximate description of the present experimental situation as discussed and demonstrated in ref 21.

Recalling that \( W_{\text{DEP}} \propto \alpha U_{\text{AC}}^2 \), we can rewrite eq 2 as

\[
\ln \tau = \text{const.} + c\alpha U_{\text{AC}}^2/kT
\]

The (a priori unknown) proportionality factor \( c \) in eq 3 may be viewed as a way to characterize the particular escape process out of the dielectrophoretic traps. Inspection of the experimental data indicates very weak variations of the escape process with differences in DNA length or conformation, buffer conditions, and static voltages \( U_{\text{DC}} \) (i.e., indistinguishable from experimental and statistical uncertainties in our procedure) but a strong dependence on the driving frequency \( \omega \). Therefore, we can derive valid scaling behaviors from our measurement procedure as

\[
\tau = \langle t \rangle / N
\]

where \( \langle t \rangle \) and \( \langle t \rangle_0 \) are the mean traveling times the DNA molecules need for crossing \( N \) spatial periods in the microstructure, when the dielectrophoretic traps are active, and when the traps are switched off \( (U_{\text{AC}} = 0 \text{ V}) \), respectively. This suggestive relation can be rigorously derived using techniques from probability theory;\(^{47}\) see Supporting Information.

\( ^{(46)} \) Hornick, C.; Well, G. *Biopolymers* 1971, 10, 2345–2358.
Experimentally, the fluorescence intensity $I(t_i)$ of DNA molecules in a given region of interest is recorded for a sequence of time instances $t_i$. (See Supporting Information, inset in Figure S-1 for an example.) The location of the region of interest is chosen such that the DNA has to pass $N = 4$ spatial periods (traps) before reaching it. The mean traveling time is evaluated via

$$
\langle t \rangle = \frac{1}{1} \sum_{t_i} t_i H(t_i)
$$

Such experiments are performed for specific values of $U_{AC}$, in particular $\langle t \rangle^0$ is obtained from a measurement with $U_{AC} = 0$ V. Then, $\tau$ can be calculated from eq 4. The trapping times $\tau$ determined in this “automatic” way agree with the trapping times obtained by manual particle tracking within the error bars (see Supporting Information, Figure S-1).

**EXPERIMENTAL SECTION**

**Materials.** The negative photoresist SU-8 (50), SU-8 developer, and thinner GBL were obtained from Microresist, Germany. Poly(dimethylsiloxane) (PDMS) Sylgard 184 was purchased from Dow Corning, Midland, Michigan. Glass microscope slides were obtained from Menzel, Germany, and 0.4 mm Pt wire was from VWR, Germany. Disodium hydrogen phosphate dihydrate, sodium chloride, $\beta$-mercaptoethanol, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, Germany. YOYO-1 for DNA staining was obtained from Molecular Probes, USA. Aminopropyltriethoxysilane (APTES) was obtained from Aremco, New York. Poly(dimethylsiloxane) (PDMS) Sylgard 184 was purchased from Dow Corning, Midland, Michigan. Glass microscope slides were obtained from Menzel, Germany, and 0.4 mm Pt wire was from VWR, Germany. Disodium hydrogen phosphate dihydrate, sodium chloride, $\beta$-mercaptoethanol, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, Germany. YOYO-1 for DNA staining was obtained from Molecular Probes, USA. Aminopropyltriethoxysilane (APTES) was obtained from Aremco, New York. Poly(dimethylsiloxane) (PDMS) Sylgard 184 was purchased from Dow Corning, Midland, Michigan. Glass microscope slides were obtained from Menzel, Germany, and 0.4 mm Pt wire was from VWR, Germany. Disodium hydrogen phosphate dihydrate, sodium chloride, $\beta$-mercaptoethanol, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, Germany. YOYO-1 for DNA staining was obtained from Molecular Probes, USA. Aminopropyltriethoxysilane (APTES) was obtained from Aremco, New York.

**DNA and Buffer Solutions.** The quality of the plasmids was checked by agarose gel electrophoresis (1% agarose, run time 1 h, 120 V, 40 mM Tris, 20 mM acetic acid, 1 mM EDTA) and capillary gel electrophoresis (parameters given in ref 31). The supercoiled DNA samples were composed of at least 82% supercoiled DNA. (See Supporting Information Table S-1.) For on-chip experiments, DNA samples had concentrations of 20 pM (except for conformational separation with 24 and 42 pM for linear and supercoiled DNA, respectively) and approximately one YOYO-1 molecule per 10 basepairs in 10 mM phosphate solution (pH 8.3, 2 mM NaCl, 1 mM EDTA, 0.2% (v/v) $\beta$-mercaptoethanol, 0.1% POP-6). Such DNA samples were used for measuring DNA polarizabilities and for performing the separation experiment in the microchip, which before was filled with the 10 mM phosphate solution.

A DNA concentration of 20 pM corresponds to a highly diluted solution such that interactions between molecules are negligible and the DNA molecules do not significantly influence solution properties.

**Soft lithography and Microfluidic Chip.** The microfluidic device (see Figure 1) was fabricated from poly(dimethylsiloxane) (PDMS) by soft lithography as reported in ref 49. Briefly, the inverted relief of the microstructure was created on a Si-wafer using contact photolithography of SU-8. PDMS was cast on that master wafer, and after curing for 4 h at 85 °C, the structured PDMS slab was peeled off and reservoir holes were punched (2 mm diameter). The PDMS slab and a glass slide were spin coated with a thin layer of PDMS, then cleaned in acetone, ethanol, and deionized water in an ultrasonic bath, oxidized in a home-built oxygen plasma chamber for 30 s (50 kV, 500 kHz, 0.1 mbar oxygen, distance of electrodes 6.2 cm), and brought into contact. A polymethylmethacrylate (PMMA) block (56 $\times$ 80 $\times$ 5 mm$^3$) with integrated reservoirs and Pt electrodes was placed on top of the PDMS device. The channel walls were coated with a solution of 3 μM SIL-3400 for 15 min. Afterward, the device was flushed with 10 mM phosphate solution (pH 8.3, 2 mM NaCl, 1 mM EDTA, 0.2% (v/v) $\beta$-mercaptoethanol, 0.1% POP-6). Then, for performing the experiments, reservoirs 1, 2, and 3 were filled with phosphate solution and reservoir 4 was filled with fluorescently labeled DNA (20 pM in the same phosphate solution, see also previous paragraph).

**Fluorescence Image Acquisition.** An Axiovert 200 microscope (Zeiss, Germany) with a motorized x/y-stage (99S008, Luidl Electronic Products, USA), a 100x oil-immersion objective (Plan Neofluar, NA 1.30 Zeiss, Germany), a mercury arc lamp (HBO 100) for excitation of stained DNA molecules, a gray filter (transmittance 25%) for reduction of incident light intensity, and a fluorescence filter set (BP 450–490, FT 510, BP 515–565, Zeiss, Germany) were used for single DNA molecule observation. A CCD interline-transfer camera (Imager3LS, LaVision, Germany) with corresponding grabber card and software (DaVis 6.2) was used for data acquisition with an 8 by 8 binning and 10 frames per second (fps) for polarizability measurements and 5 fps for conformational separation. Note that the observed region of interest only captures DNA molecules within about half of the total channel width in the y direction.

**Applied Voltages.** The voltage signal was created via a LabView 6i program and function generator DS345 (Stanford Systems, USA). The output signal was amplified by a high voltage amplifier (AMT-1B60-L Matsusada Precision, Japan) and applied to the Pt electrodes. All experiments were performed with constant $U_{BC} = 12$ V and $\omega = 60$ Hz except for Figure S-3 (Supporting Information), where the frequency dependence is studied.

**DNA Polarizability.** Defined plugs of DNA fragments (20 pM DNA; for buffer conditions, see DNA and Buffer Solutions) were injected into the microstructured channel via a two step pinched injection protocol. First step: voltages of about 10–15 V were applied at reservoirs 1, 3, and 4 while reservoir 2 was grounded.

Second step: a time dependent voltage signal \( U(t) \) was applied to reservoir 1, while reservoirs 2, 3, and 4 were grounded. With every injected DNA volume, a different \( U_{AC} \) was applied with constant \( U_{DC} = 12 \) V and \( \omega = 60 \) Hz. The migration of DNA molecules through the post array was observed, and a fluorescence intensity profile was recorded at a predefined position in the microstructured channel. (See inset of Figure S-1 (Supporting Information), rows 5–8 of the post array visible in the microscopy image.) Afterward, all molecules in channel 3 were transported into reservoir 2, before a new DNA plug was injected.

Conformational DNA Separation. A defined plug of DNA (24 pM linear DNA, 42 pM ccc DNA; for buffer conditions, see DNA and Buffer Solutions) was injected into the microstructured channel via pinched injection. During DNA migration through the post array, \( U_{AC} \) was increased from 270 to 360 V in steps of 6 V every 13 s (constant \( U_{DC} = 12 \) V and \( \omega = 60 \) Hz). The site of observation was chosen at the end of the post array. Shortly before DNA fragments reached the end of the post array, \( U_{DC} \) was set to zero and \( U_{AC} \) was set to 420 V to permanently trap all DNA molecules. Then, channel 3 was scanned for fluorescence signals with a scanning velocity of 10 \( \mu \)m/s. The spatial position along the x axis (see Figure 7) was determined using the scanning velocity and the defined starting point.

Diffusion Coefficients and Radius of Gyration. DNA molecules at a concentration of 20 pM were injected via pinched injection into channel 3 (for buffer conditions, see DNA and Buffer Solutions). All voltages were switched off. First, it was assured that no net fluid flow could be observed. Then, the Brownian motion of the noninteracting DNA molecules was imaged with 10 fps for 60 s in the microstructured region. Only trajectories unaffected by the posts were considered. After observation, the fragments were flushed into reservoir 2 and a new plug of DNA was injected. This procedure was repeated 10–15 times. The trajectories of the single molecules were extracted with the plugin MTrack 2 for ImageJ (1.41o; at least 50 molecules). Using the relation \( D = \langle \tau^2(t) \rangle / 4 t \) (for 2 dimensions), with \( \tau(t) = \tau(x(t), y(t)) \) being the position at time \( t \) (position \( x(t) = 0 \), \( y(t) = 0 \) for time \( t = 0 \) with \( \tau(0) = 0 \))\(^2 \), the diffusion coefficient can be extracted from the slope of the plot \( \tau^2(t) \) against \( t \). Employing the Einstein–Smoluchowski relation \( D = k T / (6 \pi \eta R_g) \) with Boltzmann’s constant \( k \), room temperature \( T = 295 \) K, and the viscosity of the solution \( \nu = 1 \) mPa·s, the hydrodynamic radius \( R_g \) can be calculated. The latter is related to the radius of gyration by \( R_g = 1.51 \cdot R_h \).\(^9\)

AFM Imaging. AFM images of the plasmids were taken on mica silanized with APTES. Sample volumes of 2 \( \mu \)L of DNA were incubated on the surface for 4 min at a sample concentration of 1 \( \mu \)g per mL. The images were taken in tapping mode with a NanoScope IIIa multimode AFM (Digital Instruments - Veeco, USA).

**RESULTS AND DISCUSSION**

**DNA Polarizability.** Polarizabilities of supercoiled DNA molecules (7–21 kbp) were determined according to the procedure described above. In the case of linear DNA, the data for four fragments published in ref 21 are completed by an additional linear fragment of length 12.2 kbp, which is studied here in its supercoiled conformation as well. The results are summarized in Table 1. Our findings constitute a direct comparison of DNA polarizabilities for different conformations measured with the same technique under identical experimental conditions.

The polarizabilities for linear and supercoiled DNA are of the same order of magnitude. In the case of the two 12.2 kbp DNA fragments with different conformation, for instance, they are similar within the error margins. Nevertheless, these two 12.2 kbp fragments are distinguishable via DEP effects as demonstrated below by their dielectrophoretic separation (see Application: Conformational DNA Separation).

The polarizabilities obtained from our measurement procedure are consistent with data reported in the literature for linear DNA in the size range studied here (5.5 \( \times \) 10\(^{-31} \) Fm\(^2\) to 3 \( \times \) 10\(^{-27} \) Fm\(^2\) for 4.4 to 40 kbp), although they have been obtained by completely different techniques like transient electric birefringence, conductivity dispersion, or dielectric response, under different buffer conditions and for different frequency regimes (Hz to MHz). Probably most comparable to our study, Tuukkanen et al.\(^18\) determined the polarizability of linear DNA (8.4 kbp) from dielectrophoretic trapping experiments in a microelectrode array. Their approach is not based on measuring trapping times, but instead, they assume the dielectrophoretic potential barrier to be equal to the thermal energy for voltages at which DNA trapping is just observed.\(^18\) Considering the different buffer conditions (3 mM Hepes, 2 mM NaOH, pH 6.9), our results correlate reasonably well with their measurement (2 \( \times \) 10\(^{-30} \) Fm\(^2\)). For supercoiled DNA, we compare to Bakewell et al.,\(^27\) who studied 12 kbp DNA at 137 kHz using time domain reflectometry. Their result for the polarizability (7.88 \( \times \) 10\(^{-30} \) Fm\(^2\)) again is in reasonable agreement with our findings.

For both, linear and supercoiled conformation, the polarizability \( \alpha \) increases with molecular weight, see Table 1. The dependence of the polarizability on the number of base pairs can be analyzed by a scaling law, \( \alpha \sim N^\gamma \). Different exponents \( \gamma \) ranging from 1 to 3 are discussed in the literature for short linear DNA fragments between 120 and 5000 bp. For the linear DNA sizes we studied, a scaling exponent \( \gamma = 0.4 \pm 0.1 \) is obtained, close to the Flory exponent of 0.5 with negligible excluded volume effects. (See Figure 3a.) As shown in Figure 3b, the supercoiled DNA demonstrates a different scaling behavior with \( \gamma = 2.0 \pm 0.4 \).

In obtaining this exponent, we omitted the data point for the 21 kbp plasmid. This “special treatment” of the 21 kbp DNA is discussed and justified below, but here, we already point out that it has almost the same polarizability as the 15.5 kbp plasmid (see also Table 1) despite its notably different size.

**DNA Diffusion.** In order to obtain information about the spatial extension of the linear and supercoiled DNA fragments used in this study, we also determined their diffusion coefficients;
For the 12.0 and 12.2 kbp fragments (exponent for the linear DNA of length (10.3 kbp with \(\nu\) of a different supercoiled configuration. (See main text.)

Diffusion coefficient of 1.98 DNA fragment of length 11.1 kbp, Robertson et al. obtained a due to the fact that the proportion of supercoiled DNA in the

\[ D = \frac{1.08 \pm 0.04 \text{ m}^2/\text{s}}{12.2} \]

(Samples used in our study contained 82–94% supercoiled DNA; see Supporting Information Table S-1.)

The results are summarized in Table 2. They compare very well to the diffusion coefficients measured with video tracking methods by Robertson et al.\(^{13}\) For instance, they found 1.17 m^2/s for a linear 11.1 kbp fragment (corrected for the higher viscosity of 1.2 mPa·s used in ref 13), in good agreement with our result for the 12.0 and 12.2 kbp fragments (\(D = 1.36 \pm 0.10 \text{ m}^2/\text{s}\) and \(D = 1.09 \pm 0.07 \text{ m}^2/\text{s}\), respectively). For the supercoiled DNA fragment of length 11.1 kbp, Robertson et al. obtained a diffusion coefficient of 1.98 m^2/s, which is somewhat larger than our finding for the supercoiled fragment of comparable length (10.3 kbp with \(D = 1.32 \pm 0.05 \text{ m}^2/\text{s}\) and 12.2 kbp with \(D = 1.08 \pm 0.04 \text{ m}^2/\text{s}\)). This slight discrepancy may be (partly) due to the fact that the proportion of supercoiled DNA in the sample used by Robertson et al. was only about 50–80%.\(^{13}\) (Samples used in our study contained 82–94% supercoiled DNA; see Supporting Information Table S-1.)

The scaling of the diffusion coefficients with the number of base pairs is shown in Figure 4. Recalling that \(D\) scales inversely proportional to the radius of gyration \(R_g\),\(^9\) we find a Flory exponent for the linear DNA of \(\nu = 0.45 \pm 0.05\). For comparison, exponents from \(\nu = 0.48 \pm 0.53\) have been obtained by light scattering methods (fragment lengths between 16 and 293 kbp),\(^{31,52}\) whereas Robertson et al.\(^{13}\) found \(\nu = 0.571 \pm 0.014\) for fragment sizes ranging from 5.9 to 287.1 kbp.

It is further worth noting that the Flory exponent of 0.45 determined from our measurements of the diffusion coefficients indicates a scaling behavior without major contributions from excluded volume effects. This is consistent with an approximate Debye length of about 3 nm for our experimental conditions.\(^{12,13,53}\)

For such a Debye length, Marko and Siggia\(^{12}\) estimated a marginal contribution of excluded volume effects, only for fragments >100 kbp.

For the supercoiled DNA fragments studied here, we find a Flory exponent \(\nu = 1.5 \pm 0.1\). (See Table 2.) Remarkably, Robertson et al.\(^{13}\) found a completely different scaling exponent of \(\nu = 0.571 \pm 0.057\) for supercoiled fragment sizes from 11.1 to 287.1 kbp. In the following section, we discuss these puzzling findings in more detail.

**Discussion and Interpretation.** First, we focus on the linear DNA fragments. Motivated by the scaling behavior of their polarizabilities with an exponent similar to the Flory exponent, we relate the polarizability directly to the radius of gyration \(R_g\) which is derived from the diffusion coefficients. As shown in Figure 5, we find an exponent of 0.9 \pm 0.1, i.e., a nearly linear relationship between the polarizability and the radius of gyration.

This result strongly supports the scaling with the end-to-end distance proposed by Bowers and Prud’homme.\(^{54}\) Therefore, a polarized linear DNA fragment (in the length range studied here) can be imagined to look like a large dipole with a size similar to the diameter (2\(R_g\)) of the globular DNA fragment. A classical dipole moment is defined as \(\vec{p} = q \cdot \vec{l}\) where \(\vec{p}\) is the dipole and \(q\) the charge separated by \(\vec{l}\). Combining this with the induced dipole moment \(\vec{p} = \alpha \vec{E}\), the displaced charge can be calculated when approximating \(\vec{l} = 2R_g\). We find that only 10 elementary charges make up the induced dipole at a voltage \(U_{AC} = 100\) V regardless of fragment size. Hogan et al.\(^{45}\) estimated 11 charges to be displaced along a 230 bp (78 nm) DNA fragment. A similar number of 18 charges has been derived in ref 25, however, with a charge displacement over the Kuhn length of only 130 nm (two times the persistence length) determined within a 40 kbp fragment.

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For supercoiled DNA, the situation is more complex, in particular in view of the puzzling results obtained for the longest plasmid fragment with 21 kbp, which are not compatible with the data for the shorter fragments; see Tables 1 and 2 and Figures 3b and 4b. In order to get more information about their actual spatial configuration, we examined the supercoiled DNA fragments by atomic force microscopy (AFM). (See Figure 6.) The AFM images show that the three smaller fragments (7, 10.5, 15.3 kbp) have a branched plectonemic structure, i.e., a star-like spatial arrangement with clearly visible “arms”, whereas the 21 kbp plasmid shows a more random coil like configuration. For comparison, we imaged another slightly longer plasmid with 23 kbp and again found a more coil like configuration. (See Supporting Information, Figure S-2.) A transition from a plectonemic to a random coil like structure is theoretically expected in the range of about 25 kbp. Therefore, both the polarizability and the diffusion coefficient apparently differentiate less strongly between “linear” and “supercoiled” but more pronounced between “random coil like” and “plectonemic” configurations. Actually, the results for α and D of the supercoiled 21 kbp plasmid with its more coil like configuration would perfectly fit into the scaling plots in Figures 3a and 4a for the linear DNA fragments. This interpretation justifies why we did not consider the 21 kbp for the scaling behavior of the plasmid fragments. Moreover, it may also explain the considerably different scaling exponent of D versus the number of base pairs we obtained for the supercoiled fragments in comparison to the result by Robertson et al. These authors considered supercoiled DNA fragments larger than 25 kbp in their scaling analysis (except for one fragment of length 11.1 kbp) and indeed found an exponent identical to what was found for linear (random coil like) DNA.

The scaling of the polarizability with the radius of gyration for the plectonically supercoiled DNA fragments is best described with an exponent of 1.6 ± 0.2. (See Figure 5b.) This scaling behavior is remarkably different from that found for the linear fragments and, thus, hints to a different mechanism of polarization. We speculate that the polarization process in this case involves a significant deformation of the plectonemic configuration, e.g., a

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**Figure 4.** Log–log plot of the diffusion coefficient versus the number of base pairs of (a) linear DNA, with an experimentally determined Flory exponent of $\nu = 0.45 \pm 0.05$ (apart from a negative sign which is due to plotting $D$ instead of $R_g$) and (b) ccc DNA with scaling exponent $\nu = 1.5 \pm 0.1$. Data of the 21 kbp DNA (×) is omitted in the fitting procedure because of a different supercoiled configuration. (See main text.)

**Figure 5.** Log–log plot of the polarizability versus the radius of gyration as determined from the diffusion coefficients (a) for linear DNA, indicating a linear relation in the scaling behavior according to $\alpha \sim R_g^{0.9 \pm 0.1}$; (b) for supercoiled DNA fragments, we find $\alpha \sim R_g^{1.6 \pm 0.2}$. Data of the 21 kbp DNA (×) is omitted in the fitting procedure because of a different supercoiled configuration. (See main text.)
The first three samples (7, 10.3, and 15.5 kbp) show a clear branched plectonemic structure ("stars"). The 21 kbp DNA fragment is also supercoiled but has a random coil like configuration.

Our findings for the polarizability and its scaling behavior have been obtained by analyzing the escape times of the DNA molecules from the dielectrophoretic traps. As already mentioned, these escape times are the combined result of the molecules’ polarizability and the details of the escape dynamics out of the traps, which both in general depend on the frequency ω of the applied AC voltage component. Provided all measurements are performed at identical, fixed frequency (ω = 60 Hz in our case), these two contributions can be disentangled by the approximative approach described in Quantifying Dielectrophoretic Trapping (and in ref 21), allowing a systematic comparison of polarizability data for different DNA sizes and conformations. Studying different frequencies, Chou et al. observed a nonmonotonic dependence of the trapping force (which is proportional to ΔW_{DEP} in eq 2) for several DNA fragments within a frequency range of 0–1000 Hz. A simple, overview-like measurement, which we performed for frequencies between 0 Hz and 160 kHz in order to determine an optimal trapping frequency for the detailed studies presented above, yields similar results; see Supporting Information. However, a more thorough and systematic study of the frequency dependence of the polarizability over a broad frequency range would require considerable refinement of the theoretical model (taking into account the influence of frequency on the escape process out of the dielectrophoretic traps) and of the experimental setup (since electrodeless techniques are not well suited for high frequency measurements, e.g., in the MHz range) and is beyond the scope of the present manuscript.

**Application: Conformational DNA Separation.** The general problem in separating DNA is the independence of the electro-

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**Figure 6.** AFM images of four different supercoiled DNA fragments. The first three samples (7, 10.3, and 15.5 kbp) show a clear branched plectonemic structure ("stars"). The 21 kbp DNA fragment is also supercoiled but has a random coil like configuration.

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**SUMMARY**

Polarizabilities and diffusion coefficients were determined for linear and supercoiled DNA species. Different power law scaling behaviors could be observed with respect to DNA conformation. For linear fragments, the results imply a scaling of the polarizability with the radius of gyration. For supercoiled DNA, the situation is more complex. As unraveled with the help of AFM images, the polarizability as well as the coefficient of diffusion

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critically depend on the actual spatial conformation of the super-coiled DNA fragments, i.e., plectonemic or random coil like. The differences in the dielectrophoretic properties found among the distinct DNA species were finally exploited to separate DNA topoisomers according to their conformation within a separation time of 210 s.

ACKNOWLEDGMENT

Donation of the plasmid samples by Dr. Martin Schleef and Dr. Marco Schmeer from PlasmidFactory GmbH & Co. KG, Bielefeld, is gratefully acknowledged. Help from Henning Höfemann, who determined the frequency dependence of the super-coiled DNA fragments, is gratefully acknowledged. We thank Rafael Szczepanowski for preparation of the PJP2 plasmid and Lisa Czaja and Robert Ros for AFM imaging. Special thanks are dedicated to Prof. Peter Reimann and Prof. Friederike Schmid for fruitful discussions. Financial support from the Deutsche Forschungsgemeinschaft within the collaborative research project SFB-613 (project D2) is gratefully acknowledged.

SUPPORTING INFORMATION AVAILABLE

Information available concerning materials, the derivation of the mean traveling time, the frequency dependence of trapping, and a spiking experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review March 1, 2010. Accepted July 7, 2010.

AC1005475